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Structural aspects of the ribosome evolution and function

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Structural aspects of the ribosome evolution and function

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Résumé

En 2000, les structures à hautes résolutions des deux sous-unités ribosomiques ont finalement été mises à la disposition du public. L'année suivante, la structure aux rayons X de l'ensemble du ribosome bactérien a été publiée. Ces grandes réalisations ont ouvert une nouvelle ère dans l'étude des mécanismes de la synthèse des protéines. Dès lors, il est devenu possible de relier différents aspects de la fonction du ribosome à des éléments particuliers de sa structure tertiaire. L'établissement de la relation structure-fonction peut toutefois être problématique en raison de l'immense complexité de la structure du ribosome. En d'autres termes, pour que les données cristallographiques sur la structure tertiaire du ribosome soient vraiment utiles à la compréhension du fonctionnement du ribosome, ces données devraient elles-mêmes faire l'objet d'une analyse approfondie. Le travail, présenté ici, peut être vu comme une tentative de ce genre. En appliquant l'analyse systématique des structure cristallographiques du ribosome disponibles, nous avons essayé de résoudre deux problèmes fondamentaux de la biologie ribosomale concernant (1) la nature des réarrangements du ribosome qui ont lieu à différentes étapes de son cycle de fonctionnement et (2) la possibilité de reconstitution de l'évolution du ribosome du monde-à-ARN jusqu'à nos jours.

Dans le premier projet, nous avons systématiquement comparé les structures du ribosome disponibles et de sa sous-unité afin d'identifier les domaines rigides, qui ont toujours la même conformation, et les régions flexibles dont la conformation peut varier d'une structure de ribosome à une autre. Il y a deux types de réarrangements structuraux connus dont nous voulions comprendre les mécanismes: le « ratchet-like movement » et la «fermeture de domaines ». Le premier a lieu au cours de la translocation du ribosome et est plus ou moins perçu comme une rotation d'une sous-unité par rapport à l'autre. Le deuxième se produit dans la petite sous-unité et est associé à la reconnaissance codon-anticodon au site A.

La comparaison des conformations ribosomales disponibles a révélé les mécanismes spécifiques des deux réarrangements. Bien que la sélection de l'aminoacyl-ARNt appropriée au site A et la translocation du ribosome n'ont jamais été considérés comme ayant quelque chose en commun, nous démontrons ici que les réarrangements de la structure des ribosomes associés au premier processus répète les réarrangements associés au deuxième mais dans l'ordre inverse. En d'autres termes, pendant le cycle d'élongation, la fermeture de domaine et le « ratchet » peuvent être considérés comme un mouvement de va-et-vient, qui renvoie finalement le ribosome à sa conformation initiale.

Dans le second projet, nous avons fait une tentative de reconstitution de l'évolution de l'ARNr 23S, du monde-à-ARN jusqu`à nos jours. Ici nous nous sommes basés sur la supposition que l'évolution de cette molécule a procédé par des insertions aléatoires des régions relativement courtes dans différentes parties de la chaîne poly-nucléotidique. Pour cela, nous avons élaboré des critères de l'intégrité de la structure ribosomale et présumé que lors de l'évolution, la structure du ribosome s’est toujours adaptée à ces standards. Nous avons examiné l'interaction de type A-mineur, un arrangement fréquent dans la structure de l’ARN ribosomique, constitué d'un empilement d’adénosines non-appariées, attachées à une double hélice. Nous avons supposé que dans toutes les interactions A-mineurs existantes dans le ribosome, la double hélice est apparue avant ou au moins simultanément avec la pile d’adénosines correspondantes. L'application systématique de ce principe à la structure tertiaire de l’ARN 23S a permis d'élucider de manière progressive l'ordre dans lequel les parties différentes de l’ARN 23S ont rejoint la structure.

Pris ensemble, les deux projets démontrent l'efficacité de l'analyse systématique *in-silico* de la structure tertiaire du ribosome et ouvrent la voie à de futures découvertes.

Mots clés: évolution, la structure du ribosome tertiaire, l'ARN ribosomal, le mouvement de cliquet, la fermeture de la petite sous-unité

SUMMARY

In the year 2000, the first high-resolution structures of the individual ribosomal subunits became available to the public. The following year, the X-ray structure of the complete bacterial ribosome was published. These major achievements opened a new era in studying the mechanisms of protein synthesis. From then on, it became possible to attribute different aspects of the ribosome function to particular elements of its tertiary structure. However, establishing the structure-function relationships is problematic due to the immense complexity of the ribosome structure. In other words, in order to make the crystallographic data on the ribosome tertiary structure really useful for understanding of how the ribosome functions, it must be thoroughly analyzed. Here, based on systematic analysis of the available X-ray conformations of the ribosome we have tried to resolve two fundamental problems of the ribosome biology: concerning (1) the nature of rearrangements in the ribosome that take place at different steps of its functional cycle, and (2) the reconstruction of the ribosome evolution from the RNA world to present time.

In the first project, we systematically compared the available structures of the ribosome and its subunits to identify rigid domains, which always have the same conformation, and flexible regions, where the conformation can vary from one ribosome structure to another. There were two known types of structural rearrangements whose mechanisms we wanted to understand: the ratchet-like motion and the so-called domain closure. The ratchet-like motion takes place during the ribosomal translocation and is roughly seen as a rotation of one subunit with respect to the other. The domain closure occurs in the small subunit and is associated with the cognate codon-anticodon recognition in the A-site.

Comparison of the available ribosome conformations revealed the detailed mechanisms of both rearrangements. Although the selection of the cognate amino-acyl-tRNA in the A-site and of the ribosomal translocation have never been thought to have anything in common, we demonstrate that the rearrangements in the ribosome structure associated with the first process repeat in reverse order the rearrangements associated with the second process. In other words, during the ribosome elongation cycle, the domain closure and the ratchet-like motion can be seen as a back-and-forth movement, which eventually returns the ribosome to the initial conformation.

In the second project, we attempted to reconstruct the evolution of the 23S rRNA from the RNA world to present time based on the presumption that the evolutionary expansion of this molecule proceeded though random insertions of relatively short regions into different regions of the polynucleotide chain. We developed criteria for integrity of the ribosome structure and presumed that during the evolutionary expansion, the ribosome structure always matched to these standards. For this, we specifically considered the A-minor interaction, a frequent arrangement in the rRNA structure consisting of a stack of unpaired adenosines tightly attached to a double helix. We presumed that in all A-minor interactions present in the ribosome, the double helix emerged before or at least simultaneously with the corresponding adenosine stack. The systematic application of this principle to the known tertiary structure of the 23S rRNA allowed us to elucidate in a step-vise manner the order in which different part of the modern 23S rRNA joined the structure.

Taken together, the two projects demonstrate the effectiveness of the systematic *in-silico* analysis of the ribosome tertiary structure and pave the way for future discoveries.

Key words: evolution, ribosome tertiary structure, ribosomal RNA, ratchet-like motion, small subunit domain closure

Table of contents

page

List of Tables

page

Chapter 3

List of Figures

page

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of abbreviations

16S	rRNA of 30S subunit
23S and 5S	rRNAs of 50S subunit
30S / 40S	SSU in prokaryotes / eukaryotes
50S / 60S	LSU in prokaryotes / eukaryotes
70S / 80S	ribosome in prokaryotes / in eukaryotes
Å	angstrom (1^{-10} m)
aaRS	aminoacyl tRNA synthetase
aa-tRNA	aminoacyl-tRNA (tRNA that carried amino acid)
AGPM	along-groove packing motif
A-site	ribosomal aa-tRNA binding site
ASL	anticodon stem-loop in tRNA
ATP	adenosine triphosphate
cryo-EM	cryo electron microscopy
DNA	deoxyribonucleic acid
E-site	ribosomal “exit” site for deacylated tRNA
GTP	guanosine triphosphate
GTPase	enzyme, requiring GTP as a cofactor in functioning
h44	helix in SSU, carrying essential part of subunit interface
h44c	central part of helix 44
LSU	large ribosomal subunit
LUCA	Last Universal Common Ancestor
microsome	another name for the ribosome
mRNA	messenger RNA
poly-Lys	poly-lysine
poly-Phe	poly-phenylalanine
poly-U	polyuridylic acid

P-site	ribosomal p-tRNA binding site
PTC	peptidyl-transferase center in LSU
p-tRNA	peptidyl-tRNA (tRNA that carried peptide chain)
RLM	ratchet-like motion
RMSD	root-mean square deviation
RNA	ribonucleic acid
r-protein	ribosomal protein
rRNA	ribosomal RNA
S	Svedberg (unity of sedimentation)
SSC	domain closure in SSU
SSU	small ribosomal subunit
tRNA	transfer RNA
$\text{tRNA}^{\text{fMet}}$	formylmethionyl-tRNA
tRNA^{Ala}	alanyl tRNA,
WC	Watson-Crick base pairing

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Chapter 1. Introduction

The life on Earth is based on the protein synthesis. Proteins catalyze practically all chemical reactions in the cell. They play key roles in cellular metabolism and perform numerous structural and regulatory functions. Proteins are ubiquitous: a human cell can contain up to 10,000 different types of proteins, some of which are present in billions of copies (*Yonath-2010*). To fulfill the cellular need for proteins, they must be continuously synthesized by the ribosome, a universal cellular machine existing in all organisms (*Spirin-1999*) (Figure 1). It is due to the ribosome that life on Earth in its present form has become possible.

In mid-50s, when the studies of the mechanisms of protein synthesis began, the ribosome was nothing more than a "spot on the gel". Our understanding of what the ribosome looks like and how it functions was accumulating rather slowly. The breakthrough in the study of the ribosome structure and function was made due to the progress in the ribosome crystallisation, which eventually resulted in the atomic level of elucidation of the ribosome tertiary structure. The available crystallographic data opened a new era in the analysis of the mechanism of protein synthesis and in the understanding of the nature of life on our planet.

Below, we describe how the concept of the ribosome as the universal factory of proteins gradually evolved up to present days. We will see how the scientific achievements in this area lead to the research presented in this thesis.

Microsomes, RNA and the cell activity in protein synthesis

Since late 1930-s, a number of studies demonstrated that protein synthesis is somehow linked to the nucleic acid content of the cell (*Caspersson-1939, Brachet-1941, Jeener-1941, Jeener-1942, Chantrenne-1943*). In 1944, McCartney and colleagues showed that DNA was the substance of inheritance (*Avery-1944*) and Beadle published his "one gene – one enzyme" hypothesis (*Beadle-1945*). The increased content of cytoplasmic RNA in rapidly growing cells pointed to a link between synthesis of cellular RNA and protein synthesis (*Brachet-1947, Caspersson-1947, Ehrich-1949*).

At the same time, Albert Claude observed small ribonucleic particles (*Claude-1941*), which he named "microsomes" (*Claude-1943*). These particles, which are now known as ribosomes, were originally found in tissues with high enzymatic activity (*for review, see Crick-1958, Zamecnik-*

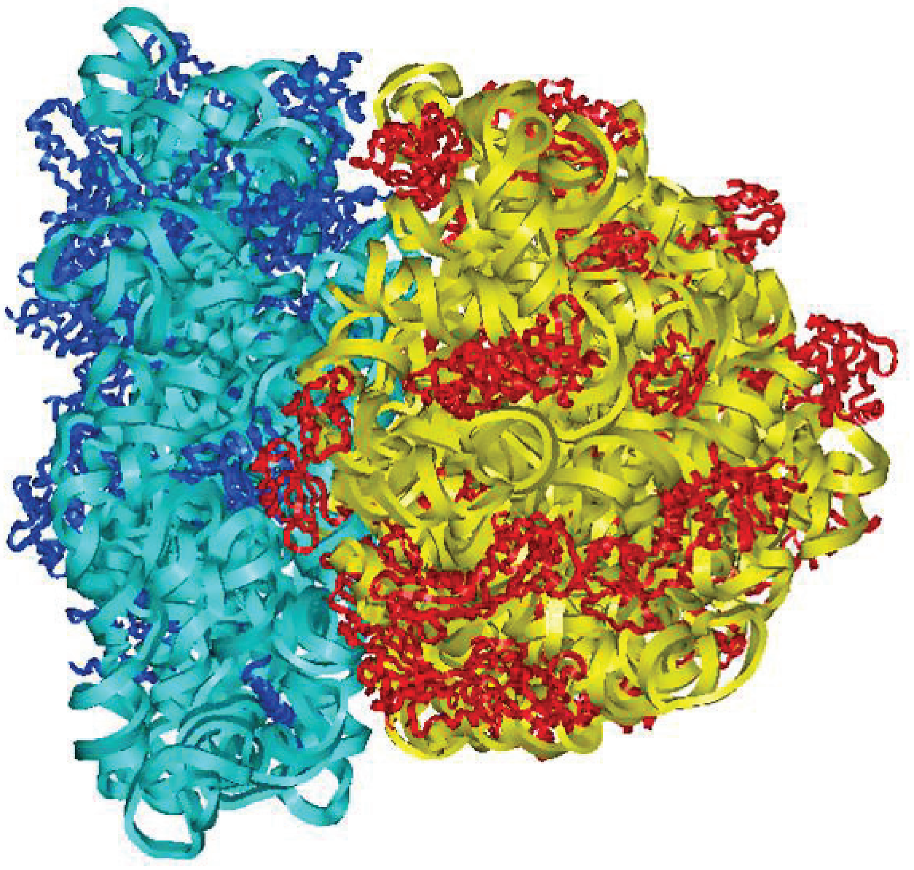


Figure 1.

The tertiary structure of modern bacterial ribosome (ribbon representation of a crystal structure by J.H.Cate (see Zhang-2009)

The ribosomal subunits: SSU - on the left (shown in blue), LSU - on the right. The rRNA of SSU is colored light-blue; that of LSU is yellow. The r-proteins of SSU are dark-blue and those of LSU are red. The pdb access codes for the two subunits are: **3i1S (SSU)** and **3i1T (LSU)**)

1969). Numerous experiments consisting in injection of radioactively-labeled amino acids into such tissues demonstrated a very high rate of amino acids incorporation into new proteins in the presence of microsomes (*Borsook-1950, Hultin-1950, Siekevitz-1952, Allfrey-1953*). These results established a strong link between microsomes and protein synthesis. However, it remained unclear whether the microsomes performed all steps of protein synthesis, or they were involved only in some intermediate steps of the process (*see for example Alfrey-1953*). In 1951, Philip Siekevitz noticed that the incorporation of amino acids occurred in the presence of ATP (*Siekevitz-1951, Siekevitz-1952*). He correlated the ATP consumption with peptide-bond formation.

In 1954, Keller, Zamecnick and Loftfield demonstrated that incorporation of amino acids into proteins occurred *almost entirely* in microsomes (*Keller-1954, Littlefield-1955*). The following year, George Palade published photographs of the microsome made with use of electron microscopy. In these photographs, the microsome consisted of two asymmetric components of about 200 Å in diameter (*Palade-1955*).

DNA, RNA template, transfer RNA and the adaptor hypothesis

The elucidation of the special role of microsomes coincided with the discovery of the double helical conformation of DNA by Watson and Crick. In 1953, these researchers published a model of the double-helical structure of DNA (*Watson-1953a*) and a model of its replication (*Watson-1953b*). This discovery elucidated the mechanism for the storage and copying of genetic information.

Shortly after these two publications, Watson and Rich suggested that DNA is involved in regulation of protein synthesis through formation of RNA intermediates (*Rich-1954*). Later, this hypothesis was presented in a form of The Central Dogma of Molecular Biology (*Crick-1958*, *Crick-1970*) and was further developed by Lockingen and De Busk (*Lockingen-1955*). According to the latter researchers, the “template RNA” was synthetized as a complement to one of the two strands of the DNA double helix and was later used as a template for the synthesis of the corresponding protein.

Strictly speaking, the idea that RNA could function as the template in protein synthesis had been suggested a few years earlier (*Caldwell-1950, Dounce-1952*). In these early works, the formation of the polypeptide chain was proposed to take place directly on the template RNA.

Furthermore, Dounce believed (*Dounce-1952*) that the selection of a particular amino acid depended on three adjacent nucleotides of the template, which inferred the existence of the triplet genetic code (*Gamow-1954*).

By the second half of 1950s, it had still been unclear which cellular RNAs function as genetic templates and how exactly the genetic information is encoded. It had also been unclear how the amino acids are delivered to the site of protein synthesis and which type of energy propels this process.

Between 1956 and 1958, a series of brilliant discoveries was made by the group of Paul Zamechnik. The researchers identified a class of enzymes catalyzing the formation of the aminoacyl-adenylate in animal cells (*Hoagland-1955, Hoagland-1956*). These enzymes, presently known as aminoacyl-tRNA synthetases (aaRS), were also found in yeast (*Berg-1956*), in bacteria (*De Moss-1956, Nismann-1957*) and in plants (*Webster-1957*). Each amino acid appeared to have its own synthetase (*Beljanski-1958, Lipmann-1958, Hoagland-1958b*).

In 1957, Zamechnik and colleagues discovered a special class of so-called *soluble* RNA that in the presence of GTP transferred amino-acids to the microsome (*Hoagland-1957*). Two years later, the soluble RNAs were given a more appropriate name, the "transfer RNAs" (*Smith-1959*) or tRNAs.

In 1958, Zamechnik and co-workers found that each amino acid was carried by a particular tRNA (*Hoagland-1958*). Zamechnik believed that tRNAs could also participate in the incorporation of the cognate amino acids into the synthesized polypeptide (*Hoagland-1958*).

In 1958, at the XII Symposia of the Society for Experimental Biology in London, Crick presented the so-called "adapter hypothesis" (*Crick-1958*). He suggested that the synthesis of a peptide was mediated by a special RNA-adaptor. Crick's adaptor was a three-nucleotide molecule with the nucleotide sequence corresponding to a particular amino acid (*Crick-1958*). Such a molecule, with the attached amino acid, interacted with the complementary three-nucleotide segment in the template, thus positioning the amino acid at the synthesized protein sequence (*Crick-1957, Crick-1958*). The proposed three-base-pair interaction between the template and the adaptor referred to the model suggested by George Gamow in 1954 (*Gamow-1954*), in which each triplet of the template coded for a particular amino acid.

The adaptor hypothesis was a very important step in understanding of the mechanism of protein synthesis. On the other hand, it was later found to be not absolutely correct. Crick thought that the adaptor was formed through splitting of the corresponding tRNA (*Crick-1958, Hoagland-1959*). In other words, according to the Crick's hypothesis, the role of tRNA was reduced to serving as the precursor for the real adaptor (*Crick-1958, Hoagland-1959*).

The discovery of the messenger-RNA and the recognition of the adaptor role of tRNA

In the 1950s, the role of the template in the protein synthesis was thought to be played by the microsomal RNA (*Brachet-1955, Loftfield-1957*). Correspondingly, a given microsome in the cell was responsible for the synthesis of a particular protein (*Brachet-1955, Loftfield-1957, Crick-1958, Hoagland-1959*). The chemical stability of the microsomal RNA was believed to guarantee the synthesis of the protein whenever it was required for the cell.

However, this hypothesis appeared to be wrong. All microsomal RNAs in a given cell were alike, which was contradiction to the known diversity of the genetic pool (*Belozersky-1958*). The latter fact was inconsistent with the proposed role of the microsomal RNA as a genetic template. Besides, the microsomes isolated from different tissues had almost the same size (*Hall -1959*). It was also demonstrated that some damages in DNA affected the synthesis of proteins without affecting the microsomal RNAs. Such a situation would have been impossible if microsomal RNAs represented real templates (*Pardee-1958, Riley-1960*).

In 1958, Arthur Pardee (*Pardee-1958*) questioned the role of the stable microsomal RNA as a protein template. According to Pardee, one template coded for only one copy of the protein (*Pardee-1954, Pardee-1958*). Therefore, the observed stability of the microsomal RNA was not an advantage for its potential role as a template. Instead, the real template had a short lifespan (*Pardee-1958*). A similar idea was suggested by François Jacob and Jacques Monod (*Jacob-1961*), who believed that the real role of the *ribosomal* RNA* was structural, while the genetic information was supplied by a special "messenger" RNA (mRNA) of a different type. The article of Jacob and Monod was still in press, when two groups of researchers reported the experimental evidence of such a messenger (*Brenner-1961, Gros-1961*).

At about the same time, the adaptor hypothesis of Crick was proven. However, the adaptor function was shown to belong to the whole intact tRNA, which was thus recognized as the true adaptor. First, it was demonstrated that the tRNA was never split in smaller pieces. Then, it was shown that after the amino acid incorporation, tRNAs quits the ribosome unaffected (*Hoagland-*

[*The term *ribosome* was proposed by Richard B Roberts and replaced the term *microsome* (*Roberts-1958, p.viii*)]

1960, Zamecnik-1960).

Later, the adaptor hypothesis got a direct confirmation. A cysteine residue attached to its cognate tRNA was converted to alanine and then successfully incorporated into the peptide chain in response to the poly-UG messenger (*Chapeville-1962*).

Genetic code, wobble hypothesis and cloverleaf

The concept of messenger RNA led to a series of experiments with homo-polynucleotides and more complex synthetic mRNAs (*Littauer-1957*) that were used for studying the specificity of different genetic triplets. In the proposed *in vitro* experiments, the ribosome was mixed with a particular polynucleotide followed by determination of the amino acid sequence of the synthesized peptide (*Lamborg-1960*).

The first successful experiment was made by Nirenberg and Matthey (*Nirenberg-1961*). In this case, the mRNA represented a poly-uridine chain (poly-U) and promoted synthesis of poly-phenylalanine. A similar experiment with the poly-A mRNA provided poly-lysine (*Lengyel-1961*). In 1963, important progress was achieved in deciphering the genetic code (*Ochoa-1963, Nirenberg-1963*). However, the real breakthrough happened when Khorana and colleagues found a way to synthesize a messenger RNA having a given nucleotide sequence (*Falaschi-1963, Nishimura-1964*). By the mid-60s, the whole table of the genetic code had been decrypted (*Nirenberg-1965, Khorana-1966*). As expected (*Gamow-1954, Crick-1957*), the genetic code was highly redundant.

In 1966, Francis Crick published the so-called wobble hypothesis (*Crick-1966*) in an attempt to rationalize the redundancy of the genetic code. According to Crick, in the first two positions of the triplet, only the standard Watson-Crick base pairs can be formed, while for the third base pair some wobble in base pairing is allowed (*Crick-1966*). At about the same time, the complete nucleotide sequence of the $tRNA^{Ala}$ was determined (*Holley-1965*). The authors suggested the secondary structure of the tRNA molecule, the famous "cloverleaf", and located the position of the codon in the tRNA structure.

Studies of ribosome morphology in 1960s

From the early 1960s, the ribosome had become an object of extensive analysis (*Tissieres-1960*). Previously, it had been demonstrated that the bacterial and eukaryotic ribosomes, although being of different sizes, have a lot of similarities (*Tissieres-1958*). In particular, each of the two types of ribosomes was composed of two asymmetric components characterized by the sedimentation constants 50S and 30S in bacteria, and 60S and 40S in eukaryotes; RNA occupied two thirds of the ribosome volume and the rest was taken up by proteins; both the activity and the structure of the ribosome were strongly dependent on the presence of magnesium.

In 1960, Huxley and Zubay published electron microscopy images of the asymmetric components of the ribosome (*Huxley-1960*) naming them the large and small subunits (LSU and SSU, respectively). Each subunit was shown to contain one monolithic RNA of a high molecular weight: in the bacterial ribosome, the SSU contained 16S rRNA, while the LSU contained 23S RNA (*Littauer-1959, Littauer-1961, Spirin-1961*), the eukaryotic analogs of these RNA chains were 18S and 28S RNA, respectively (*Hall-1959, Littauer-1961, Maeda-1961*). Soon after, it became clear that LSU contained more than one RNA molecule: a small 5S RNA was found in both prokaryotes (*Rosset-1963*) and eukaryotes (*Marcot-Queiroz-1965, Comb-1965, Galibert-1965*). In contrast, the number of the detected ribosomal proteins in the two subunits exceeded 30 (*Spitnik-Elson-1963, Spitnik-Elson-1964*).

Ribosome function

The activity of the ribosome in protein synthesis was also extensively analyzed. In 1962, Warner, Rich and Hall published the electron-microscope image in which several ribosomes were attached to the same mRNA (*Warner-1962*). Such a cluster of ribosomes was named the "polysome". This study explained the mechanism of simultaneous production of several copies of the same protein (*Warner-1962, Wettstein-1963*).

In 1963, Okamoto and Takanami showed that the mRNA binding site was localized on the SSU (*Okamoto-1963, Takanami-1963*). Conversely, the binding site of the nascent peptide was localized on LSU (*Takanami-1962, Gilbert-1963*). The nascent peptide was covalently linked to the tRNA that delivered the latest amino acid (*Nathans-1961, Takanami-1962, Gilbert-1963*). It was also shown that a molecule of puromycin (*Yarmolinsky-1959*), when added to the ribosome, was able to accept the polypeptide chain from the peptidyl tRNA (*Allen-1962*). It became clear that during protein synthesis, the ribosome can simultaneously contain two tRNAs. Following this study, Gilbert and colleagues postulated (*Cannon-1963*) the existence of a "center of peptide bond formation" in the LSU. The hypothetical center contained two sites for tRNA binding. The two sites were later denoted as: the "P-site" for the peptidyl-tRNA (henceforth, p-tRNA) and the "A-site" for the aminoacyl-tRNA (henceforth, aa-tRNA) (*Watson-1964*). It was suggested the growing peptide was transferred from the p-tRNA to the aa-tRNA. The transfer was accompanied by the addition of the new amino acid to the C-terminus of the nascent protein (*Cannon-1963*).

The hypothesis of Gilbert suggested that after the peptide transfer, the p-tRNA moves from the A- to P-site, thus emptying the A-site for the next aa-tRNA. This led Watson to propose the idea of the mRNA translocation after each peptide transfer, making the next codon ready for decoding (*Watson-1963*) (Figure 2). The p-tRNA was supposed to move together with the mRNA (*Watson-1964*).

The concept of two binding sites obtained wide experimental support (*Arlinghaus-1964, Traut-1964, Warner-1964, Wettstein-1965, Suzuka-1965, Bretscher-1966*). The suggested center of the peptide bond formation (peptidyl-transfer center, or PTC) was localized on the 50S subunit (*Monro-1967b, Pestka-1967*).

Initiation, elongation, and termination in protein synthesis

In 1967, Ghosh and Khorana first described the initiation of protein synthesis in bacteria (*Ghosh-1967*). According to their data, a special initiator formyl-methionyl tRNA ($tRNA^{fMet}$) (*Marcker-1964, Adams-1966, Clark-1966, Webster-1966*) together with mRNA and the SSU formed the initiation complex (*Nomura-1967a*). Then, the complex joined the LSU (*Nomura-1967b*), providing the active 70S ribosome ready to accept the first aa-tRNA (*Hawtrey-1964*).

Initiation in eukaryotes was found to follow the same general path (*Bishop-1966, Brown-1970*), although the role of $tRNA^{fMet}$ was not confirmed (*Caskey-1967, Wilson-1970, Heywood-1970*). Instead, the non-formylated $tRNA^{Met}$ was suggested as the initiator tRNA (*Housman-1970, Jackson-1970, Wigle-1970*).

At the same time, the $tRNA^{fMet}$ appeared to be the universal initiator tRNA in mitochondria and in chloroplasts (*Smith-1968, Burkard-1969*), which is logical given that these organelles are evolutionary descendants of bacteria (*Cohen-1970*).

The elongation of the peptide chain was expected to proceed until a signal in the mRNA for the protein completion is reached. This signal would induce the release of the nascent peptide

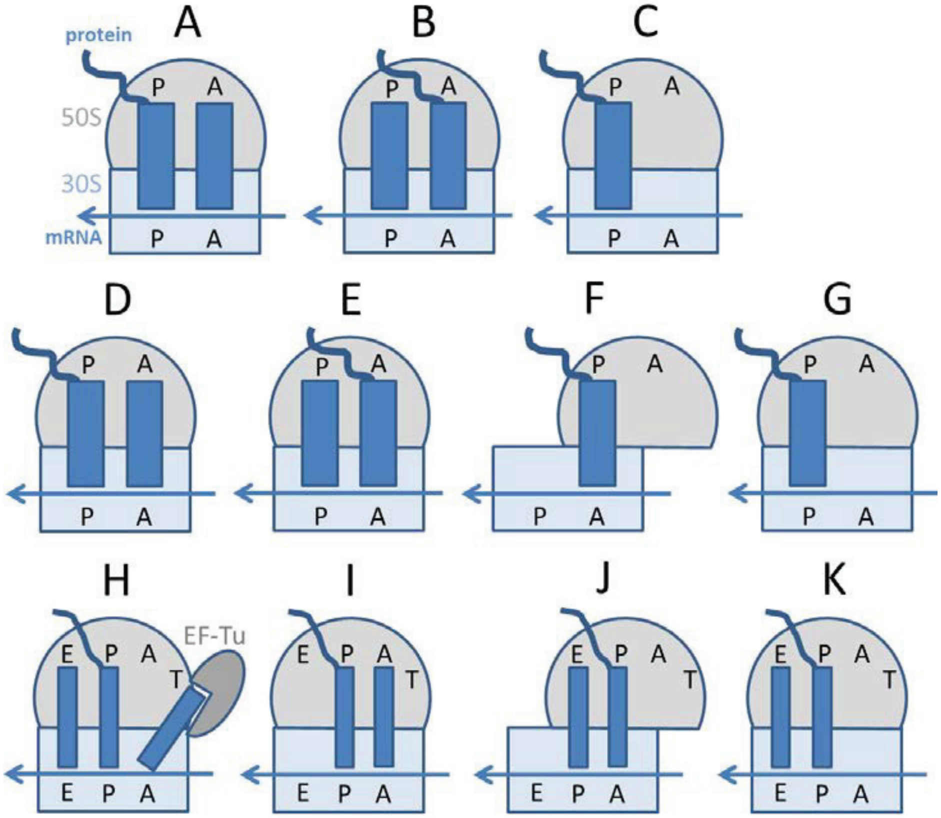


Figure 2. Models of the ribosome translocation

(Upper row) **Model of Gilbert-Watson:** (A) the aa-tRNA in the A-site, the p-tRNA in the P-site; (B) transpeptidation: the p-tRNA in the A-site, the deacylated tRNA in the P-site; (C) translocation: the deacylated tRNA quits the ribosome while the p-tRNA (together with mRNA) shifts to the P-site, thus liberating the A-site for the next aa-tRNA.

(Middle row) **Model of Bretscher:** (D) the aa-tRNA is in the A/A (LSU/SSU) position, the p-tRNA is in the P/P position; (E) transpeptidation: the p-tRNA is in the A/A position, the deacylated tRNA is in the P/P position; (F) the 1st step of the translocation: the deacylated tRNA quits the ribosome, while SSU with the attached p-tRNA moves relative to LSU resulting in the P/A position for the p-tRNA; (G) the 2nd step of translocation: LSU with the attached p-tRNA (together with mRNA) moves relative to LSU, resulting in the P/P position of the p-tRNA and the empty A-site.

(Lower row) **Model of Nierhaus-Noller:** (H) the aa-tRNA recognition: the aa-tRNA (attached to the EF-Tu) is in the A/T position, the p-tRNA is in the P/P position, the deacylated tRNA is in the E/E position; (I) the aa-tRNA is in the A/A position, the p-tRNA is in the P/P position, the deacylated tRNA quits the ribosome; (J) transpeptidation and spontaneous translocation in LSU: the p-tRNA is in the P/A position, the deacylated tRNA is in the E/P position; (K) translocation in SSU: the p-tRNA is in the P/P position, the deacylated tRNA is in the E/E position, the A-site is empty.

to the cytosol (*Ganoza-1966, Tissieres-1960*). In the mid-1960s, three stop codons were identified: UAG (*Sarabhai-1964, Stretton-1965*), UAA (*Brenner-1965*) and UGA (*Brenner-1967*).

Because the initiation of a new round of translation requires that the ribosomal subunits be isolated, it was deduced that the translational termination is followed by the dissociation of the ribosomal subunits (*Kaempfer-1968, Colombo-1968, Subramanian-1968, Kaempfer-1969*).

At the same time, evidence suggested that each step of protein synthesis is assisted by a particular protein factor (*Lucas-Lenard-1971*).

Translation factors

Elongation factors

Already in 1958 it was initially observed that the transfer of amino acid to the ribosome was accompanied by the GTP consumption (*Hoagland-1958c*). The subsequent experiments on eukaryotic cells showed that this GTP consumption was linked to the enzymes involved in the elongation (*Grossi-1959, Zamecnik-1960, Bishop-1961, Fessenden-1961, Hardesty-1963*). The two identified enzymes were called "transfer factors" TF-1 and TF-2 (*Arlinghaus-1963*, *Arlinghaus-1964*). Their prokaryotic analogs were extensively studied in the mid-60s (*Nakamoto-1963, Allende-1964, Conway-1964, Nishizuka-1966A*). The bacterial analog of TF-1 was named T, thus underlining its role in the transfer of tRNA; the analog of TF-2 was named G to underline its GTPase activity (*Nishizuka-1966B*). The T-factor was then separated into two fractions: Tu (thermally unstable) and Ts (thermally stable) (*Lucas-Lenard-1966*). The factors demonstrated high affinity for each other (*Miller-1969, Miller-1970*). The Tu-factor formed a ternary complex with GTP and aa-tRNA (*Gordon-1967, Ravel-1967, Gordon-1968, Cooper-1969*). This complex was found to mediate the aa-tRNA association with the ribosome (*Ravel-1968, Ravel 1968b, Gordon-1969, Ravel-1969, Lucas-Lenard-1969*). The ternary complex was never formed with the initiator $tRNA^{fMet}$ (*Ono-1968*).

The G-factor was found to promote the translocation of the p-tRNA (*Nishizuka-1966a, Tanaka-1968*). However, the role of the G-factor was erroneously linked to the formation of the peptide bond (*Haenni-1968*).

Bacterial and eukaryotic elongation factors showed notable similarity. Like in prokaryotes, the eukaryotic aa-tRNA reached the ribosome within the ternary complex (*Ibuki-*

1966, Rao-1967, Jerez-1969). Besides, the tertiary complex in which the eukaryotic factor TF-1was replaced by its bacterial analog was able to bring the aa-tRNA to the ribosome (*Krisko-1969*).

Peptidyl-transferase

For many years, the peptide bond formation was attributed to the activity of an enzyme (*Nakamoto-1963, Nishizuka-1966b*), presumably to the G-factor (*Haenni-1968*). However, in mid-1960s it was shown that the reaction of transpeptidation could be catalyzed by the LSU alone. It did not require elongation factors, the SSU or mRNA (*Zamir-1966, Gottesman-1967, Monro-1967, Monro-1967b, Monro-1968*). Many researchers suggested that the peptidyl-transferase activity was an intrinsic property of the LSU (*Skogerson-1968, Maden-1968, Haenni-1968, Staehelin-1969*).

Initiation factors

In the second half of 1960s, the initiation of translation was also found to be factor-dependent (*Revel-1966, Stanley-1966, Miller-1968*). In bacteria, three initiation factors, IF1, IF2 and IF3, were identified (*Revel-1968, Wahba-1969*). It was proposed that IF2 stimulated the association of mRNA to SSU (*Revel-1968*) and, together with IF1, mediated the attachment of $\text{tRNA}^{\text{fMet}}$ to complex SSU-mRNA (*Salas-1967, Hershey-1969*). Factor IF3 was suggested to stimulate the formation of the initiation complex (*Wahba-1969, Berisd-1971*). The initiation was accompanied by GTP hydrolysis (*Leder-1967, Ohta-1967, Allende-1967*): the IF2 was a GTPase (*Kolakofsky-1968, Chae-1969, Lelong-1970*).

Translation termination factors

Until the mid-1960s, the mechanism of termination of translation remained unclear (*Takanami-1965, Ganoza-1966, Lengyel-1966, Fox-1968, Bretscher-1968b*). Some researchers suggested the existence of a special tRNA that recognized the stop-codon and released the nascent peptide from the p-tRNA (*Brenner-1965*). On the other hand, the termination could be also mediated by an enzyme (*Tissieres-1960*).

In 1967, Mario Capecchi isolated a two-component fraction that was able to recognize the stop codons (*Capecchi-1967*). The R1-component had affinity to codons UAA and UAG, while the R2-component recognized codons UAA and UGA (*Scolnick-1968*). Both factors had a common binding site on the ribosome (*Scolnick-1969*) and their activity required the presence of both ribosomal subunits (*Weisblum-1968*). The activity consisted in the transfer of the nascent peptide from the p-tRNA to a water molecule (*Tompkins-1970*). In addition to R1 and R2, a GTP-dependent factor S was found (*Milman-1969, Goldstein-1970b*), which was suggested to stimulate the activity of the R-factors (*Milman-1969*).

At the end of 1960s, a termination factor was also discovered in eukaryotes (*Goldstein-1970*). This factor demonstrated an enzymatic activity similar to that of the prokaryotic R-factors. At the same time, it was a GTPase, like the S-factor.

Ribosome Recycling Factor

It was already known that the initiation of protein synthesis requires the existence of the free ribosomal subunits. Correspondingly, the forced dissociation of the ribosome to the isolated subunits at the end of protein synthesis was originally attributed to one of the initiation factors (*Subramanian-1968, Miall-1970, Subramanian-1970*).

In early 1970s, two laboratories independently discovered an enzyme that was suggested to liberate the ribosome from mRNA (*Hirashima-1972, Subramanian-1973*). The new factor was named the ribosome release factor (RRF). The following separation of the subunits was presumably accomplished by IF3 (*Hirashima-1973*).

The RRF was found to act together with EF-G and GTP (*Hirashima-1973, Ogawa-1975*). Later, Ehrenberg and colleagues demonstrated that RRF, together with EF-G and GTP, was responsible for the subunit dissociation, while IF3 stimulated the removal of the deacylated tRNA from the ribosome (*Karimi-1999*).

In 1994, RRF was renamed to the ribosomal recycling factor (*Janosi-1994*), to distinguish it from the known release factors that liberate the nascent peptide.

Peptidyl-tRNA hydrolase

Once in a while it happens that the p-tRNA quits the ribosome because of a premature abortion of translation. In the late 1960s, several laboratories independently discovered a rescue enzyme, which released such a p-tRNA to the cytosol (*Cuzin-1967, Kössel-1968, Menninger-1976*). The enzyme hydrolyses the bond between the p-tRNA and the nascent polypeptide chain and is thus named the hydrolase (*Kössel-1969*). Contrary to the release factors (Capecchi-1967), the hydrolase did not affect the p-tRNA bound to the ribosome (*De Groot -1968, De Groot -1969, Menninger-1970*) and was inert to the initiator tRNA^{fMet} (*Kössel-1968, Schulman-1975*). The hydrolase activity was found to prevent contamination of the cytosol by the potentially abandoned p-tRNAs (*Menninger-1978, Atherly-1978*), whose accumulation is lethal to the cell (*Menninger-1979*).

“Non-enzymatic” translation

In the late-1960s, it was demonstrated that translation can proceed in the absence of the elongation factors (*Kuriki-1967, Pestka-1969, Gavrilova-1971*). An aa-tRNA can bind to the ribosomal A-site without assistance of the EF-Tu (*Kuriki-1967, Sokolff-1972, Glukhova-1975*). The translocation, in turn, can occur without the EF-G (*Gavrilova-1971b*). Although the factor-free translation was less efficient than in the presence of the elongation factors (*Pestka-1969, Zamir-1971, Spirin-1973, Glukhova-1975*), it demonstrated the central role of the ribosome in protein synthesis.

Also, in the early 1970-s, Spirin and co-workers found that the ribosomal protein S12 plays a role of an inhibitor of factor-free translocation (*Gavrilova-1972, Gavrilova-1974, Gavrilova-1974b, Glukhova-1975, Gavrilova-1976*). Many years later, Rachel Green and co-workers showed that S12 acts in collaboration with S11 and S13 (*Southworth-2002, Cukras-2003*).

In the 1970s, “non-enzymatic” protein synthesis was extensively studied in different laboratories (*Pestka-1974, Cabañas-1980, Kemkhadze-1981, Bergemann-1983*). Among other findings, it was shown that translocation can proceed even in the absence of mRNA (*Belitsina-1981, Belitsina-1982*), being dependent exclusively on the ribosome and the tRNAs.

Early models of functional ribosome

At the end of the1960s, the mechanism of protein synthesis on the ribosome remained unclear. A few models of the functioning ribosome were suggested at that time (*Bretscher-1968, Spirin-1969, Hill-1969, Skoultchi-1969, Woese-1970*). In the models proposed by Bretscher and Spirin, the translating ribosome constantly fluctuated between two conformational states (*Bretscher-1968, Spirin-1969*).

Bretcher postulated that a reciprocal motion of the ribosomal subunits permitted the translocation of the p-tRNA (with mRNA). According to Bretcher, each subunit has its own A- and P-sites (Figure 2). During the translocation, the tRNA moves from the A/A position (A site on both subunits) to the P/P position through a transient P/A position (Figure 2). In some sense, Bretcher predicted that the tRNA translocation proceeds through formation of hybrid states, although on the detailed level, the predicted states were different from those known nowadays (see below).

In the Spirin's model, the ribosome oscillated between the so-called "locked" and "unlocked" states (*Spirin-1969*). The unlocked state enables the aa-tRNA to enter the ribosomal A-site and to form the codon-anticodon interactions. It also allows the p-tRNA to be translocated from the A- to P-site and the deacylated tRNA to quit the ribosome from the P-site. The locked state, on the other hand, guarantees that both the aa- and p-tRNAs are properly fixed in the A- and P-sites, respectively, which would allow the reaction of transpeptidation.

In the 1970s, the ribosome motion between two conformational states was confirmed in several laboratories (*Schreier-1971, Chuang-1971, Tritton-1978*). In 2000, Frank and Agrawal visualized the ribosome rearrangement by cryo-EM methods (*Frank-2000*).

Ribosomal morphology studies in 1970s

During the 1970s, important progress was achieved in the definition of the ribosomal protein and RNA contents. The technology of isolation of individual proteins from the bacterial ribosome (hence, r-proteins) had already been developed in the previous decade (*Nomura-1965, Lerman-1966, Traut-1966, Gavrilova-1966*). It allowed systematic analysis of the known ribosomal proteins (*Traut-1969, Stöffler-1971, Kaltschmidt-1970a*) and identification of new ones both in the prokaryotic (*Kaltschmidt-1970b, Dzionara-1970*) and eukaryotic (*Collatz-1977, Tsurugi-1978*) ribosomes. The primary sequences of all known r-proteins were determined in the *E.coli* ribosome (*Brimacombe-1978*). Several laboratories published the assembly maps of the bacterial ribosome (*Kurland-1969, Nomura-1969, Mizushima-1970, Schaup-1970, Held-1974*).

The rRNA was also extensively analyzed. By 1970, it had become evident that the 5S rRNA exists in all organisms (*Attardi-1970*) and its complete sequence was determined (*Brownlee-1967, Forget-1967, Williamson-1969*). Another small rRNA with sedimentation coefficient 5.8S was also discovered in the LSU of eukaryotes (*Pene-1968, Sy-1970, Udem-1971, Payne-1971, Woledge -1974, Nazar-1975, Nazar-1975b, Nazar-1976*).

By the end of 1970s, comparative analysis of rRNA primary sequences from different organisms (*Brosius-1978, Carbon-1979, Brosius-1980, Schwarz-1980, Eperon-1980, Rubtsov-1980*) allowed modelling of the secondary structures of the rRNAs from bacteria (*Woese-1980, Noller-1981, Stiegler-1981*), eukaryotes (*Veldman-1981, Man'kin-1981, Michot-1982, Olsen-1983, Hadjiolov-1984, Chan-1984*), organelles (*Branlant-1981, Köchel-1982*) and the newly discovered archaea (*Gupta-1983*). The results showed **a strong evolutionary conservation of the rRNA secondary structure through all evolutionary domains** (*Zwieb-1981, Glotz-1981, Cammarano-1982, Gutell-1985*) (Figure 3).

Shine-Dalgarno and Kozak sequences

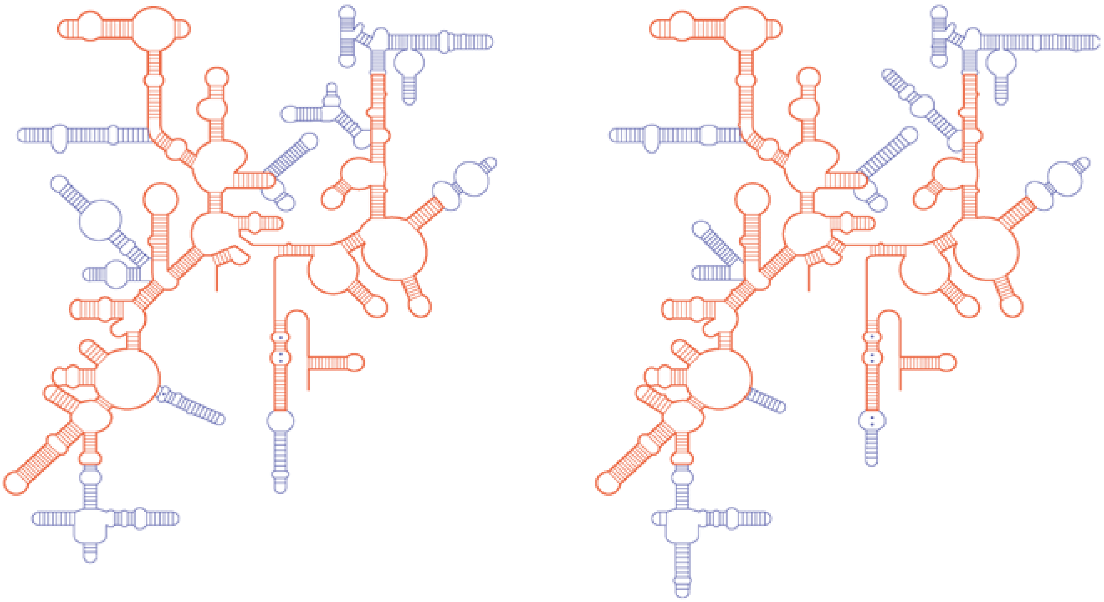
Prior to the mid-1970s, the mechanism for initial positioning of the initiator tRNA and the mRNA at the decoding region of SSU remained unclear (*Thach-1966*). In 1975, John Shine and Lynn Dalgarno found that each bacterial mRNA had a short purine segment located, on average, 3-5 nucleotides upstream of the start codon (*Shine-1974, Shine-1975*). The matching pyrimidine sequence was found at the 3'-end of 16S rRNA (*Shine-1974, Shine-1975*). The interaction between the 3'-terminal sequence of 16S rRNA and the determined sequence in mRNA guaranteed the proper positioning of the $\text{tRNA}^{\text{fMet}}$ and mRNA before the beginning of the elongation of translation. The interacting regions in mRNA and in 16S tRNA were named Shine-Dalgarno and "anti-Shine-Dalgarno" regions, respectively.

In eukaryotes, however, the mRNAs had no common segment close to the 5'-terminus (*Firtel-1979, Montgomery -1980, Chin-1981, McKeown-1981*). Nevertheless, in 1981, Marilyn Kozak found a particular nucleotide sequence pattern around the initiation codon (*Kozak-1981, Kozak-1981b*). She found that the third nucleotide upstream of the initiator codon AUG was usually A, while the base following the AUG sequence in most cases was G.

New Elongation Factor: EF- P

Another translational factor, EF-P, was discovered in the mid-1970s (*Glick-1975, Glick-1976*). The EF-P was suggested to mediate the formation of the very first peptide bond (*Glick-1979, An-1980, Aoki-1991, Aoki-1997, Ganoza-2000*). For a long time, it was believed that at the

(a) Bacteria (b) Archaea



(c) Eukarya (d) Plant mitochondria

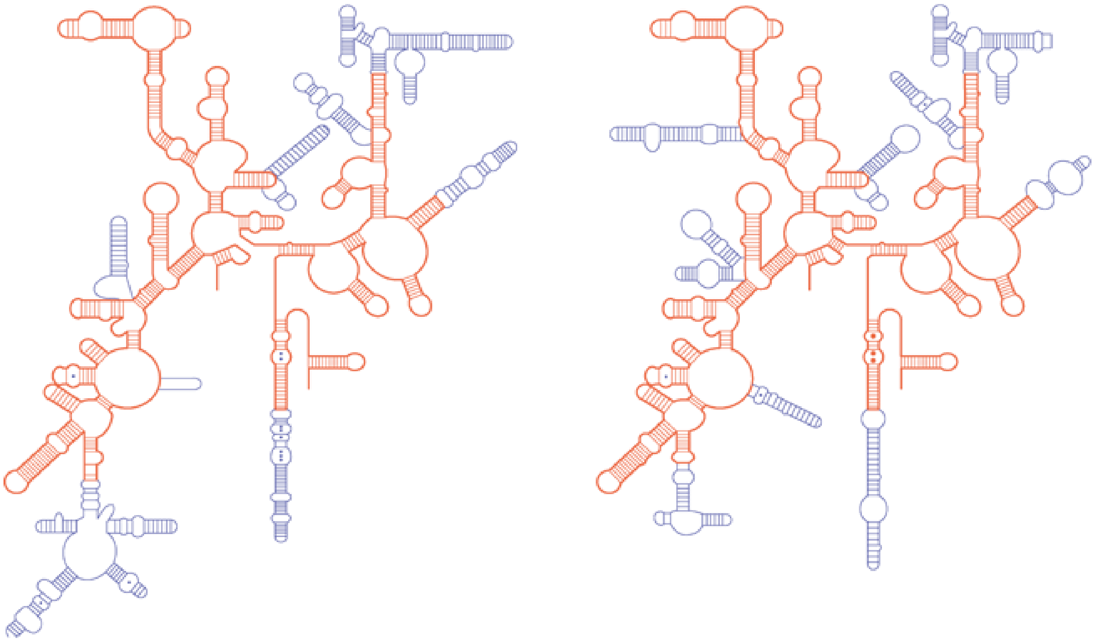


Figure 3.

The secondary structures of 16S-type rRNAs from various species (red lines show common structural core)

(**a**) E. coli; (**b**) Halobacterium volcanii; (**c**) yeast, cytoplasmic ribosomes; and (**d**) mitochondria of plants (maize). (from *Gutell -1985*) beginning of translation, factor EF-P occupies the A-site of the LSU thus driving the initiator tRNA to the P-site (*Aoki-2008*). This opinion was based on the similarity between the shapes of the EF-P and tRNA (*Hanawa-Suetsugu-2004*). However, the recent crystallographic studies made in the laboratory of T. Steitz showed that EF-P is positioned between the P- and E-sites (*Blaha-2009*). Hence, the role of EF-P is suggested to stabilize the position of the initiator tRNA in the P-site before the formation of the first peptide bond (*Blaha-2009*).

Translational fidelity

In the early 1980s, the level of misincorporation of amino acids during translation was estimated as $5x10^{-4}$ (*Thompson-1982b*). The major causes of mistakes were related to the tRNA aminoacylation and the selection of the cognate aa-tRNA at the ribosomal A-site (*Zaher-2009*). The misacylation of the tRNA was linked to the way of how the aaRS recognizes the cognate tRNA. The mechanism of this recognition was first studied in the 1960s (*Berg-1961, Kiselev-1964, Jacobson-1964, Yarus-1967*). Originally, it was thought that aaRS discriminate the tRNA by the anticodon (*Hayashi-1966*). However, the following experiments showed that this is not always true (*Schimmel-1979, Normanly-1986, Schimmel-1987, McClain-1988*).

In the late 1980s, the recognition elements were found in different parts of the tRNA molecule (*De Duve-1988, Hou-1988, Moras-1993, Becker-1996*). The recognition elements that unambiguously determines the identity of a particular tRNA was named the "second genetic code" (*De Duve-1988*). In general, the level of errors in translation associated with mis-aminoacylation was shown not to exceed 10^{-4} (*Loftfield-1972*).

The selection of the cognate aa-tRNAs at the A-site is related to the formation of the cognate codon-anticodon double helix (*Lucas-Lenard-1971*). For the cognate and near-cognate tRNAs, the energies of the codon-anticodon association must be different. This difference should provide the required level of accuracy in the admission of a new amino acid into the growing protein. However, kinetic studies made in the mid-1970s showed that the difference in the stability of the cognate and near-cognate codon-anticodon double helices was insufficient to discriminate the cognate tRNA with the known level of fidelity (*Ninio-1974, Ninio-1975, Hopfield-1974*). The two researches independently proposed the existence of an additional "proofreading" step during the aa-tRNA identification.

The proofreading step must be separated from the initial recognition of the aa-tRNA by an irreversible step associated with GTP hydrolysis and departure of the Tu-factor from the ribosome (*Thompson-1977, Thompson-1982*). During the proofreading step, most non-cognate aa-tRNAs that successfully pass the initial recognition step should be expelled from the ribosome (*Thompson-1981, Kurland-1984*). In the first half of the 1980s, mutagenesis experiments demonstrated that a higher ribosome accuracy during the tRNA selection led to a lower rate of protein synthesis (*Thompson-1982b, Bohman-1984, Ruusala-1984*). It was suggested that the cell, in principle, is able to maintain the balance between the speed and accuracy of translation (*Thompson-1986*).

The "three binding sites" model

Before the 1980s, it was thought that the ribosome had only two tRNA binding sites, A and P (*Watson-1963, Watson-1964*). In such a "two-site" model, the deacylated tRNA was ejected to the cytosol directly from the P-site. In 1981, Knud Nierhaus suggested the existence of the third "exit" site (E-site), which could harbor the deacylated tRNA until the next elongation cycle (*Rheinberger-1981*). The "three-site model" was initially criticized (*Schmitt-1982*), but soon received experimental support from several laboratories (*Grajevskaja-1982, Kirillov-1983, Lill-1984, Parfenov-1985, Rodnina-1988*). Since then, Nierhaus has actively promoted the three-site model in a number of papers (*Rheinberger-1983, Rheinberger-1986, Hausner-1988, Remme-1989, Geigenmüller-1990*) and the three tRNA-sites were visualized by both cryo-EM data (*Agrawal-1996*) and by X-ray crystallography (*Yusupov 2001*).

In an attempt to further develop the "three-site model" Nierhaus proposed the "three-site allosteric hypothesis" (*Rheinberger-1983, Rheinberger-1986b, Gnirke-1989, Nierhaus-1990*). He suggested that the presence of the deacylated tRNA in the E-site reduces the affinity of the A-site to the aa-tRNAs. On the other hand, the presence of the tRNA in the A-site pushes the deacylated tRNA to the cytosol. Therefore, only two tRNAs were allowed to be associated with the ribosome at any moment. However, experiments in other laboratories did not confirm the exclusion principle of Nierhaus (*Lill-1984, Rodnina-1988, Semenkov-1996 , Petropoulos-2012*). In fact, the third, "exit" site for the deacylated tRNA was first hypothesised in 1965 by Wettstein and Noll (*Wettstein-1965*). However, the idea was not accepted by the scientific community: the presence of a deacylated tRNA on the ribosome was considered redundant (*Cannon-1967*).

The hybrid states of tRNA on the ribosome

In 1989, Moazed and Noller suggested that the translocation of tRNAs in the ribosome proceeds in two steps (*Moazed-1989*) (Figure 2.). After the transpeptidation, the acceptor terminus of the p-tRNA spontaneously moves in the LSU from the A to P-site, while in the SSU, its anticodon arm remains attached to the A-site. Such movement results in formation of a hybrid A/P state for the p-tRNA. Simultaneously, the deacylated tRNA moves to the P/E hybrid state. Then the EF-G factor promotes the shift of both anticodons on the SSU, providing for the P/P and E/E tRNA states, respectively. Another hybrid state was described for the aa-tRNA when it is still attached to the Tu-factor (*Moazed-1989b*). This hybrid state was denoted as A/T, because the anticodon half of such aa-tRNA occupies the A-site of the SSU, while its acceptor stem is bound to the Tu-factor (Figure 2).

The ribozymes and the concept of RNA world

In the early 1980s, Cech and colleagues found that the pre-ribosomal intron of *Tetrahymena* was able to perform a series of self-splicing reactions (*Kruger-1982*). Soon after this, experiments in the Altman laboratory showed that the catalytic activity of the nucleoprotein RNase P was based on its RNA part rather than on the protein part (*Guerrier-Takada-1983*).

In 1984, Noller and co-workers found that the ribosomal RNA of the prokaryotic LSU was directly involved in the peptide bond formation (*Barta-1984*). The r-proteins, on the contrary, were unable to perform this catalytic reaction in the absence of rRNA (*Nierhaus-1973, Sonenberg-1973, Howard-1974, Ballestd-1974, Nierhaus-1974, Moore-1975, Barta-1984*). It is also important that the peptide bond formation did not require an energy source (*Pestka-1969*) in addition to the energy provided by the aa-tRNA (*Krayevsky-1975*). These results suggested that the ribosome is also an RNA-based enzyme (*Moore-1988*).

The existence of RNA enzymes or “ribozymes” led to the hypothesis of the so-called “RNA-world” (*Gilbert-1986*). According to this hypothesis, early life on Earth was exclusively RNA-based, while proteins appeared at later stages of evolution (*Gilbert-1986, Ganem-1987, Moore-1988, Joyce-1989, Benner-1991, Moore-1993*).

Thus, the ribosome could be a relic of the RNA world (*Moore-1988*).

The tertiary structures of ribosomal components

By mid-1980s, the shape of the ribosomal subunits and their location in the ribosome had been roughly determined (*Wittmann-1983*). In the following decade crystallographic studies provided high-resolution structures of several r-proteins (*Leijonmarck-1980, Wilson-1986, Ramakrishnan-1992*) and as well as of some translational factors, including the EF-Tu (*Berchtold-1993, Nissen-1995) and EF-G (AEvarsson-1994, Czworkowski-1994*). Some rRNA fragments were determined by the nuclear-magnetic resonance spectroscopy (*White-1992, Szewczak-1993, Wimberly-1993*). However, a structure of the whole ribosomal particle at atomic level resolution remained unresolved due to their enormous size and difficulties in obtaining crystals of a sufficient quality (*Yonath-1987*).

In the 1990s, the only available method for visualization of complete ribosomal complex was by cryo-EM reconstruction, which provided a structure at approximately 25Å resolution (*Frank-1991, Frank-1995, Stark-1995*). Nevertheless, this method allowed Frank and colleagues to obtain images of the ribosome complexed with three tRNAs (*Agrawal-1996*) and the ribosome complexed with EF-G (*Agrawal-1998*).

In 2000, the cryo-EM data allowed Frank and Agrawal to demonstrate that a rearrangement of the ribosome structure occurred during translocation (*Frank-2000*). The phenomenon was described as the reciprocal “ratchet-like motion” (RLM) between the two subunits (*Frank-2000*), or, simply, “ratcheting” (Figure 4 a, b).

During the past decade, cryo-EM techniques have rapidly progressed. The cryo-EM reconstruction of the eukaryotic ribosomes at 5.5Å resolution has become possible (*Armache-2010*). In spite of limitations of the method (*Frank-2009*), cryo-EM remains a powerful tool for structural studies. Sometimes, it is the only source of structural information. For example, the structures of the ribosomes from mitochondria (*Maslov-2006, Sharma-2009*) and chloroplasts (*Sharma-2007*) currently exist only in the form of three-dimensional reconstructions from cryo-EM density maps.

The ribosome crystallographic studies

A breakthrough in the ribosome crystallography occurred at the end of 1990s. In 2000, T. Steitz and co-workers published the crystal structure of the 50S subunit at the 2.4Å resolution (*Ban-2000*). This structure showed that the PTC does not contain proteins (*Nissen-2000*), thus

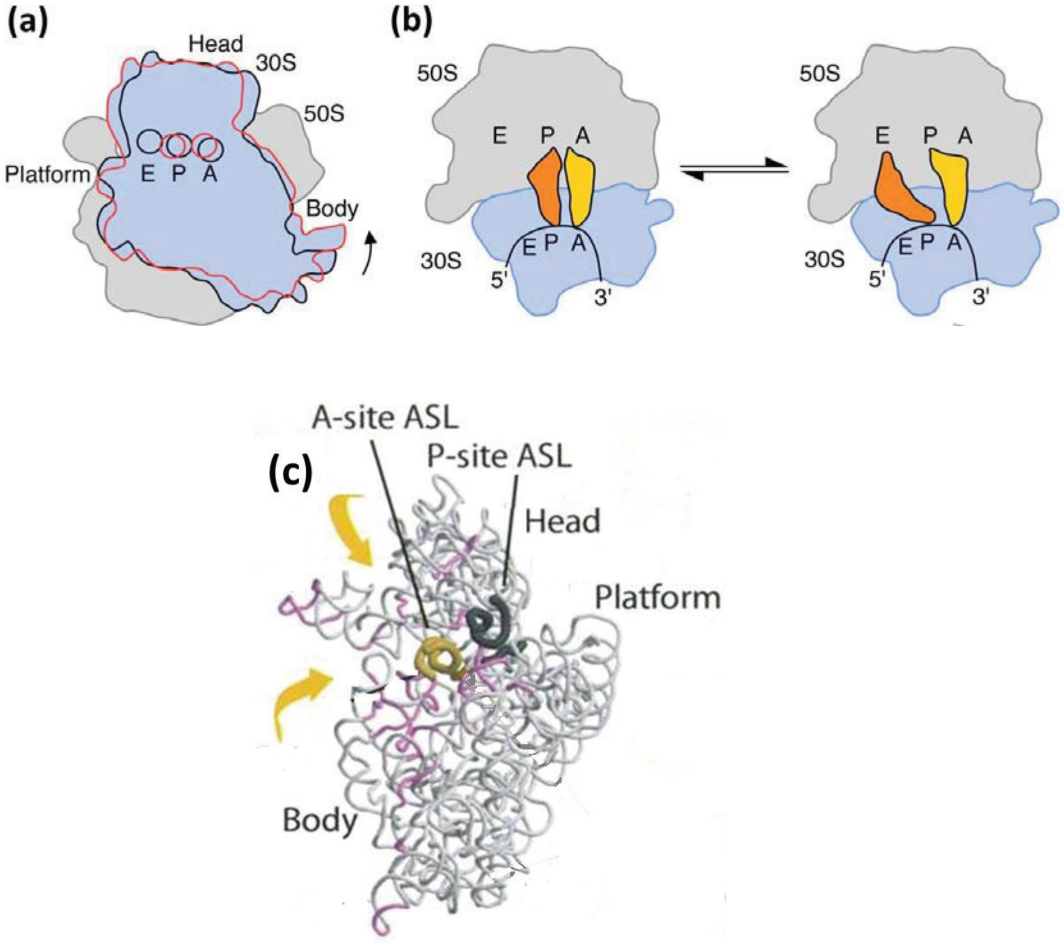


Figure 4

Global structural rearrangements on the ribosome

(**a, b**) **reciprocal ratchet-like motion of the ribosomal subunits (ratcheting)** (from *Zhang-2009*)

(**a**) View of the bacterial 70S ribosome, composed of the SSU (blue) and the LSU (grey). In the SSU, the three domains - Head, Body and Platform - are indicated.

The SSU rotates from a non-ratcheted conformation (black outline) to a ratcheted conformation (red outline) relative to the LSU. Letters indicate the positions of the aminoacyl (A), peptidyl (P), and exit (E) tRNA binding sites.

(**b**) Schematic of tRNA binding states on the ribosome. (The view of the ribosome is rotated 90° (face-down) from that in (a)). In the transition of the ribosome to the ratcheted state, tRNAs shift from binding in the A/A and P/P sites (SSU/LSU, respectively) to occupy hybrid binding sites (A/P and P/E sites).

(c) The SSU closure (modified from *Ogle-2002)*

Movements of 16S RNA backbone phosphorus atoms (*T. thermophilus* numbering) after superposition of the SSU complexed with a cognate ASL (*Ogle-2002*) on the *apo* structure (*Wimberly-2000*).

Regions moving by more than the estimated standard deviation of the individual atoms are highlighted in pink, and arrows are added to indicate general directions of movement.

confirming that the ribosome is a ribozyme (*Cech-2000, Steitz-2003*). This structure also provided important details about the peptide exit tunnel (*Nakatogawa-2002, Berisio-2003*). At about the same time, the group of Ramakrishnan published the structure of the 30S subunit (*Wimberly-2000*). The crystal structure of the SSU provided a detailed view of the subunit interface helix h44 and the decoding region at the top of this helix. The following year, Yusupov and colleagues published the structure of the entire 70S ribosome (*Yusupov-2001*) (Figure 4). In spite of a somewhat lower resolution (5.5Å), this structure provided a detailed picture of the subunit interaction (Figure 5). In this structure, the ribosome was simultaneously complexed with a mRNA and three tRNAs in the A, P and E sites, providing an additional argument against the exclusion principle of Nierhaus (*Rheinberger-1983*).

The newly obtained crystallographic data provided extensive information on the structure of ribosomal RNA (*Klein-2004*) and its interaction with r-proteins (*Klein-2004b*). Systematic examination of the rRNA tertiary structure allowed for identification of new structural motifs (*Klein-2001, Nissen-2001, Gagnon-2002, Szép-2003, Steinberg-2007a, Steinberg-2007b, Boutorine-2012*).

The progress in the ribosome crystallography and biochemistry allowed realization of more complex tasks. The high resolution structures of the complete bacterial ribosome in the apo-form (*Schuwirth-2005, Selmer-2006*) and complexed with different translational factors (*Pai-2008, Gao-2009, Schmeing-2009, Blaha-2009, Jin-2010, Korostelev-2010, Jin-2011, Zhou-2012*) were published in the last decade.

Two findings were essential for the research presented in this thesis. In 2002, V. Ramakrishnan observed that the SSU can undergo a specific rearrangement in the small subunit that he described as a domain closure (henceforth, SSC) (*Ogle-2002*). The SSC can take place when the new aa-tRNA forms the cognate codon-anticodon complex the in A-site (*Ogle-2003*) (Figure 4c). The other finding was made in the group of J. Cate, who published in 2009 the structure of the ribosome in the ratcheted state (*Zhang-2009*). In the presented thesis, these data were used for understanding the principles of the ribosome dynamics.

Today at least 7 different laboratories perform crystallographic studies on the ribosome (of Stetz, Ramakrishnan, Yonat, Yusupov, Cate, Noller and Ban). Their data are extremely important for understanding the mechanistic details of protein synthesis. Based on their data,

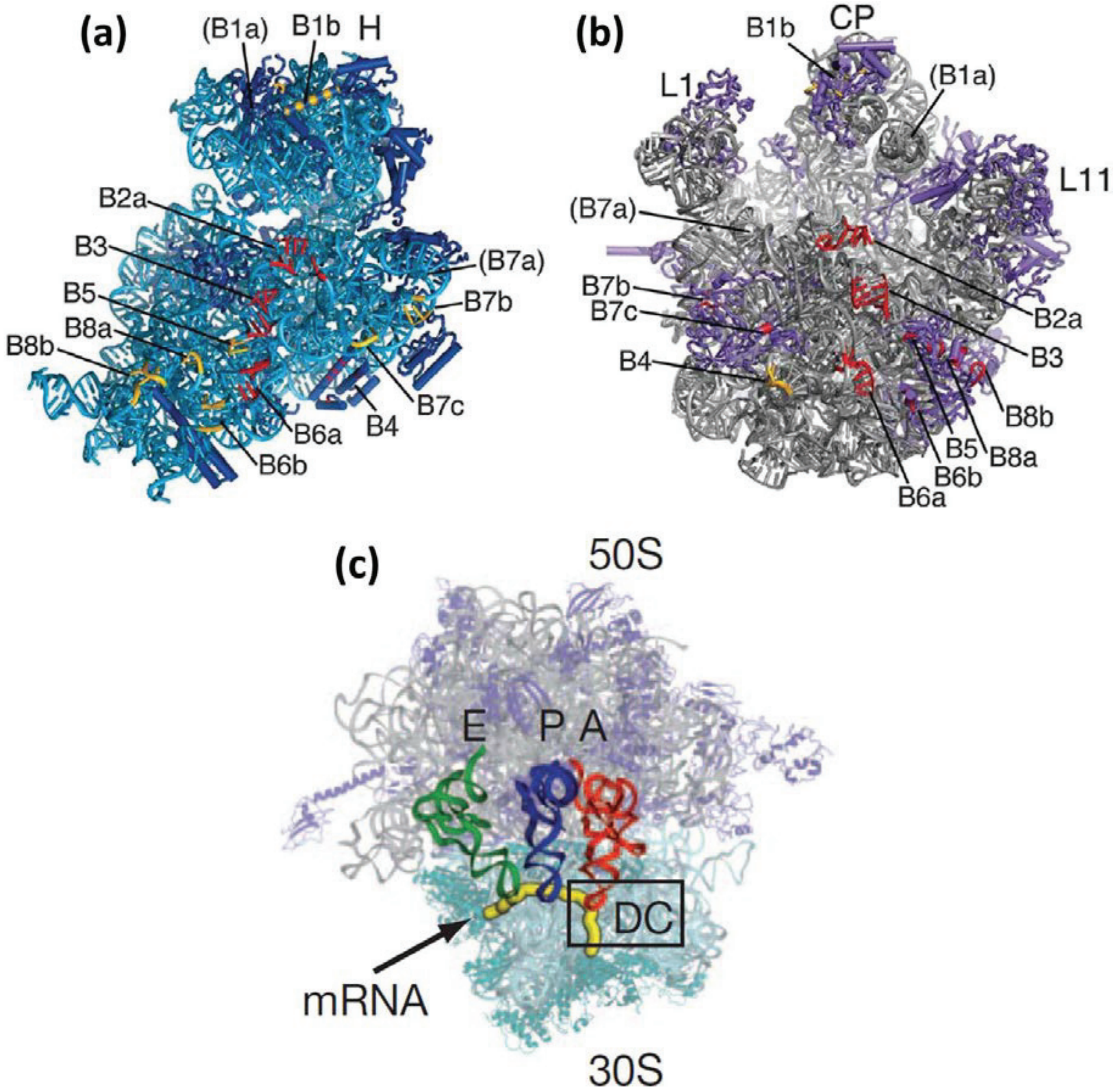


Figure 5.

Interactions between the ribosomal subunits and the position of the three tRNAs on the ribosome

(**a, b**) **Interaction between the subunits** (**a, b**) (from *Duncle-2011*): on both subunits corresponding intersubunit bridges have the same numbering and colored in red and yellow. On the SSU, the head domain is marked H. On the LSU, the central protuberance, the L1 stock and the L11 stock are marked CP, L1 and L11 correspondingly.

(**a**) The SSU, as it is seen from the LSU; the rRNA is colored in light blue, the r-proteins are in dark blue

(**b**) the SSU, as it is seen from the LSU; the rRNA is colored in grey, the r-proteins are in magenta

(**c**) **The binding sites of the three tRNAs (c)** Letters indicate the positions of the aminoacyl (A), peptidyl (P), and exit (E) tRNA binding sites. (from *Demeshkina-2012*) hundreds of researchers in the world bring the missing elements into the huge puzzle of the protein synthesis process.

We hope that the results presented in this thesis will be also helpful for filling a few empty spots in this puzzle.

Problems addressed in the thesis

Evolutionary model for 23S rRNA

So far, it is unknown how protein life emerged on our planet. On the other hand, the modern process of protein synthesis in the cell is centered in the ribosome, which is the universal machinery of protein synthesis in all organisms (*Spirin-1999*). Thus, it has been suggested that the emergence of this machinery preceded the emergence of proteins.

However, the modern ribosome is a complex of RNA and proteins. Before proteins, how could such ribosomes work? This question was answered with the discovery, that the ribosome is a ribozyme (see earlier in the text). The early ribosome could be an RNA only complex.

The rRNA of modern bacterial ribosome consists of about five thousand nucleotides (the eukaryotic rRNA is even bigger). Spontaneous reshuffling of this huge chain in a right fold, providing the complex ribosome functionality seemed improbable due to the enormous time required for finding that unique fold. An extraterrestrial emergence of the rRNA (in the context of the Panspermia Hypothesis (see for example *Bada-2001, Belbruno-2012*)) seems to be a much better explanation.

Simultaneously, there was a simple idea of gradual evolution of the ribosome. Originally, it might emerge as a small RNA-molecule (*Woese-2001*) that was able to link two amino acids through the peptide bond (*Noller-2004*). The oligo-peptides with random amino acid sequences interacted with RNA molecules, increasing the variability of the early RNA-based life (*Szathmáry-1997, Noller-2004*). This process continued until the ribosome became efficient enough to perform the template-dependent synthesis of proteins. Then, the first r-proteins appeared in the ribosome structure (*Polacek-2005*).

Hence, the LSU with peptidyl-transfer center (PTC) was suggested to emerge before the SSU (*Schimmel-1998*). Correspondingly, the PTC is believed to be the most ancient element of the ribosome (*Polacek-2005, Hury-2006*).

However, the way how the tertiary structure of rRNA evolved remained unknown due to the absence of adequate research methods. The structural core of rRNA is extremely conserved in all living organisms (*Gutel-1994, Olsen-1997, Wuyts-2001*) proving that the rRNA in this conformation existed before the splitting of Tree of Life into branches (*Doolittle-1994, Woese-1998, Joyce-2002*). As such, the comparison of nucleotide sequences of existing rRNA (*Cavalli-Sforza-1967*) appears useless for the studying of early ribosome evolution.

In the absence of ready-to use methods, we invented our own approach. The approach is based on the assumption that the ribosome evolved in a stepwise manner. Originally, the proto-ribosome could appear as a small RNA molecule that was able to perform a certain function related to the modern ribosome. The following development of this RNA proceeded through emergence of new elements above the existing structure. Each element emerged randomly but only those that could be adapted to the existing ribosome structure had a chance to propagate in evolution.

If the emergence and adaptation of new elements happened in a similar way, the traces of such an evolutionary pathway might exist in the structure of contemporary rRNA. Thus, systematic analysis of the ribosome crystal structure could help to decipher the evolution of the ribosome.

This approach led us to an evolutionary model, which explains how the proto-ribosome could emerge to the contemporary 23S rRNA. The description of the model formed the basis of the second and third chapters of this thesis.

Studies of the ribosome dynamic

At different steps of its functional cycle, the ribosome undergoes global structural rearrangements, such as the RLM (*Frank-2000*) and the SSC (*Ogle-2002*).

The RLM was originally observed by cryo-EM methods and described as a reciprocal rotation between two ribosomal subunits (*Frank-2000*). The RLM was suggested to promote translocation (*Horan-2007*). When the ribosome acquired the “ratcheted” (rotated) conformation, the p-tRNA and deacylated tRNA move to the hybrid A/P and P/E states (*Julián-2008*).

The SSC was originally related to the selection of new aa-tRNA (*Ogle-2002*). The closure of the SSU takes place when the codon-anticodon interaction is cognate (*Ogle-2003*). It should also be mentioned that the SSC was discovered when a high-resolution structure of the entire ribosome did not exist. Correspondingly it was not known whether the domain closure of the SSU can affect the LSU. The possibility of a relation between the SSC and RLM has never been discussed.

In 2009, Cate and co-workers determined the structures of the *E.coli* ribosome in intermediate states of ratcheting (*Zhang-2009*). Each crystal contained two co-crystallized ribosomes rotated at about 6° relatively to each other. In spite of the rotation, the central bridges at the subunit interface remained intact. It could be due to the proximity of the bridges to the center of subunit rotation. On the other hand, it would be possible if part of the SSU containing these bridges remained immobile relatively the LSU in ratcheting. The second scenario did not seem improbable because of the existence of domain closure that implied the mobility of the SSU structure.

Based on these data, we developed a strategy for systematically comparing the ratcheted and non-ratcheted ribosome conformations. This strategy allowed us to elucidate the pattern of ribosome rearrangement during the RLM and to understand how these rearrangements can be regulated. The mechanism of regulation of RLM was found to use the same elements of the SSU structure that had already been known to regulate SSC. Further analysis showed the virtual identity of the rearrangements that take place during RLM and SSC. This analysis is presented in the fourth chapter of this thesis.

Chapter 2. Article:

A hierarchical model for evolution of 23S ribosomal RNA

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Konstantin Bokov : participated in preparation of experimental design, did experiments, analyzed results, participated in preparation of manuscript, prepared figures

Sergey V. Steinberg : developed general experimental design, analyzed results, prepared manuscript, participated in preparation of figures

ABSTRACT

The emergence of the ribosome constituted a pivotal step in the evolution of life. This event happened nearly four billion years ago, and any traces of early stages of ribosome evolution are generally thought to have completely eroded away. Surprisingly, a detailed analysis of the structure of the modern ribosome reveals a concerted and modular scheme of its early evolution.

INTRODUCTION

The ribosome is an RNA–protein complex performing protein synthesis in all living cells (*Stillman-2001*). It is generally accepted that the ribosome originated from the so-called 'RNA world' when proteins did not exist and the primordial chemical reactions of life were catalysed by RNA (*Crick-1968, Gilbert-1986*). Although the contemporary ribosome contains several dozen proteins (*Yusupov-2001, Ban-2000, Harms-2001, Selmer-2006, Schuwirth-2005*), the two major functions of the ribosome—the selection of the proper amino acid and the transpeptidation—are performed by RNA (*Ogle-2001, Noller-1992, Nissen-2000*), whereas proteins have only an auxiliary role. Structurally, RNA forms the core of the ribosome, whereas proteins are mostly located at the periphery. Hence, the problem of the origin of the ribosome concerns the origin of ribosomal RNA. Because in all living organisms the core of the ribosome has a very similar structure, it must have formed before the split of the tree of life into three phylogenetic domains (*Gutell-1994, Doudna-2002*). Consequently, the comparison of the available nucleotide sequences of rRNA is not sufficient for the deduction of how the ribosome emerged.

However, the ribosome tertiary structure could provide key clues about the details of this process.

Our initial observation was that, compared to other domains of the 23S rRNA secondary structure, A-minor interactions in domain V (*Cannone-2002*) follow a very specific pattern. A-minor is a frequently found RNA arrangement consisting of a stack of unpaired nucleotides, predominantly adenosines, that pack with a double helix (*Nissen-2001, Doherty-2001*). In the A-minor interactions that domain V forms with other parts of 23S rRNA, the double helix almost exclusively belongs to domain V, whereas the adenosine stack usually belongs to the rest of the molecule (Fig. 1). This characteristic distinguishes domain V from other domains of 23S rRNA, in which the proportion of the adenosine stacks and the double helical regions that form A-minor interactions is reversed.

To explain this abnormality of domain V, we suggest that it reflects the order in which different parts were added to 23SrRNA as it evolved. In the A-minor motif, the conformational integrity of the adenosine stack depends on the presence of the double helix, whereas the helix can maintain a stable conformation without interaction with its counterpart. Presuming that the integrity of the ribosome structure has been maintained throughout its entire evolution, adenosine stacks should not have appeared in rRNA before the corresponding double helices. Because domain V contains the peptidyl-transferase centre (PTC) (*Polacek-2005*), which performs the central function of the ribosome, we expect it to be among the most ancient elements of the ribosome structure. Thus, the abnormality of domain V can be explained by the formation of the A-minor interactions between double helices of a more ancient domain V and the adenosine stacks of more recently acquired parts of 23S rRNA.

THE MODEL

The ability of the A-minor motif to serve as an indicator of the relative age of its moieties can be used to determine the order in which different elements were added to the ribosome structure during its evolution. To demonstrate such ordered assembly, we developed a strategy of systematically dismantling the ribosome structure through elimination of those elements that could be considered as most recent acquisitions. As an element, we considered an individual double helix or a domain of stacked nucleotides that on addition to the ribosome structure would form a stable compact arrangement. We suggested a general principle that an element could not be a recent addition if its removal compromised the integrity of the remaining parts of the ribosome. The 5' and 3' ends of a removed element must be structurally close enough to each other to be considered a local insertion. This would guarantee that, after the fragment is removed, the remaining RNA chain maintains its structural integrity. Also, because the integrity of each strand of a double helix depends on the presence of the other strand, a removed element must contain both strands of the same helix. Finally, if a removed element forms the A-minor motif with the remaining ribosome, it must contain the stack of unpaired nucleotides that form this interaction, and not the double helix. Because our analysis was focused on A-minor interactions, the exact location of the boundaries between different elements was not essential, as long as all adenosine stacks and all corresponding double helical regions remained intact. Additional requirements imposed on elements are discussed in Supplementary Data 1.

Analysis of the tertiary structure of the Escherichia coli 23S rRNA (*Schuwirth-2005*) revealed 19 elements for which elimination does not compromise the integrity of the remaining part of the structure. These elements form level 1 in Fig. 2b; their location in the 23S rRNA secondary structure is shown in Fig. 2a and their complete description is given in Supplementary Data 1. The identified elements form a total of 13 A-minor interactions with regions located in the remaining part of the molecule (see Supplementary Data 2 and Supplementary Notes 1). In all of these interactions, the adenosine stacks belong to the identified elements, whereas the double helices are located in the remaining part of 23S rRNA. Thus, the elements of layer 1 could be considered the final generation of acquired elements. We then identified an additional 11 elements, the presence of which is essential for the integrity of only the elements of level 1. Accordingly, we describe these elements as constituting the penultimate generation of added elements (elements of level 2 in Fig. 2b). We repeated the same procedure ten more times and identified a total of 59 elements.

The position and the conformation of each identified element depend on the presence of only the elements of the preceding generations. In Fig. 2b, each dependency of element P on the presence of element Q is shown as arrow Q→P. There are two types of dependencies, D1 and D2. A D1 dependency indicates that the removal of Q before P would split the whole molecule into two separate parts. A D2 dependency indicates that the removal of Q before P would compromise the conformation of P. In total, we identified 59 D1 dependencies and 56 D2 dependencies. Out of all D2 dependencies, 54 were based on the formation of A-minor interactions. The remaining 2 D2 dependencies corresponded to two non-local pseudoknots (discussed later).

The removal of the 12 generations of acquired elements eliminated 93% of the original 23S rRNA. The remaining part, located in domain V, is shown in Fig. 2a by the blue and red lines; its central loop forms the PTC. Recently, it was observed that this region consists of two consecutive parts having practically identical secondary and tertiary structures (*Nissen-2000, Agmon-2005*) (blue and red parts in Fig. 2a; see also Supplementary Figs 1 and 2). The blue and red parts are arranged symmetrically to each other and form binding sites for the CCA-3'-termini of transfer RNA molecules in the P- and A-sites, respectively. Moreover, there is a very close correspondence between the positions of the nucleotides of both parts involved in the fixation of the equivalent elements of both tRNAs (*Nissen-2000, Agmon-2005, Samaha-1995, Kim-1999, Hansen-2002*).

The similarity between both parts is so high that it is logical to suggest that they originated by a duplication of the same RNA fragment. From this point of view, the evolution of 23S rRNA started with an initial fragment of about 110 nucleotides, which, probably, was able to bind the CCA-3'-terminus of what would later be tRNA. The duplication of this fragment allowed the resulting molecule to bind simultaneously two CCA-3'-termini. Within this arrangement, the two CCA-3'-termini associated with both parts are juxtaposed in space to allow for the transpeptidation reaction. Most probably, this dimer was already able to synthesize oligopeptides with random amino acid sequences, which would allow us to call it proto-ribosome. This view is supported by the fact that in-vitro-selected small RNA molecules resembling the PTC are able to perform transpeptidation (*Zhang-1997*), thus demonstrating that this reaction does not require any other elements of the ribosome structure. All other elements of 23S rRNA were gradually added to the structure, one element at a time, in essentially the same way. Each element could appear only when all elements that were required for its proper positioning had already been placed. New elements were added as insertions containing all necessary details to dock with the surface of the evolving ribosome without disturbing already existing parts. The most common way for a new element to be fixed on the ribosome surface would be through the formation of an A-minor interaction with an already existing double helix.

JUSTIFICATION OF THE MODEL

For justification of the suggested evolutionary model, we analysed those features of the 23S rRNA tertiary structure for which the 59 elements could be consecutively removed without damaging the integrity of the remaining part. Our analysis shows that removal of these elements is possible if and only if the arrows representing D1 and D2 dependencies do not form cyclic structures—that is, cases where a chain of several consecutive arrows arranged head-to-tail starts and finishes at the same element. A mathematically rigorous proof of this statement and the explanation of why the absence of cycles of dependence is essential for dismantling the 23S rRNA structure are given in Supplementary Notes 2.

The absence of cycles built of D1 dependencies reflects the hierarchical topology of the secondary structure of 23S rRNA in which the removal of remote elements of each domain would not compromise the integrity of the remaining RNA chain. Such topology could be disrupted by pseudoknots, several of which exist in 23S rRNA (*Gutell-1994*). However, most pseudoknots are arranged in the same region of the secondary structure and can be removed as a single element. Only two pseudoknots between the loops of elements 27–39 and 33–40 are not local (Fig. 2a). However, in both cases it was possible to split the two strands of the inter-loop double helix on the grounds that the conformational integrity of only one of the two loops requires the presence of the other loop (Fig. 2b and Supplementary Notes 1).

For the given 23S rRNA secondary structure, the absence of cycles involving D2 dependencies is a consequence of the particular orientation of many A-minor interactions. For example, dependency 41→6 (Fig. 2b) stands for the A-minor interaction between the double helix of element 41 and the adenosine stack of element 6. In the opposite situation, if the double helix occurred in element 6, while the adenosine stack was provided by element 41, four elements would have formed cycle 41→33→23→6→41. Similar cycles would have occurred in many other parts of 23S rRNA if the orientations of A-minor interactions were different. The existence of any such cycle would have arrested the procedure of dismantling the 23S rRNA structure before it reached PTC.

To demonstrate how unlikely the absence of cycles really is, we calculated the probability for the 23S rRNA structure to be cycle-free if the orientations of all A-minor interactions were chosen randomly. Our analysis presented in Supplementary Notes 2 shows that the total probability of a cycle-free arrangement in this case would be $P < 10^{-9}$. Such low probability excludes the possibility that the absence of cycles of dependence in 23S rRNA has occurred by chance. Instead, it strongly supports a hierarchical scenario for its evolution, according to which the integrity of each element of 23S rRNA depends only on the presence of more ancient elements of its structure. The absence of cycles in the 23S rRNA tertiary structure does not depend on the way we defined individual elements, but instead represents a fundamental property of this molecule.

MAJOR PERIODS IN THE 23S RRNA EVOLUTION

The scheme of dependencies presented in Fig. 2b can help us to elucidate some details of the evolution of the large ribosomal subunit after the emergence of the proto-ribosome. Our analysis shows that stabilization of the proto-ribosome tertiary structure was a major aspect of the 23S rRNA evolution in the post-proto-ribosome era. In Fig. 3 the structure of the proto-ribosome is shown without other parts of 23S rRNA (Fig. 3a) and with the gradually increasing number of added elements (Fig. 3b–e). The elements forming each structure are shown in Supplementary Fig. 3. The first 8 elements added to the proto-ribosome form a foundation that closely interacts with the bottom part of the proto-ribosome and effectively supports its conformation (Fig. 3b). Further addition of 12 elements makes this foundation wider and more massive (Fig. 3c). Finally, after the addition of a total of 50 elements, the proto-ribosome became surrounded by added elements on all sides except the side from which PTC must be reached by tRNAs (Fig. 3d). The added elements were arranged so they did not interfere with the release of the nascent peptide, leading to the formation of the exit channel (4 in Fig. 3f).

The emergence of the foundation provided new functional opportunities. In particular, it allowed the formation of the area of contact with the small ribosomal subunit (Fig. 3e), which was essential for the integration of this subunit into the ribosome. Another consequence of the lateral expansion of the proto-ribosome foundation was that it allowed the formation of the three protuberances (yellow in Fig. 3e–g). In Fig. 2b, the elements forming these protuberances are positioned at the upper levels (see Supplementary Fig. 3f). Correspondingly, the particular functions associated with the protuberances—namely, the assistance in the selection of the proper aminoacyl-tRNA and the GTPase reaction (*Savelsberg-2000, Kavran-2007*) (the L7/L12 protuberance) as well as in the release of the deacylated tRNA from the E-site (*Nikulin-2003*) (the L1 protuberance)—should be relatively late acquisitions of the ribosome.

Our results also demonstrate that, despite its visible complexity, the structure of 23S rRNA follows a rather simple principle and could have evolved in a relatively short time on the evolutionary scale. Each new insertion emerged randomly and was accommodated only if it made the ribosome more stable and effective as a transpeptidase. At early stages of evolution, the ribosome existed exclusively as an RNA body. Later, when the ribosome functioning became sufficiently effective to produce proteins, the latter started playing an important part in the ribosome structure. We can argue that, among all structures shown in Fig. 3, the structure in Fig.

3b corresponds most closely to the moment when the RNA world changed for the protein-based world. This conclusion is based on the fact that although ribosomal proteins interact with the structure in Fig. 3b only marginally, they form extensive contacts with later structures (not shown). Whether indeed the structure in Fig. 3b corresponds to the end of the RNA world and thus represents the most effective all-RNA ribosome, however, requires further experimental analysis.

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REFERENCES

Agmon, I., Bashan, A., Zarivach, R. & Yonath, A. Symmetry at the active site of the ribosome: structural and functional implications. Biol. Chem. **386**, 833–844 (2005).

Ban, N. et al. The complete atomic structure of the large ribosomal subunit at 2.4A° resolution. Science **289**, 905–920 (2000).

Cannone, J. J. et al. The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics **3**, 2 (2002).

Crick, F. H. The origin of the genetic code. J. Mol. Biol. **38**, 367–369 (1968).

Doherty, E. A., Batey, R. T., Masquida, B. & Doudna, J. A. A universal mode of helix packing in RNA. Nature Struct. Biol. **8**, 339–343 (2001).

Doudna, J. A. & Rath, V. L. Structure and function of the eukaryotic ribosome: the next frontier. Cell **109**, 153–156 (2002).

Gilbert, W. The RNA world. Nature **319**, 618 (1986).

Gutell, R. R., Larsen, N. &Woese, C. R. Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. Microbiol. Rev. **58**, 10–26 (1994).

Hansen, J. L., Schmeing, T. M., Moore, P. B. & Steitz, T. A. Structural insights into peptide bond formation. Proc. Natl Acad. Sci. USA **99**, 11670–11675 (2002).

Harms, J. et al. High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. Cell **107**, 679–688 (2001).

Kavran, J. M. & Steitz, T. A. Structure of the base of the L7/L12 stalk of the Haloarcula marismortui large ribosomal subunit: analysis of L11 movements. J. Mol. Biol. **371**, 1047–1059 (2007).

Kim, D. F. & Green, R. Base-pairing between 23S rRNA and tRNA in the ribosomal A site. Mol. Cell **4**, 859–864 (1999).

Nikulin, A. et al. Structure of the L1 protuberance in the ribosome. Nature Struct. Biol. **10**, 104–108 (2003).

Nissen, P. et al. The structural basis of ribosome activity in peptide bond synthesis. Science **289**, 920–930 (2000).

Nissen, P. et al. RNA tertiary interactions in the large ribosomal subunit: the A-minor motif. Proc. Natl Acad. Sci. USA **98**, 4899–4903 (2001).

Noller, H. F., Hoffarth, V. & Zimniak, L. Unusual resistance of peptidyl transferase to protein extraction procedures. Science **256**, 1416–1419 (1992).

Ogle, J. M. et al. Recognition of cognate transfer RNA by the 30S ribosomal subunit. Science **292**, 897–902 (2001).

Polacek, N. & Mankin, A. S. The ribosomal peptidyl transferase center: structure, function, evolution, inhibition. Crit. Rev. Biochem. Mol. Biol. **40**, 285–311 (2005).

Samaha, R. R., Green, R. & Noller, H. F. A base pair between tRNA and 23S rRNA in the peptidyl transferase centre of the ribosome. Nature **377**, 309–314 (1995).

Savelsbergh, A. et al. Stimulation of the GTPase activity of translation elongation factor G by ribosomal protein L7/12. J. Biol. Chem. **275**, 890–894 (2000).

Schuwirth, B. S. et al. Structures of the bacterial ribosome at 3.5 A° resolution. Science **310**, 827–834 (2005).

Selmer, M. et al. Structure of the 70S ribosome complexed with mRNA and tRNA. Science **313**, 1935–1942 (2006).

Stillman, B. (ed.) The Ribosome. Cold Spring Harbor Symposia on Quantative Biology (Cold Spring Harbor Laboratory Press, 2001).

Yusupov, M. M. et al. Crystal structure of the ribosome at 5.5A resolution. Science **292**, 883–896 (2001).

Zhang, B. & Cech, T. R. Peptide bond formation by in vitro selected ribozymes. Nature **390**, 96–100 (1997).

FIGURES

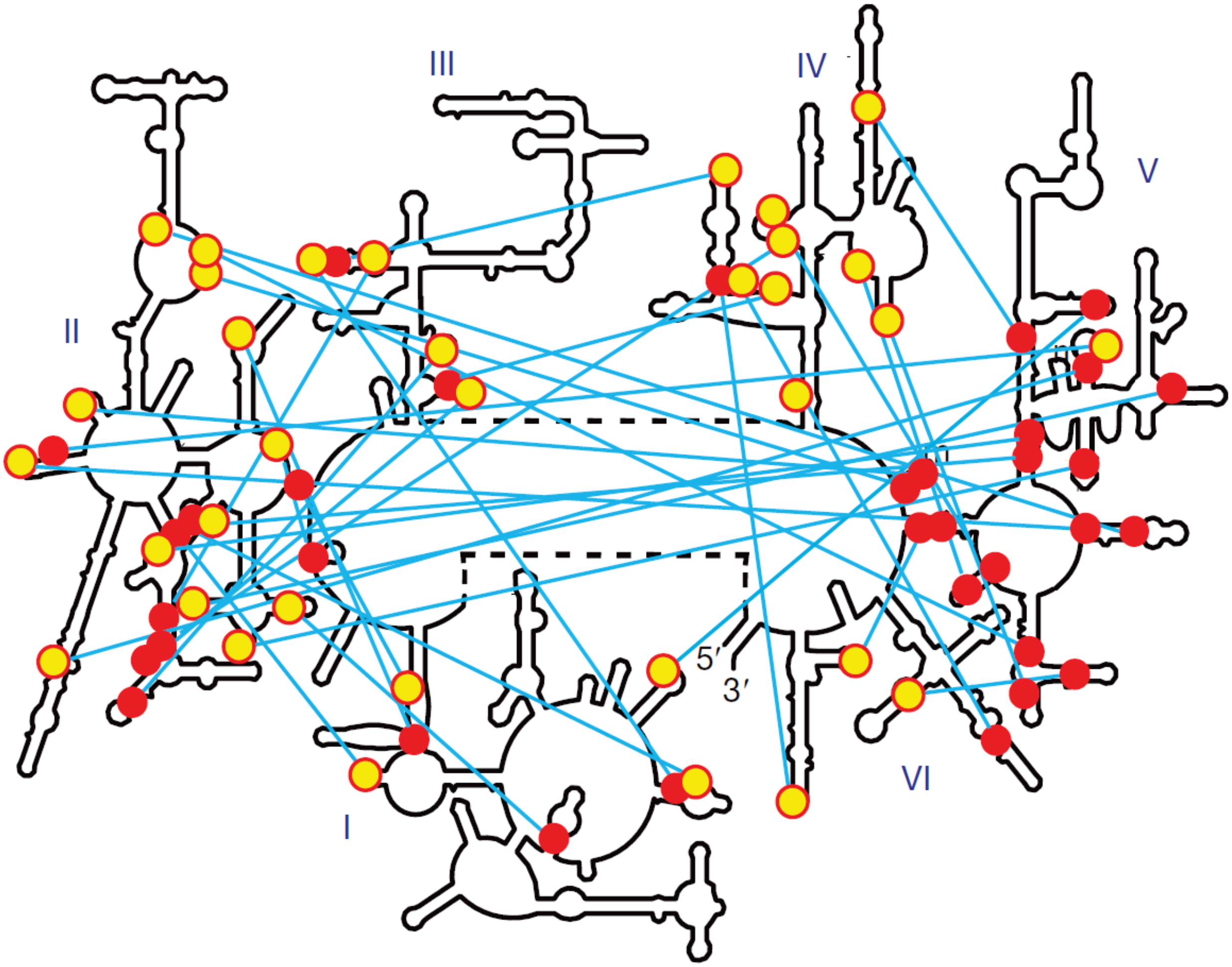


Figure 1

Location of inter-domain A-minor interactions in the secondary structure of the E. coli 23S rRNA.

The secondary-structure domains are marked by roman numerals. Each A-minor interaction is shown by a cyan line connecting the double helix (red circle) and the corresponding adenosine stack (yellow circle). Unlike other domains, domain V almost exclusively forms these interactions using double helices and not adenosine stacks.

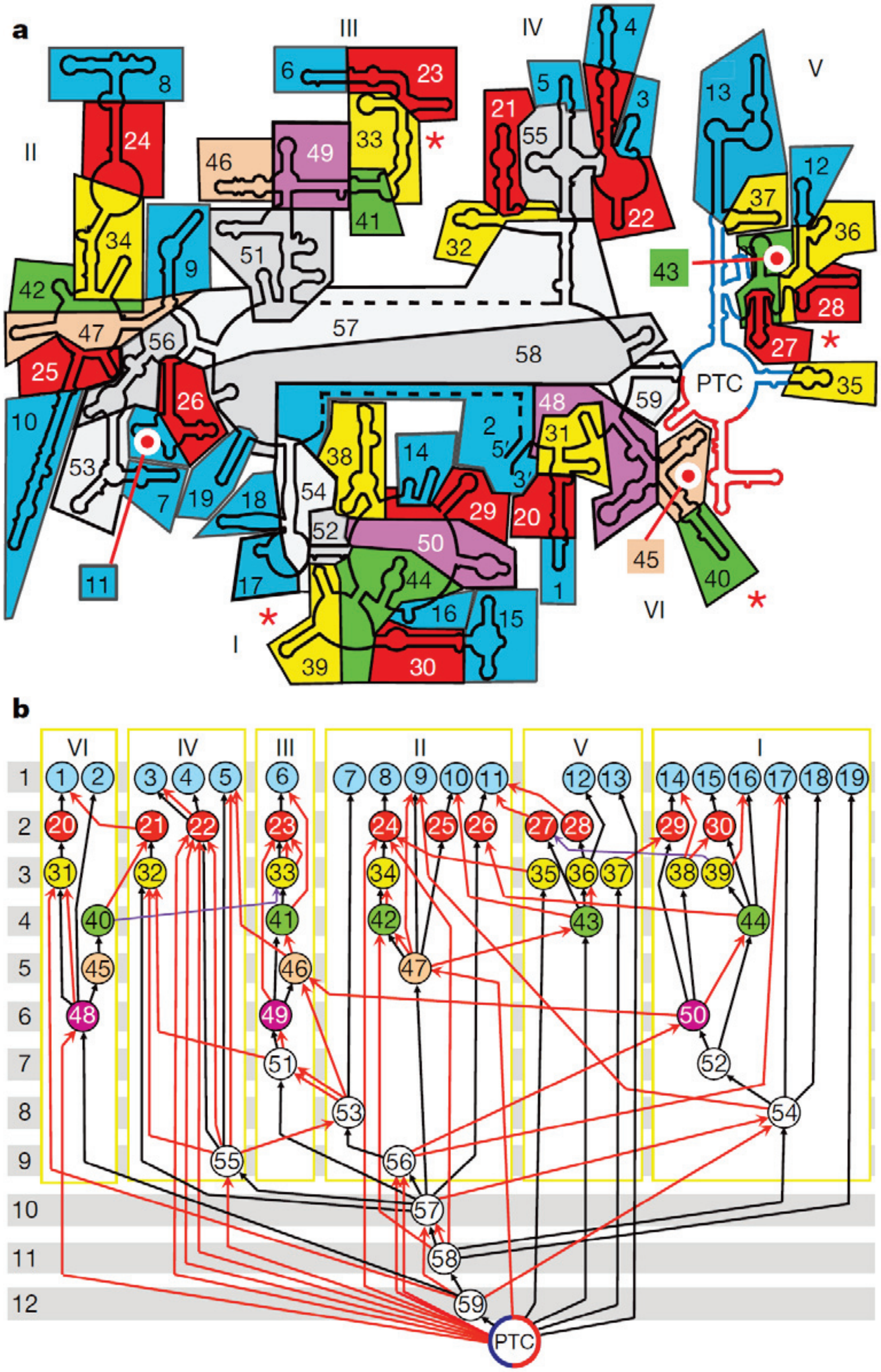


Figure 2

The location of the identified elements in the E. coli 23S rRNA secondary structure (a) and the network of D1 and D2 dependencies between them (b).

(the legend is provided on the next page)

Legend of Figure 2 (see previous page)

Each element has the same colour in (**a)** and (**b)**. The roman numerals indicate secondary-structure domains. PTC stands for the symmetrical arrangement in domain V containing the peptidyl-transferase centre (the proto-ribosome).

(a), The two halves of the proto-ribosome are blue and red. Red asterisks indicate the four elements that form two non-local pseudoknots 27–39 and 33–40.

(b), An arrow connecting two elements Q→P indicates that the position of P depends on the presence of Q. Black and coloured arrows represent D1 and D2 dependencies, respectively. Red arrows Q→P represent A-minor interactions formed by a double helix of element Q and a nucleotide stack of element P. Two violet arrows originate from the dissection of two non-local pseudoknots (see Supplementary Notes 1). The numbers of levels are shown on the left. The detailed description of all elements and of all D2 dependencies is given in Supplementary Data 1 and 2.

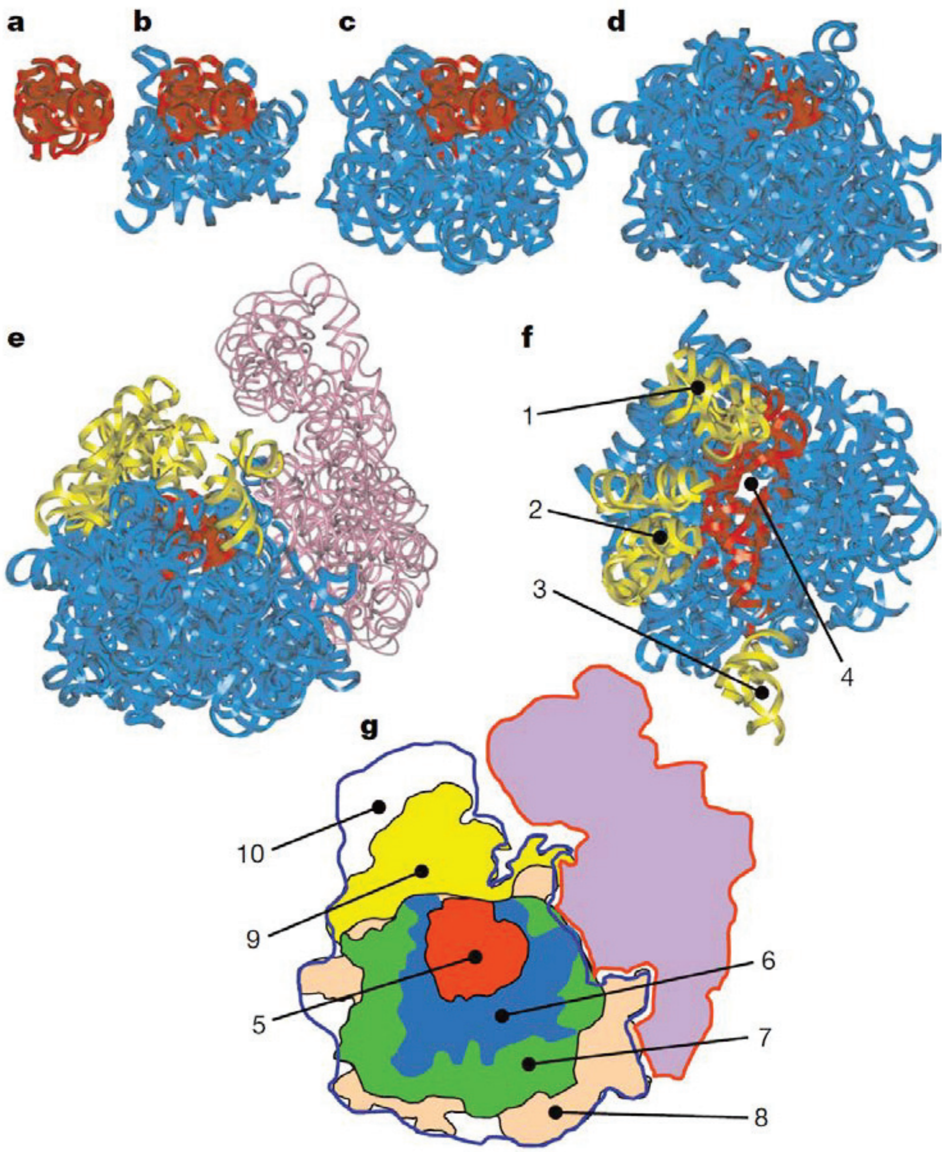


Figure 3 The aggrandizement of the 23S rRNA structure during its evolution.

a–e, the proto-ribosome with 0 (**a**), 8 (**b**), 20 (**c**), 50 (**d**) and all 59 (**e**) elements added. The proto-ribosome is red, elements forming the protoribosome foundation are blue, the protuberances are yellow, and 16S rRNA is purple. The complete list of the elements forming structures a–e is given in Supplementary Fig. 3. f, The top view of the 23S rRNA structure shown in e. g, The positions of the parts of 23S rRNA shown in a–e in the context of the whole ribosome. The structures of the 50S and 30S subunits are contoured by the blue and red line, respectively. 1–3 are the L7/L12, central and L1 protuberances, respectively; 4 is the exit channel; 5–9 are the structures shown in a–e, respectively; 10 is the part of 50S subunit that does not include 23S rRNA. This part is formed by ribosomal proteins and 5S rRNA.

SUPPLEMENTARY INFORMATION

1. Supplementary Data 1: The complete description of all elements identified in *E.coli* 23S rRNA

The structure analyzed

For the analysis, we took the structure of the *E.coli* ribosome (pdb identifies 2AW4, see *Ogle-2001*).

What is element?

As an element, we considered an individual double helix or an arrangement of stacked nucleotides, which upon addition to the ribosome structure would form a stable compact arrangement. The position of each element in the 23S rRNA is shown in Fig 2a of the main text.

The boundaries between elements

The boundaries between all elements were arranged to make each element a putative insertion, having the 5´- and 3´-termini close to each other.

For A-minor interactions, the double helix and the adenosine stack belonged to two different elements. Also, the boundaries between elements were arranged to split neither adenosine stack, nor the corresponding part of the double helix. As a result, each moiety of an A-minor motif kept its integrity. These rules were not applicable to those A-minor interactions in which the adenosine stack and the double helix were not separated by at least one base pair (see Supplementary Data 2). The latter A-minor interactions were out of scope of this research.

In most cases, the boundary between two elements coincided with the boundary between two domains of stacked nucleotides. In some cases, more than one helix was included in the element. Such cases mostly pertained to local pseudoknots. For these pseudoknots, both double helices forming the pseudoknot were included in the element (see the main text). Also, if a new element was unable to form a stable contact with the ribosome surface, it could be extended to the degree that such contact became possible.

We should note, however, that because our analysis was focused on A-minor interactions, the exact location of the boundaries between different elements was not essential, as long as all adenosine stacks and all corresponding double helical regions remained intact.

The description of each element in the following list

The number of each motif corresponding to that in Fig 2 of the main text is red.

Each element is composed of one or several fragments of the 23S rRNA polynucleotide chain. Each fragment is enclosed in brackets. For each fragment, the identity and the number of the first and the last nucleotides is provided.

Each element as a result of a local insertion

In each element, the first nucleotide of the first fragment and the last nucleotide of the last fragment are positioned close to each other in the tertiary structure. This closeness was required to qualify the element as a putative insertion. If the element consists of more than one fragment, the last nucleotide of each fragment and the first nucleotide of the following fragment are also positioned close to each other in the tertiary structure. This closeness reflects the fact that the piece of 23S rRNA enclosed between the two nucleotides has also been qualified as a putative insertion.

1 = (A2850 → U2866)
2 = ((G1 → C8) + (G2895 → U2904))

Comment: In our analysis, we treated 23S rRNA as a circular molecule, which allowed us to consider the double helix formed by the 5´- and 3´-terminal parts of the molecule as an individual element (element 2) and to remove it at a due moment.

3 = (G1906 → G1929)
4 = (U1855 → G1888)
5 = (A1802 → U1818)
6 = (G1482 → C1507)
7 = (U702 → C732)
8 = (A1048 → A1111)
9 = (G1198 → G1247)
10 = (U846 → G930)
11 = (A627 → U653)
12 = (U2297 → U2320)
13 = (A2198 → C2226)
14 = (G121 → U148)
15 = (G295 → C343)
16 = (A222 → G232)
17 = (U448 → A472)
18 = (C475 → C509)

19 = (A532 → G561)

20 = ((U2832 → U2849) + (G2867 → A2883))

21 = (G1707 → G1756)

22 = ((G1835 → C1854) + (A1889 → C1905) + (G1930 → A1970))

23 = ((U1467 → U1481) + (A1508 → G1546))

24 = ((G1026 → G1047) + (G1112 → A1134))

25 = ((G822 → A845) + (U931 → A945)

26 = ((U588 → A626) + (A654 → A670))

27 = (A2392 → C2424)

28 = (C2347 → G2382)

29 = ((A119 → U120) + (A149 → G177))

30 = ((G271 → A294) + (A344 → G367))

31 = ((G2791 → G2831) + (U2884 → G2892))

32 = ((G1681 → C1706) + (A1757 → G1763))

33 = ((G1429 → U1466) + (C1547 → G1568))

34 = ((C991 → G1025) + (C1135 → G1185))

35 = (G2464 → G2488)

36 = ((C2283 → U2296) + (U2321 → A2346) + (G2383 → G2389))

37 = (G2093 → U2197)

38 = (G51 → A118)

39 = (A371 → A423)

40 = (U2690 → G2719)

41 = ((G1416 → C1428) + (A1569 → C1582))

42 = ((G974 → A990) + G1186)

43 = ((U2259 → G2282) + (U2390 → G2391) + A2425)

44 = ((A216 → A221) + (A233 → A270) + (A368 → G370) + (G424 → C433)

45 = ((C2646 → U2689) + (U2720 → G2732))

46 = (C1350 → G1381)

47 = ((C812 → A821) + (C946 → A973) + (G1187 → C1197) + (A1248 → G1250))

48 = ((C2629 → G2645) + (A2733 → U2790) + (A2893 → U2894))

49 = ((G1341 → C1349) + (G1382 → U1415) + (A1583 → U1602))

50 = ((G46 → U50) + (G178 → G215))

51 = ((G1271 → U1340) + (A1603 → U1647))

52 = ((G35 → G45) + (U434 → C445))

53 = ((U686 → G701) + (G733 → U773))

54 = ((G9 → U34) + (G446 → A447) + (G473 → G474) + (C510 → A526))

55 = ((C1764 → A1801) + (U1819 → U1834) + (U1971 → C1990))

56 = ((C671 → A685) + (G774 → U811))

57 = ((G579 → C587) + (C1251 → C1270) + (U1648 → U1680) + (U1991 → U2017))

58 = ((C527 → C531) + (U562 → G578) + (G2018 → A2042))

59 = ((C2043 → G2057) + (C2611 → G2628))

PTC = ((A2058 → U2092) + (A2227 → C2258) + (A2426 → C2463) + (U2489 → 2610)

Comment: PTC stands for the symmetrical structure in Domain V that contains the peptidyl-transferase centre. This structure is seen in Fig 2a of the main text (blue and red parts) and in Supplementary Figs 1, 2. We argue that this structure represents the proto-ribosome

2. Supplementary Data 2: the complete list of the A-minor motifs that are used for determination of D2 dependences

For determination of D2 dependences, we used only those A-minor interactions that fitted to the following criteria:

Single-nucleotide A-minor interactions were not considered

1. The stack of unpaired nucleotides contained two or more nucleotides. There are two reasons to ignore those A-minor interactions that were formed by individual non-stacked nucleotides. First, the concept of integrity of the adenosine stack is not applicable to a single nucleotide. Second, there is a reasonably high probability that an individual non-stacked adenosine emerged after the emergence of the element that contains it. In this case, it would be deceiving to use the orientation of such A-minor-interaction for determination of the relative age of the two elements between which it is formed.

The two moieties of the A-minor motif must be separated

2. We also required that the adenosine stack and the corresponding double helical region be separated in the 23S rRNA secondary structure by at least one base pair. This requirement helped us to avoid situations when the double helix and the adenosine stack are so close to each other that most probably, emerged simultaneously.

Not only adenosines are allowed

3. The identities of the unpaired nucleotides that interact with the double helix do not need to be restricted to adenosines. Other nucleotide identities are also permitted, as long as all other aspects of A-minor interactions are preserved.

Adenosines can interact not only with the minor groove

4. Although it is generally believed that in A-minor motifs, the adenosine stack interacts exclusively with the minor groove of a double helix, in reality, it can also interact with backbones (ref. 19 in the main text). We do not limit our consideration to a particular type of contact between the unpaired nucleotides and the double helix, as long as this contact is essential for the integrity of the stack of unpaired nucleotides.

1 ← 21

A2860 ← (C1708=G1750)
G2859 ← (U1709=A1749)
C2858 ← (G1710=C1748)

3 ← 22

A1927 ← (C1837=G1903)
A1928 ← (C1836=G1904)

5 ← 46

A1809 ← (C1363=G1368)
A1810 ← (G1362=C1369)

5 ← 55

A1802 ← (U1798=A1821)
A1803 ← (G1797=C1822)

6 ← 41

A1494 ← (C1577=G1421)
A1495 ← (C1577=G1421)
A1496 ← (C1577=G1421)
A1496 ← (U1576=G1422)

9 ← 47

U1224 ← (G1193=C814)
G1225 ← (A1194=U813)
A1226 ← (G1195=C812)

9 ← 54

A1213
A1214 ← (G30=C510)

10 ← 43

A910 ← (C2264=G2276)
A911 ← (C2263=G2277)

11 ← 28

A644 ← (C2368=G2349)

U646

11 ← 27

A631 ← (U2401=G2415)

A632 ← (C2403=G2414)

14 ← 38

A126 ← (G54=C116)

A127 ← (G55=C115)

16 ← 39

A226 ← (G409=C418)

A227 ← (G410=C417)

17 ← 56

G465 ← (U683=A794)

A466 ← (G682=C795)

21 ← 40

A1754 ← (G2693=C2716)

A1755 ← (G2694=C2715)

22 ← 55

A1900 ← (C1793=G1826)

A1900 ← (A1794=U1825)

A1970 ← (G1792=U1827)

22 ← PTC

A1952 ← (U2548=A2560)

A1953 ← (G2549=C2559)

22 ← PTC

C1965 ←

A1966 ← (G2592=C2601)

22 ← PTC

A1853 ← (U2086*U2233)

A1853 ← (G2087=C2232)

A1854 ← (G2087=C2232)

A1889 ← (U2086*U2233)

A1890 ← (U2085=G2234)

23 ← 33

A1477 ← (G1435=C1557)

A1515 ← (G1436=C1556)

23 ← 33

A1528

A1544 ← (C1447=G1464)

A1545 ← (C1446=G1465)

23 ← 49

A1469 ← (C1386=G1401)

A1470 ← (A1385=U1402)

24 ← 35

A1028 ← (G2464=C2486)

A1029 ← (C2465=G2485)

24 ← 58

U1132

A1133 ← (C2025=G2038)

A1133 ← (U2026=A2037)

24 ← PTC

G1128 ← (A2516=U2568)

A1129 ← (C2515=G2569)

26 ← 44

A608 ← (C237=G260)

A608 ← (C238=G259)

A609 ← (C237=G260)

A621 ← (C238=G259)

29 ← 37

A160 ← (C2208=G2216)

A161 ← (C2207=G2217)

30 ← 38

A294 ← (G80=C106)

A345

A346 ← (C79=G107)

A347

31 ← 48

A2809 ← (G2630=C2788)

A2810 ← (G2631=C2787)

31 ← 59

A2823 ← (C2047=G2621)

C2824 ←

32 ← 55

A1690 ←

A1700 ← (G1766=C1986)

32 ← 51

A1759 ← (U1629=A1637)
C1760 ← (A1630=U1636)

34 ← 42

A1000 ← (G976=C987)
A1001 ← (G977=C986)
A1001 ← (G978=C985)
A1155 ← (G976=C987)

36 ← 43

A2327 ← (C2261=G2279)
A2388 ← (C2260=G2280)

55 ← PTC

A1785 ← (G2588=C2606)
A1785 ← (A2589=U2605)
A1787 ←

41 ← 46

A1569 ← (C1351=G1380)
A1570 ← (C1351=G1380)
A1571 ← (C1351=G1380)
A1572 ← (C1350=G1381)

42 ← 47

A983 ← (C946=G971)
A984 ← (A947=U970)

42 ← 58

A980 ← (U2026=A2037)
A981 ← (G2027=C2036)

43 ← 47

A2273 ← (G953=C964)
A2274 ← (G954=C963)

44 ← 50

G250 ← (G194=C201)
A251 ← (G194=C201)

46 ← 50

A1365 ← (G187=C209)
A1365 ← (G188=C208)
A1366 ← (G186=C210)

46 ← 53

A1353 ← (A693=U769)

A1354 ← (C692=G770)

A1378 ← (U694=G768)

47 ← PTC

A959 ← (U2457=G2484)

A960 ← (C2456=G2495)

48 ← PTC

A2740 ← (G2523=C2540)

A2741 ← (G2524=C2539)

A2764 ← (G2523=C2540)

49 ← 51

A1392 ← (C1315=G1337)

A1393 ← (C1314=G1338)

50 ← 56

A190 ← (C679=G798)

A191 ← (C678=G799)

A207 ← (C680=G797)

51 ← 53

A1272 ← (A743=U754)

A1618 ← (A743=U754)

51 ← 53

G1300

A1634 ← (C698=G763)

53 ← 55

A764 ← (U1775=A1789)

A781 ← (G1776=C1788)

Comment: this case is special. It is discussed in Supplementary Notes l

54 ← 57

A514 ← (C581=G1259)

A515 ← (U580=A1260)

54 ← 59

A14 ← (C2044=G2624)

A14 ← (C2045=G2623)

A526 ← (C2043=G2625)

56 ← PTC

A675 ← (G2067=C2443)

A676 ← (G2069=C2442)

56 ← PTC

A793 ← (C2073=G2436)
G784 ← (C2072=G2437)
A792 ← (C2072=G2437)

57 ← 59

A1654 ← (G2048=C2620)
A1655 ← (G2049=C2619)

57 ← 58

G1252 ← (A563*G578)
A1253 ← (C564=G577)

3. Supplementary Notes 1: Special cases in determination of D2 dependencies

1. Contact 53, 56 ← 55

In 23S rRNA, three elements 53, 54 and 56 form the following contact:

53, 56 ← 55

53: A764 ← (U1775=A1789)
56: A781 ← (G1776=C1788)

An unusual feature of this contact is that here, the two adenines are distant from each other in the 23S rRNA secondary structure. This aspect did not allow us to keep both adenosines within the same element. As a result, the two adenosines belong to two different elements, 53 and 56. We think that the integrity of element 56 does not depend on the presence of element 55. Indeed, A781 in element 56 is involved in loop 780-GAAA-783, which would form a stable tetraloop arrangement if the contact with element 55 did not exist. Only in the presence of element 55 and after the emergence of element 53, A781 can break from the tetraloop arrangement 780-GAAA-783 and can form a stack with A764 of element 53, which, in turn, would form the A-minor interaction with element 55.

Such a scenario of the formation of this A-minor interaction would make the integrity of only element 53 and not of 56 dependent on the presence of element 55

2. Contact PTC ← 56

There is a rather weak contact between PTC and element 56, which consists of a hydrogen bond between the amino group of A2060 (PTC) and the O2’ group of the unpaired nucleotide U807 (element 56):

PTC ← 56

A2060 ← (C673 - U807)
G2502

Although this contact can be qualified as a variant of A-minor interaction, we do not think that the integrity of PTC depends on it: in addition to the interaction with element 56, A2060 forms van der Waals contacts with the backbone of nucleotides G2444 and G2445 within PTC. The latter contacts would stabilize the position of A2060 even in the absence of element 56. Based on these considerations, we did not consider contact A2060 ← (C673 - U807) as a D2 dependency.

3. Non-local pseudoknots

23S rRNA contains two non-local pseudoknots between elements 33-40 and 27-39, which in Fig 2a of the main text are shown by red asterisks. In both cases, one element (elements 33 and 27, Fig 2b of the main text) can be removed separately from the other one (elements 40 and 39) on the ground that such removal would not compromise the integrity of the latter element.

In the 33-40 case, region 2702-2704 of element 40 forms a double helix with region 1455-1457 of element 33. In element 40, this region is a part of a tetraloop, which would have a quasi-stable structure even without the interaction with element 33. This aspect allows the dissection of the pseudoknot and the removal element 33 separately from element 40.

A similar situation occurs in the 27-39 case. Here, region 413-416 of element 39 forms a double helix with region 2407-2410 or element 27. Element 39, due to the formation of base pair C385-G411, also represents a local pseudoknot. The presence of this base pair makes the conformation of loop 411-416 stable even without interaction with element 27. This aspect allows the dissection of the pseudoknot and the removal of element 27 separately from element 39.

4. Supplementary Notes 2: Quantitative validation of the proposed model of the 23S rRNA evolution

A. Why the absence of cycles of dependence is essential?

The possibility to remove 59 elements without affecting the integrity of the remaining part of 23S rRNA is manifested by the fact that in Fig 2b of the main text all D1 and D2 dependencies are oriented from a lower to higher level. A theorem can be proven that the orientation of all dependences from a lower to higher level is possible **if and only if** D1 and D2 dependencies do not form cycles.

Below we provide the proof of this theorem formulated in terms of the graph theory. All undefined terms can be found in: J.A. Bondy and U.S.R. Murty. Graph Theory. Springer, GTM 244, 2008. ISBN: 978-1-84628-969-9.

Let D=(V,A) be a finite digraph with the vertex set V and the arc set A. We define a sequence of digraphs D_i=(V_i,A_i), i=0,1,... so that D_0=D and V_{i+1}=V_i-S_i, A_{i+1}=A_i-A'_i for all i = 0,1,... where S_i is the set of sinks of D_i and A'_i is the set of all arcs of D_i that are incident to sinks. Clearly, |V_{i+1}| is less or equal than |V_i| for all i>0. So, there exists a positive integer m such that |V_m|=|V_{m+1}|=....

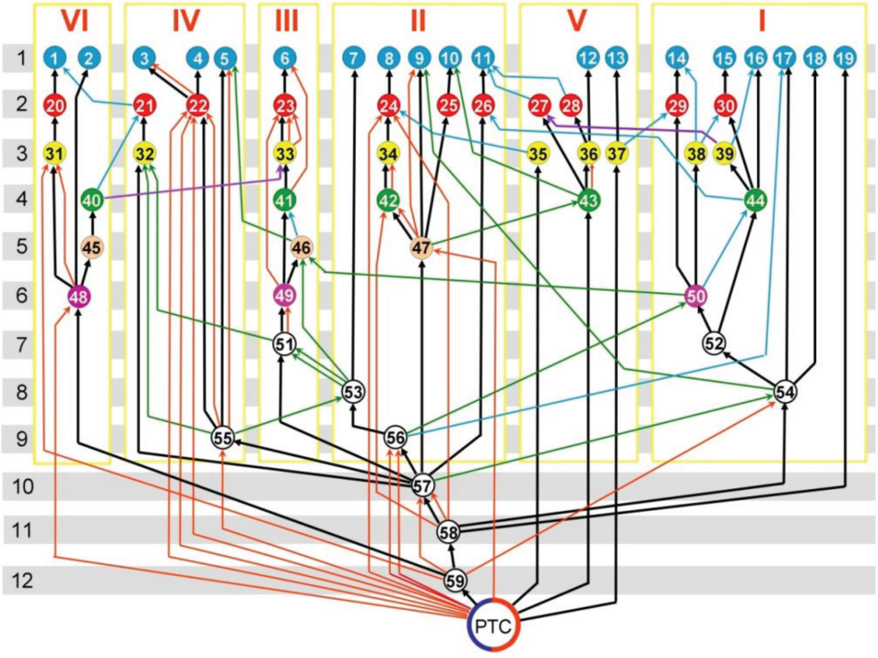
Take the minimal m with this property and set D^*=D_m.

Claim.The digraph D^* is empty (i.e. contains no vertices) if and only if the digraph D is acyclic.

Proof. **Let C_i be the set of vertices of a directed cycle of the digraph D_i for some i.** Then obviously the intersection of C_i and S_i is empty. This implies that any directed cycle of D is also a directed cycle of D^*. Thus the necessity follows. **Conversely, let the digraph D be acyclic**. Suppose that the digraph D^*=D_m is not empty. Since D_m=D_{m+1}, we conclude that set S_m is empty. Then given a vertex v of D_m there exists a vertex u such that (v,u) is the arc of D_m. It follows that D_m has a directed cycle. However this cycle is obviously the cycle of D. So D is not acyclic. **Contradiction**.

Corollary: the probability that all D1 and D2 dependencies can be arranged from a lower to higher level is equal to the probability that these dependencies do not form cycles.

B. Calculation of the probability P that D1 and D2 dependencies do not form cycles if the orientations of all D2 dependencies are chosen randomly



The calculation of probability P was based on the analysis of the scheme of D1 and D2 dependencies shown above. This scheme is equivalent to that given in Fig 2b of the main text, except that here, all D2 dependencies are divided in four classes based on their ability to form cycles. Each class is shown by its own colour. Red, green and blue dependencies correspond to A-minor interactions. Violet dependencies correspond to non-local pseudoknots.

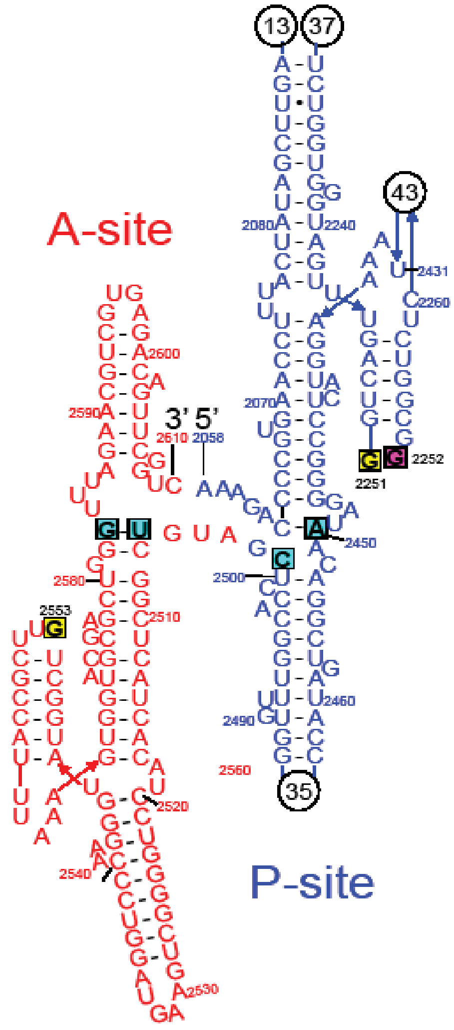
In the 28 red dependencies Q→P, element Q is positioned in the 23S rRNA secondary structure on the way between PTC and element P. For each dependency of this group, the inversion of the orientation will provide a cycle. The probability that none of these dependencies creates a cycle is thus P1 = 2^{-28}.

Then, 13 green inter-branch dependencies can form additional cycles containing two or more such dependencies. For example, a simultaneous inversion of two green dependencies 57→54 and 54→9 creates cycle 57→47→9→54→57. Therefore, the probability that these two dependencies form a cycle is ¼, and, correspondingly, the probability that they do not form a cycle is ¾. The total probability P2 that all green dependences do not form cycles was determined through consideration of all possible combinations of orientations of the green dependences. This analysis provides P2 = 207×2^{-10}.

Finally, 13 blue as well as 2 violet dependencies do not provide additional opportunities for cycling compared to the red and green dependencies.

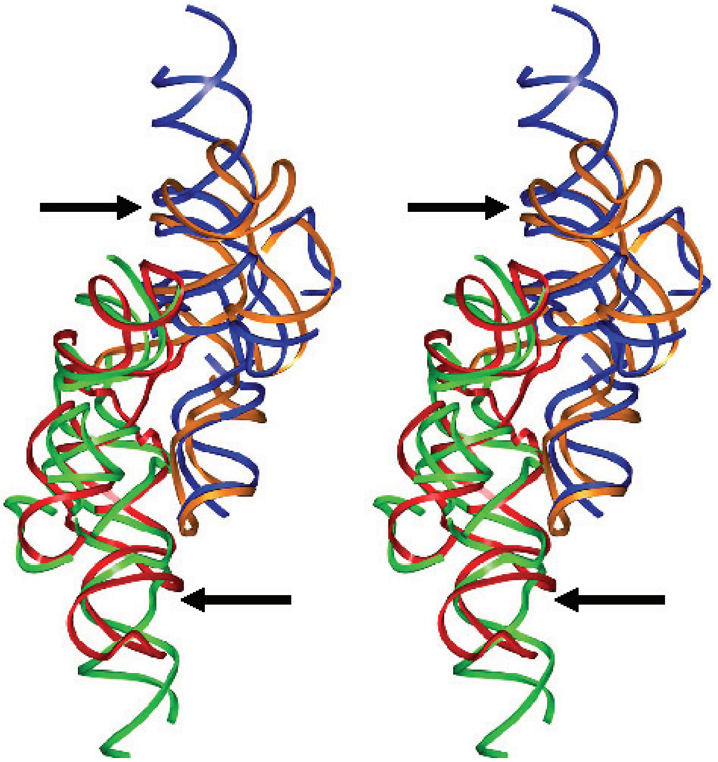
The total probability of the absence of cycles is thus $P = P1 \times P2 = 207 \times 2^{-38} \approx 7.53 \times 10^{-10}$.

5. Supplementary Figure 1: The nucleotide sequence and the secondary structure of the symmetrical region in Domain V containing PTC



The blue and red parts of the symmetrical region in Domain V correspond to the blue and red parts in Fig 2a of the main text). The boxed nucleotides are involved in the coordination of tRNA nucleotides C74 (magenta), C75 (yellow) and A76 (cyan) in the P- (blue part) and A-sites (red part) (reviewed in Norbert Polacek and Alexander S. Mankin *Critical Reviews in Biochemistry and Molecular Biology*, 40:285–311, 2005). Nucleotides A2450 and C2501 are involved in base triple C2063-A2450-C2501 (not shown). The positions of removed elements 13, 35, 37 and 43 are indicated. There is a strong symmetry between the two parts of the structure shown in this figure on the levels of secondary structure and function. However, on the level of tertiary structure, this symmetry does not include the peripheral helices [2077-2088; 2231-2243] and [2520-2545], which have different orientations. This difference is indicated by the inclination of helix [2520-2545] from the vertical orientation. Most probably, the difference in the orientation of the two helices has originated from the fact that the two helices are attached to the central part of the structure through flexible connections 2075-2076-2244 and 2518-2519-2546. Correspondingly, their orientations in the tertiary structure are dependent on their interactions with other parts of 23S rRNA.

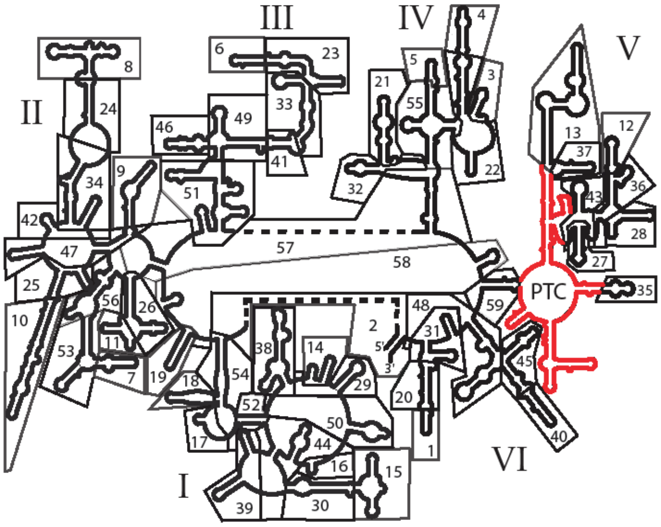
6. Supplementary Figure 2: The tertiary structure of the symmetrical region in Domain V containing PTC



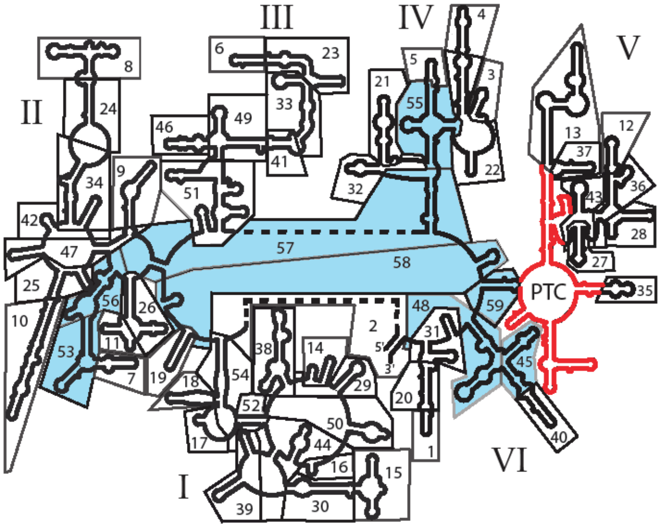
The stereo-view of the superposition of the symmetrical region with its own image rotated for 180º. The red and blue parts correspond to those in the secondary structure (see Fig 2a of the main text and Supplementary Figure 1). In the rotated image, the brown and green parts are the same as red and blue, respectively. In general, the whole structure is reasonable, well superposable with its own image. The only parts of the structure that cannot be superposed are the peripheral helices [2077-2088; 2231-2243] and [2520-2545] (shown by arrows). We suggest that the whole symmetrical region emerged due to the duplication of a 110 nucleotide fragment. However, due to the fact that the peripheral helices [2077-2088; 2231-2243] and [2520-2545] were attached to the central part of this structure through flexible connections 2075-2076-2244 and 2518-2519-2546 (see Supplementary Figure 1), the particular orientation of the two helices became dependant on the interaction with other parts of 23S rRNA.

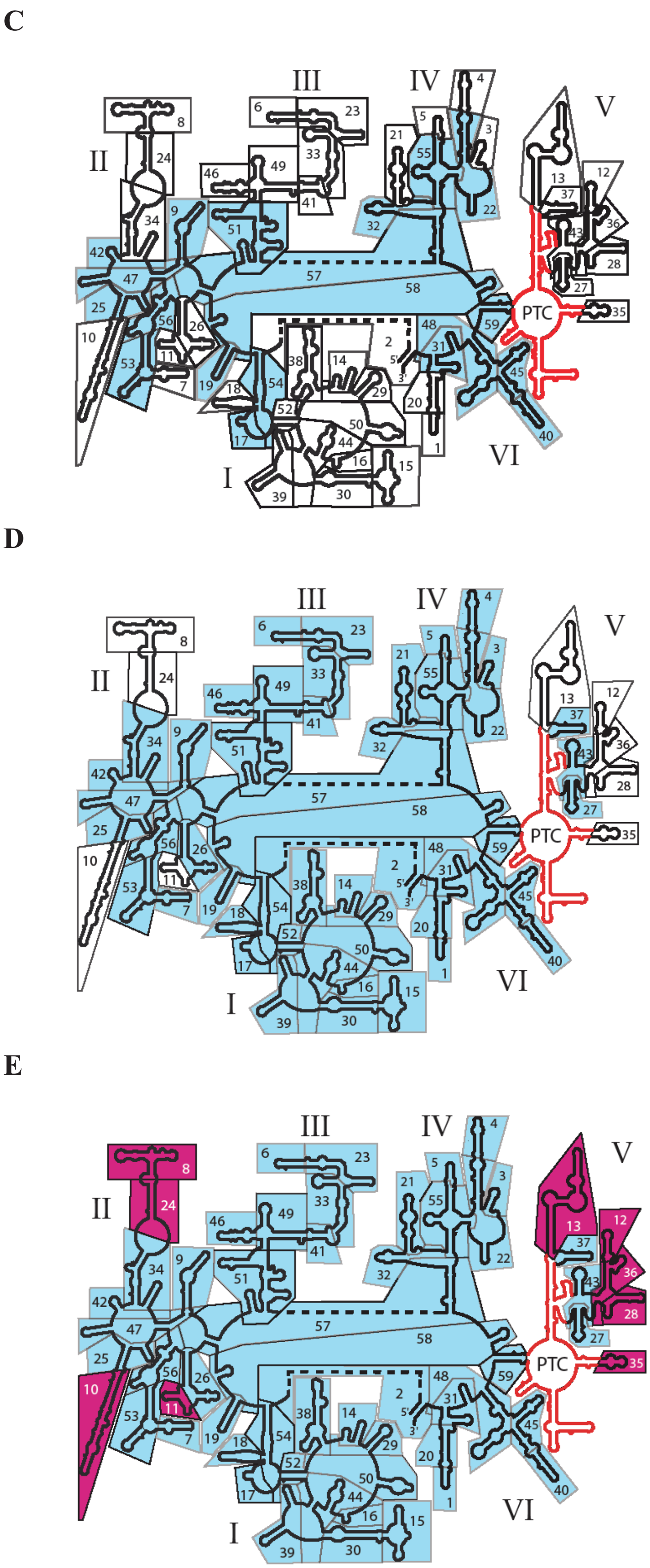
7. Supplementary Figure 3: The elements composing all structures shown in Fig. 3 of the main text A, B , C, D and E

A



B



C
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II
III
IV
V
VI
PTC
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3'
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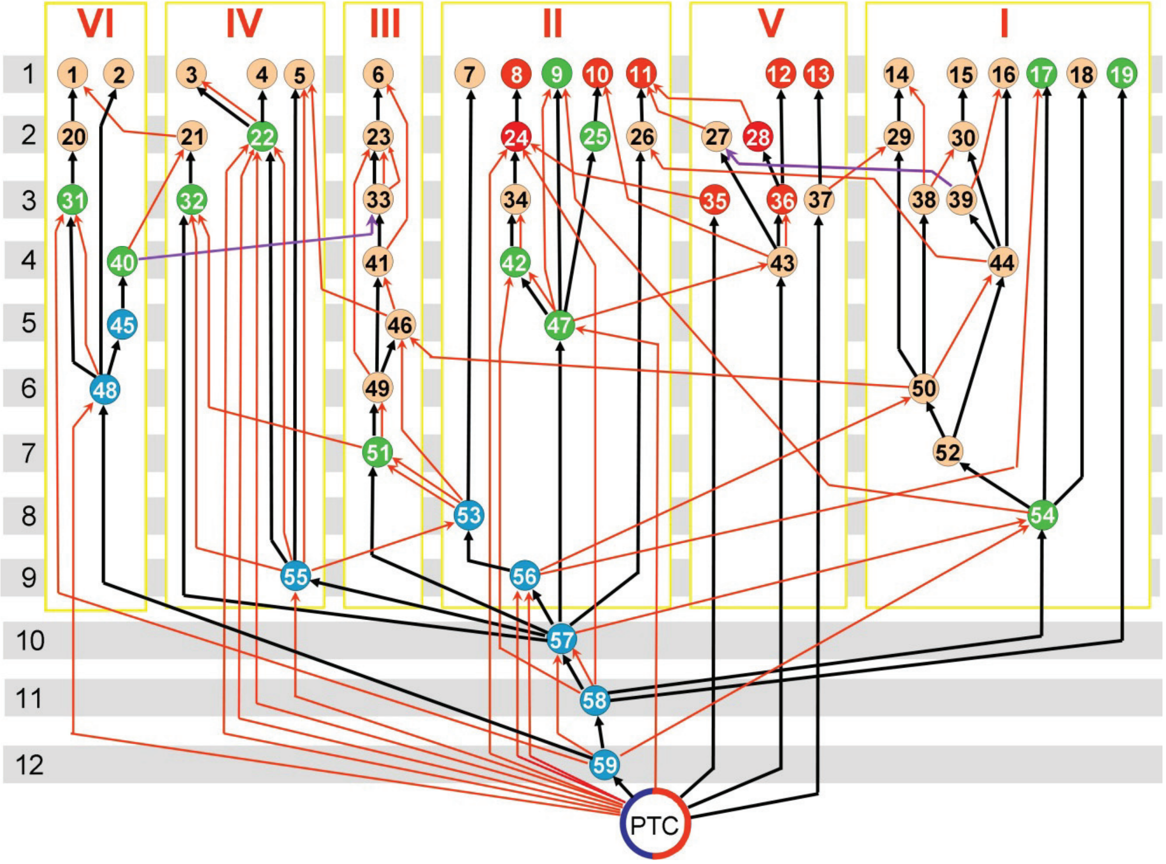
F



ABCDE:

A, B, C, D, and E correspond to the equivalent images in Fig 3 of the main text. Red: the proto-ribosome; cyan: the foundation for the proto-ribosome; magenta: the three protuberances.

F:

All added elements shown in the format of Fig. 2b of the main text. Blue, green and brown are those elements that make structures B, C, and D, respectively. The elements forming protuberances are red. Although the expansion of the 23S rRNA structure does not go strictly level-by-level, each new element appears only when all elements that are required for its proper positioning have already been placed.

Chapter 3. Article:

Molecular palaeontology as a new tool to study the evolution of ribosomal RNA

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In : M.V. Rodnina, W. Wintermeyer, R. Green, R. Eds., *Ribosomes: Structure, Function, and Dynamics* (Springer, Wien - New York, 2011).

Contribution of each author :

Sergey V. Steinberg : prepared general concept, prepared manuscript, participated in preparation of figures

Konstantin Bokov : prepared concept details, participated in preparation of manuscript, prepared figures

1. INTRODUCTION

The ribosome is a large RNA-protein complex that performs the synthesis of proteins in all living organisms. The emergence of the ribosome has been a pivotal step in the evolution of life on earth. It is generally accepted that the ribosome emerged almost four billion years ago from the RNA world, in which the primordial chemical reactions of life were catalyzed by RNA (*Crick-1968, Gilbert-1986*). Correspondingly, the ancient ribosome represented an RNA body, while proteins were added to its structure later, when the ribosome became effective enough to synthesize them. The original ribosomal RNA (rRNA) is believed to have been a rather small molecule, which gradually expanded to the modern size through addition of new elements (*Noller-2004, Hury-2006, Smith-2008*). In order to understand details of this evolutionary process, one cannot use the standard approach of aligning available nucleotide sequences of ribosomal RNA and constructing phylogenetic trees. Due to the nature of that approach, its ability to elucidate evolutionary events in the past is limited by the moment when all branches of the phylogenetic tree come together, which corresponds to the so-called Last Universal Common Ancestor (LUCA). On the other hand, because in all presently living organisms the ribosome core has essentially the same structure (*Gutell-1994, Doudna-2002*), it should have formed before the split of the tree of life in three major domains, i. e. before LUCA. This discrepancy makes the standard approach inapplicable to the problem of early ribosome evolution and necessitates the development of alternative approaches.

The determination of the tertiary structure of the two ribosomal subunits (*Ban-2000, Wimberly-2000*) and of the whole ribosome (*Schuwirth-2005, Selmer-2006*) opened new possibilities for understanding how the ribosome emerged in evolution. Several attempts have been made to approach the problem using the available structural and biochemical data (*Hury-2006, Smith-2008, Wuyts-2001*). In particular, based on the fact that the peptidyl transferase center, which is the active site of the ribosome, is positioned in the middle of the tertiary structure of the 23S rRNA (*Nissen-2000, Polacek-2005*), it was considered as the most ancient part of the ribosome (*Hury-2006*). Correspondingly, domain V of 23S rRNA, which contains the peptidyl transferase center and forms contacts with most of the other domains, was also suggested to be the most ancient domain of the molecule. Other parts of the 23S rRNA appeared later and thus were positioned farther from the center and closer to the surface of the modern ribosome. A more detailed description of the evolution of 23S rRNA was hardly possible without taking into consideration the real conformation of the ribosomal RNA, although how exactly the knowledge of the conformation could be used was not at all clear.

In this situation, we suggested that if indeed different parts of the ribosome structure emerged at different moments, it could be possible to distinguish between more recent and more ancient elements based on the way they interact with each other. More recent elements emerged after more ancient elements and are thus expected to structurally adapt for the proper interaction with the latter. Correspondingly, if we were able to detect signs of such adaptation, it would help us to determine the relative age of different elements and thus to figure out the order in which different elements joined the ribosome structure as it evolved. The idea that the evolution of rRNA could be followed not only based on the proximity of different elements to the peptidyl-transferase center, i. e. based on the global position of elements in the whole ribosome structure, but also locally, based on the interaction between neighboring elements, was eventually developed into a new approach that we call here molecular palaeontology. We tested this approach on 23S rRNA from *Escherichia coli* and surprisingly found that the tertiary structure of this molecule contains a lot of information on the early evolution of the ribosome, which has been practically undisturbed for four billion years. The analysis of this information has allowed us to develop a concerted, modular scheme of the evolution of 23S rRNA which is described in the present article.

2. RESTRAINED EVOLUTION OF THE RIBOSOME STRUCTURE

We start the description of our approach by making some general suggestions concerning the situation in which the primordial ribosome was evolving. We divide the whole process of the ribosome evolution in two major periods, before and after the emergence of the first RNA molecule able to perform a ribosome-related function. Henceforth, we will call that RNA molecule the proto-ribosome. We may not know what the exact function of the proto-ribosome was, but this function should have been essential for the organism in which it appeared. We think that the proto-ribosome was relatively small, which allowed it to emerge as the result of a single mutagenic act. Prior to the appearance of the proto-ribosome, ribosome evolution could be described as random reshuffling of RNA chains that was limited only by the capacity of the RNA-synthesizing machinery of the organism. However, when the proto-ribosome emerged and spread in the population, the maintenance of the level of ribosome function achieved so far became an essential aspect for the competitiveness and survival of organisms.

The further expansion of the size of the ribosome is expected to have proceeded through a series of insertions in different regions of the polynucleotide chain. Although the location and the nucleotide sequence of each insertion presumably were completely random, only those insertions that made the ribosome more effective would have had a chance to spread in the population and to be passed down to the following generations. This aspect allows us to formulate general constraints that an insertion should comply with to be propagated in evolution. These constraints pertain to the location of the insertion in the tertiary structure of the ribosome, to the conformation of the inserted fragment, and to the way it interacts with the existing parts of the ribosome that emerged previously.

First, the necessity to preserve the level of the ribosome function that was already achieved can be formulated on the structural level as a requirement that the insertion must not disturb the positions of nucleotides that were already present in the ribosome. This can only be possible if in the tertiary structure the 5’ and 3’ termini of the inserted fragment are positioned close to each other. Also, the inserted fragment should not interfere with the existing parts of the tertiary structure. These constraints would disfavour insertions in crowded areas (Figure 1A) and would favour those insertions that occur at the outskirts of the existing structure (Figures 1B and C). The inserted fragment is also expected to be fixed on the surface of the growing ribosome structure (Figure 1C), because only then the addition of the new structural element would not compromise the stability of the whole ribosome.

The final set of constraints imposes limits on the types of interactions that the newly emerged fragment can form with the already existing parts of the ribosome. In the tertiary structure of the rRNA, one can find many cases when one region of the polynucleotide chain can acquire a particular conformation only in the presence of another region. The most typical cases of this kind represent Watson-Crick double helices and A-minor motifs. In a double helix, the conformation of each of the two strands is fixed through base pairing with the opposite strand. In the A-minor motifs, which consist of a stack of unpaired nucleotides, predominantly adenosines, that pack with a double helix (*Nissen-2001*, *Doherty-2001*), the adenosine stack acquires a particular conformation only upon the interaction, with the double helix. For all such cases, we suggest a general principle according to which structural integrity of more ancient elements cannot be dependent on the presence of more recently acquired elements. For double helical regions, the application of this principle means that both strands of a helix should emerge simultaneously as parts of the same fragment. For A-minor motifs, it means that the adenosine stack cannot be a more ancient acquisition of the ribosome than the corresponding double helix. The latter constraint represents a type of adaption of a newly emerged element to the existing structural context and provides the asymmetry between more ancient and more recent elements of the ribosome structure.

3. DISMANTLING THE RIBOSOME STRUCTURE

Based on the constraints described above, we developed a procedure of systematic dismantling the structure of rRNA through elimination of those elements that are qualified as latest acquisitions of the ribosome. As an element, we considered an individual double helix or a domain of stacked nucleotides that would form a stable compact arrangement upon addition to the ribosome structure. The 5’ and 3’ termini of an element qualified as a latest acquisition must be structurally close to each other. A qualified element must contain both strands of the same double helix. Finally, if a qualified element forms an A-minor motif with the remaining ribosome, it must contain the stack of unpaired nucleotides that form this interaction, and not the double helix. All elements qualified as latest acquisitions of the ribosome thus constitute the last generation of acquired elements. Their removal would allow the identification of the second-last generation of acquired elements. The iterative procedure would be repeated until it reaches the proto-ribosome or until no more elements could be removed. Because this approach studies the process of development of a molecule of life through analysis of more and more ancient fossils of this molecule, it shares some features with palaeontology. Therefore, we name this approach molecular palaeontology.

The application of the described dismantling algorithm to the tertiary structure of 23S rRNA from *E. coli* (*Schuwirth-2005*) identified 59 elements which are distributed over 12 generations (*Bokov and Steinberg-2009*). In Figure 2, these elements are depicted as numbered circles, and their positions in the 23S rRNA secondary structure are shown in Figure 3A. In Figure 2, the circles are connected by arrows, all of which go from a lower to a higher level. Each arrow A → B between elements A and B indicates that B is a more recent acquisition of the ribosome than A and that the emergence of B was dependent on the presence of A. Most arrows are either black or red. Black arrows indicate that elements A and B are covalently connected in the way that the appearance of B before A would have compromised the integrity of the polynucleotide chain of 23S rRNA. Red arrows stand for the A-minor interactions between the double helix of element A and the nucleotide stack of element B. The appearance of B before A thus would have compromised the conformational integrity of element B.

There are also two magenta arrows connecting elements 40 → 33 and 39 → 27. These two arrows correspond to the so-called non-local pseudoknots and indicate a special type of dependency that we faced in our analysis. By definition, pseudoknots constitute secondary structure arrangements in which the loop of a stem-loop structure forms a double helix with a region outside this stem-loop. The requirement that in each helix both strands should be part of the same element may create problems if in one of the two helices constituting a pseudoknot the strands belong to regions distant from each other in the secondary structure. 23S rRNA from *E. coli* contains eight pseudoknots, six of which are local in the sense that both helices constituting the pseudoknot are proximal to each other in the secondary structure of 23S rRNA and can thus belong to the same element. Only two pseudoknots are not local, those in which a double helix is formed between elements 40 and 33 and between elements 39 and 27 (Figure 2). A careful analysis of the latter two pseudoknots showed that in both of them one strand of the double helix is able to keep its structural integrity without forming base pairs with the opposite strand. For example, element 40 consists of a stem capped by a tetraloop, and it is the nucleotides of this tetra-loop that form the pseudoknot double helix with element 33 (Figure 4). Given that tetraloops represent compact stable arrangements, the integrity of element 40 does not need the presence of element 33. This aspect has allowed us to eliminate element 33 without jeopardising the integrity of element 40, even though the two elements form together a double helix. A similar situation pertains to elements 39 and 27, which together also form a double helix. Here, like in the previous case, the integrity of element 39 was judged not to be dependent on the presence of element 27. These two examples show that, on rare occasions, the requirement that two strands of the same helix should have emerged simultaneously can be violated. This can happen, however, only if, due to particular circumstances, the integrity of one of the two strands can be maintained without the presence of the opposite strand.

4. THE ORIGIN OF 23S RRNA

The removal of the 59 elements identified by the analysis described above eliminated 93% of the original 23S rRNA. The remaining part, which consists of 220 nucleotides, is located in domain V (Figure 3). The central region of this 220-nucleotide fragment forms the peptidyl-transferase center. Recently, it was observed that this fragment has a symmetric structure (*Agmon-2005*). The symmetry is clearly seen on the levels of both secondary and tertiary structure (blue and red regions, Figure 3B). One half of this symmetric structure corresponds to the P site (blue), the other half to the A site (red). Moreover, there is a close correspondence between the positions of the nucleotides of the two halves that are involved in the fixation of the equivalent elements of the tRNAs in A and P sites (*Samaha-1995, Nissen-2000, Kim-1999, Hansen-2002*). In the polynucleotide chain of the remaining part, the P-site half precedes the A-site half. The similarity between the two halves is so high that it is logical to suggest that they originated by a duplication of the same RNA fragment (*Agmon-2005*). From this point of view, the evolution of 23S rRNA started with an initial fragment of about 110 nucleotides, which presumably was able to bind the CCA terminus of what would later become tRNA. The duplication of this fragment allowed the resulting molecule to bind two CCA termini simultaneously. Within this arrangement, the two CCA termini associated with the two halves are juxtaposed in space to allow for the transpeptidation reaction. Most probably, this dimer was already able to synthesize oligopeptides with random amino acid sequences, hence the designation proto-ribosome. This view is supported by the fact that in-vitro-selected small RNA molecules resembling the peptidyl-transferase center were able to perform transpeptidation (*Zhang-1997, Zhang-1998*), thus demonstrating that this reaction does not require any other element of the ribosome structure.

All other elements of 23S rRNA were gradually added to the proto-ribosome, one element at a time, in essentially the same way. Each element could appear only when all elements that were required for its proper positioning had already been placed, as defined in Figure 2. New elements were added as insertions containing all necessary details to dock with the surface of the evolving ribosome without disturbing already existing parts. The most common way for a new element to be fixed on the ribosome surface would be through the formation of an A-minor interaction with an already existing double helix. As the number of added elements grew, the proto-ribosome became larger (*Bokov and Steinberg-2009*). As a result, in the structure of the modern 23S rRNA, the age of different elements would generally correlate with their distance from the peptidyl transferase center (Figure 5). Even though this result may be considered as expected, the fact that it was not assumed at the outset of our analysis and has been obtained based solely on the consideration of local interactions between closely packed elements adds to the validity of the whole approach.

5. PROBABILISTIC QUANTIFICATION OF THE MODEL

In this section we provide a quantitative evaluation of the proposed model of 23S rRNA evolution. The question to address is whether the dismantling algorithm applied to an RNA molecule of a size of the 23S rRNA will always be able to eliminate practically the whole polynucleotide chain or, alternatively, for some structures it can be arrested before it reaches the end of the molecule. The question is equivalent to whether it is always possible to arrange all elements of an arbitrary RNA structure in the way shown in Figure 2, i.e. with all dependencies going from a lower to a higher level.

According to our analysis, the dismantling algorithm will always be able to reach the end of the molecule, as long as the dependencies between different elements existing in this molecule do not form cycles. For a given secondary structure, the presence or absence of such cycles would depend on the particular scheme of A-minor interactions. We can analyze, for example, what would happen if dependence 59 → 54 (Figure 2) had the opposite direction. This would mean that contrary to what happens in real 23S rRNA, the adenosine stack of this A-minor interaction now belongs to element 59, while the double helix is located in element 54. After such reorientation of dependence 59 → 54, the dependencies between three elements 54, 57 and 59 would form the cycle 59 → 57 → 54 → 59. The existence of this cycle will make each of the three elements 54, 57 and 59 dependent on the presence of the other two. Due to such inter-dependence, the dismantling procedure will be automatically arrested when it reaches any element of this cycle. We thus can conclude that the elimination of all 59 elements of 23S rRNA has been possible due to the fact that the tertiary structure of the molecule does not contain cycles of dependency.

Interestingly, the reorientation of an A-minor interaction does not always lead to the formation of a cycle. For example, the reorientation of A-minor interaction 21 → 1 does not create a cycle and can be accommodated to the pattern of Figure 2 through the displacement of several elements: elements 1, 20 and 31 should move down one layer, while element 21 should move up one layer. After these accommodations, all dependencies will again be oriented from a lower to a higher level.

If the evolution of 23S rRNA proceeded according to the scenario proposed here, i.e. through consecutive incorporation of local insertions and formation of A-minor motifs between adenosine stacks of more recently emerged elements and double helices of more ancient elements, the automatic outcome will be that the tertiary structure of 23S rRNA is free of cycles of dependency. An alternative explanation would be that such cycles are absent by chance, while the directions of the A-minor interactions did not play any specific role in the evolution of 23S rRNA. To discriminate between the two hypotheses, we analyzed the scheme presented in Figure 2 and showed that, if the orientations of all A-minor motifs in 23S rRNA were not essential for evolution and were chosen randomly, the probability that such structure did not contain cycles of dependency would have been $P < 10^{-9}$. Such a low probability allows us to conclude that the absence of cycles is a fundamental property of 23S rRNA that is directly related to the particular trajectory of its emergence.

6. THE SIMPLICITY OF THE RIBOSOME STRUCTURE

23S rRNA is a large molecule with a rather complex tertiary structure. However, the analysis we have performed indicates that a molecule of the size of 23S rRNA could have a much more complex tertiary structure. The strict constraints under which 23S rRNA has been evolving, namely the requirement for systematic incremental increase of ribosome efficiency, do not provide too much freedom for the appearance of exceedingly complex structures. In the following, the indicators of the relative simplicity of the tertiary structure of 23S rRNA are summarized:

(A.) The molecule is built based on the same simple concept applied to the structure over and over again. As a result, the tertiary structure of the whole molecule is characterized by global order, as illustrated by the common orientation of all A-minor motifs (Figure 2).

(B.) The requirement for the ribosome to have a stable structure after the addition of each new element limits the size of elements to be added. Indeed, for a large element that emerged spontaneously, the probability that it properly adapted to a rather heterogenic surface of the existing ribosome and became stably integrated would have been rather low. As a result, the average length of inserted elements was only 45 nucleotides and the length exceeded a hundred nucleotides only for two elements.

(C.) The structure of 23S rRNA is packed with A-minor interactions. The universal presence of the A-minor motif in the molecule can be explained by the relatively small size of newly emerged elements and the necessity to fix their positions on the surface of the ribosome. The fixation of an element with the help of an A-minor interaction does not require any special arrangement in more ancient elements except for the existence of a short double helical region. Given that double helices are common elements of RNA structure, such regions will always have been present in the vicinity of a new insertion. Also, a stack of unpaired adenosines can be arranged in almost any place of a newly emerged element, regardless of its structure. The A-minor motif thus seems to be the easiest way for the integration of a new element into the existing ribosome structure.

(D.) The necessity for the ribosome to maintain the stability of its structure after addition of each new element precludes the existence of long unpaired regions. This aspect explains the high percentage of nucleotides of 23S rRNA that are involved in double helices.

(E.) The prohibition of long unpaired regions eliminates the possibility for the emergence of non-local pseudoknots in which fragments belonging to distant parts of the secondary structure form a long double helix. In the two non-local pseudoknots present in the 23S rRNA, this helix contains only three or four base pairs. Moreover, in both pseudoknots the conformation of one strand of this helix can be stabilized by the particular structural context. The presence of such context seems to be essential for the ability of this strand to emerge before the appearance of the opposite strand.

7. THE DETERIORATING EVOLUTION OF MITOCHONDRIAL RIBOSOMES

The suggested model of the evolution of 23S rRNA is mainly based on the assumption of a systematic incremental increase of ribosome efficiency, which does not allow mutations rendering the ribosome less effective to propagate in evolution. This principle prohibits the emergence of elements whose structural integrity would require the emergence of additional elements in the future. It also prohibits deletions, thus providing for the incremental growth of the size of the ribosomal RNA. However, if at some moment the priorities changed and the incremental increase of the ribosome efficiency became no longer essential for the competitiveness of the organism, the evolutionary path could have taken a dramatic turn. A case of this type seems to have happened with mitochondrial ribosomes from protozoa, which, compared to bacterial ribosomes, have lost essential parts of their rRNA (*O' Brien-2002*, *Mears-2002*). We associate this phenomenon with the fact that the function of mitochondrial ribosomes in these organisms has become limited to the synthesis of only a dozen mitochondrial membrane proteins encoded in the mitochondrial genome. Given that membrane proteins have a substantially longer lifespan than most soluble proteins and thus are not needed to be renewed every few hours, the requirements for the efficiency of the mitochondrial ribosomes are less stringent. As a result, the length of the RNA of these ribosomes can be reduced, and still the organism will remain perfectly functional.

One may suggest that the deterioration of the rRNA in these mitochondria repeats in the reverse order the same steps that took place during the primary emergence of the ribosome. We do not support this suggestion and think that the two processes have very little, if anything, to do with one another. They have also taken place in essentially different conditions: the deleted parts of RNA in mitochondrial ribosomes are usually replaced by proteins. This replacement would compensate, at least partly, for the loss of the RNA part, which results in a twice as high protein content in mitochondrial ribosomes compared to cytosolic ribosomes (*O' Brien-2002*).

8. TERTIARY STRUCTURE VERSUS NUCLEOTIDE SEQUENCE

The results of our analysis strongly suggest that most elements of the modern 23S rRNA have practically the same tertiary structure as they had at the moment of their emergence. This suggestion correlates with the directionality of the A-minor interaction in the modern ribosome and with the fact that the ribosome core is conserved among all organisms. However, the conservation of the secondary and tertiary structure of rRNA still allowed some drift of the nucleotide sequence which is responsible for the existing variety of nucleotide sequences of rRNA. Interestingly, the analysis of rRNA sequences showed that the most variable parts correspond to double-helical regions, while unpaired regions exhibit much stronger conservation (*Smit-2007*). This aspect further supports the idea that the preservation of tertiary arrangements, which are mostly modulated by unpaired regions, was more important for ribosome assembly and function than particular nucleotide sequences of double-helical regions.

Interestingly, while most sequence modifications of rRNA can be considered to result from neutral drift, some changes seem to have been essential. Thus, when the original duplication of the 110-nucleotide fragment created the proto-ribosome, the coexistence of two identical fragments in the same molecule should have created a problem for proper folding. In particular, those parts of the polynucleotide chain that in the original 110-nucleotide fragment formed double helices now got a possibility to form helices both within each half and between the two halves. To avoid such folding problems, there should have been a strong evolutionary pressure to make the nucleotide sequences of both halves of this structure as different as possible. The modification of the nucleotide sequences of the two halves of the proto-ribosome presumably has tuned the structure of the peptidyl transferase center toward higher efficiency of the transpeptidation reaction. As a result, in the modern ribosome, a similarity between the nucleotide sequences of the two halves of the symmetric structure in domain V is not detectable, despite the fact that the symmetry is clearly seen in both secondary and tertiary structure.

To conclude, we can say that the secondary and tertiary structure of ribosomal RNA has been substantially more conserved than the nucleotide sequence, which makes the molecular palaeontology approach described here a valuable tool for analysis of the early ribosome evolution.

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REFERENCES

Agmon I, Bashan A, Zarivach R, Yonath A (2005) Symmetry at the active site of the ribosome: structural and functional implications. Biol Chem 386: 833 – 844

Ban N, Nissen P, Hansen J, Moore PB, Steitz TA (2000) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. Science 289: 905 – 920

Bokov K, Steinberg SV (2009) A hierarchical model for evolution of 23S ribosomal RNA. Nature 457: 977– 980

Crick FH (1968) The origin of the genetic code. J Mol Biol 38: 367– 369

Doherty EA, Batey RT, Masquida B, Doudna JA (2001) A universal mode of helix packing in

RNA. Nature Struct Biol 8: 339 – 343

Doudna JA, Rath VL (2002) Structure and function of the eukaryotic ribosome: the next frontier. Cell 109: 153–156

Gilbert W (1986) Origin of life: The RNA world. Nature 319: 618

Gutell RR, Larsen N, Woese CR (1994) Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. Microbiol Rev 58: 10 – 26

Hansen JL, Schmeing TM, Moore PB, Steitz TA (2002) Structural insights into peptide bond formation. Proc Natl Acad Sci USA 99: 11 670–11 675

Hury J, Nagaswami U, Larios-Sanz M, Fox GE (2006) Ribosome origins: The relative age of 23S rRNA Domains. Orig Life Evol Biosph 36: 421– 429

Kim DF, Green R (1999) Base-pairing between 23S rRNA and tRNA in the ribosomal A site. Mol Cell 4: 859 – 864

Mears JA, Cannone JJ, Stagg SM, Gutell RR, Agrawal RK, Harvey SC (2002) Modeling a minimal ribosome based on comparative sequence analysis. J Mol Biol. 321: 215 – 234

Nissen P, Hansen J, Ban N, Moore PB, Steitz TA (2000) The structural basis of ribosome activity in peptide bond synthesis. Science 289: 920 – 930

Nissen P, Ippolito JA, Ban N, Moore PB, Steitz TA (2001) RNA tertiary interactions in the large ribosomal subunit: the A-minor motif. Proc Natl Acad Sci USA 98: 4899 – 4903

Noller HF (2004) The driving force for molecular evolution of translation. RNA 10: 1833–1837

O’ Brien TW (2002) Evolution of a protein-rich mitochondrial ribosome: implications for human genetic disease. Gene 286: 73–79

Polacek N, Mankin AS (2005) The ribosomal peptidyl transferase center: structure, function, evolution, inhibition. Crit Rev Biochem Mol Biol 40: 285 – 311

Samaha RR, Green R, Noller HF (1995) A base pair between tRNA and 23S rRNA in the peptidyl transferase centre of the ribosome. Nature 377: 309 – 314

Schuwirth BS, Borovinskaya MA, Hau CW, Zhang W, Vila-Sanjurjo A, Holton JM, Cate JH (2005) Structures of the bacterial ribosome at 3.5 A° resolution. Science 310: 827– 834

Selmer M, Dunham CM, Murphy FV, Weixlbaumer A, Petry S, Kelley AC, Weir JR, Ramakrishnan V (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. Science 313: 1935–1942

Smit S, Widnan J, Knight R (2007) Evolutionary rates vary among rRNA structural elements.

Nucleic Acids Res 35: 3339 – 3354

Smith TF, Lee JC, Gutell, RR, Hartman H (2008) The origin and evolution of the ribosome. Biol Direct 3: 16

Wimberly BT, Brodersen DE, Clemons WM Jr, Morgan-Warren RJ, Carter AP, Vonrhein C, Hartsch T, Ramakrishnan V (2000) Structure of the 30S ribosomal subunit. Nature 407: 327– 339

Wuyts J, Van de Peer I, De Wachter R (2001) Distribution of substitution rates and location of insertion sites in the ter tiary structure of ribosomal RNA. Nucleic Acid Res 29: 5017– 5028

Zhang B, Cech TR (1997) Peptide bond formation by in vitro selected ribozymes. Nature 390: 96–100

Zhang B, Cech TR (1998) Peptidyl-transferase ribozymes: transreactions, structural characterization and ribosomal RNAlike features. Chem Biol 5: 539 – 553

FIGURES

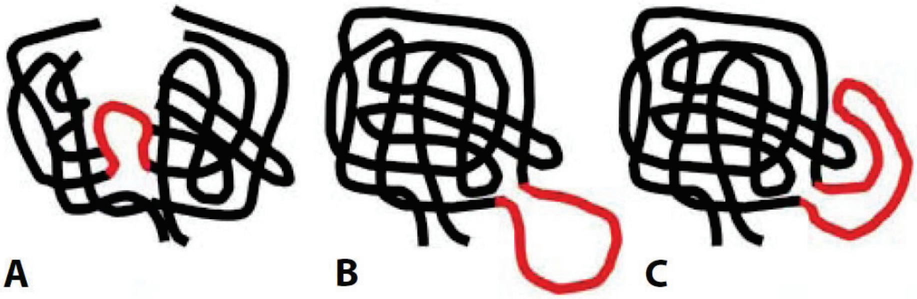

Figure 1

Different types of accommodation of a newly emerged insertion to an existing proto-ribosome.

(**A**) If the new insertion (red) occurred in a crowded area of the existing molecule (black), it could result in the breakage of the whole tertiary structure.

(**B, C**) If the insertion occurred at the outskirts of the ribosome structure, it would not disturb the tertiary structure.

(**B**) If the insertion has a loose conformation, it would create problems for folding and integrity of the ribosome structure.

(**C**) If the insertion has a compact structure and can be fixed on the surface of the ribosome, it would stabilize the whole structure and will have a chance to propagate in evolution.

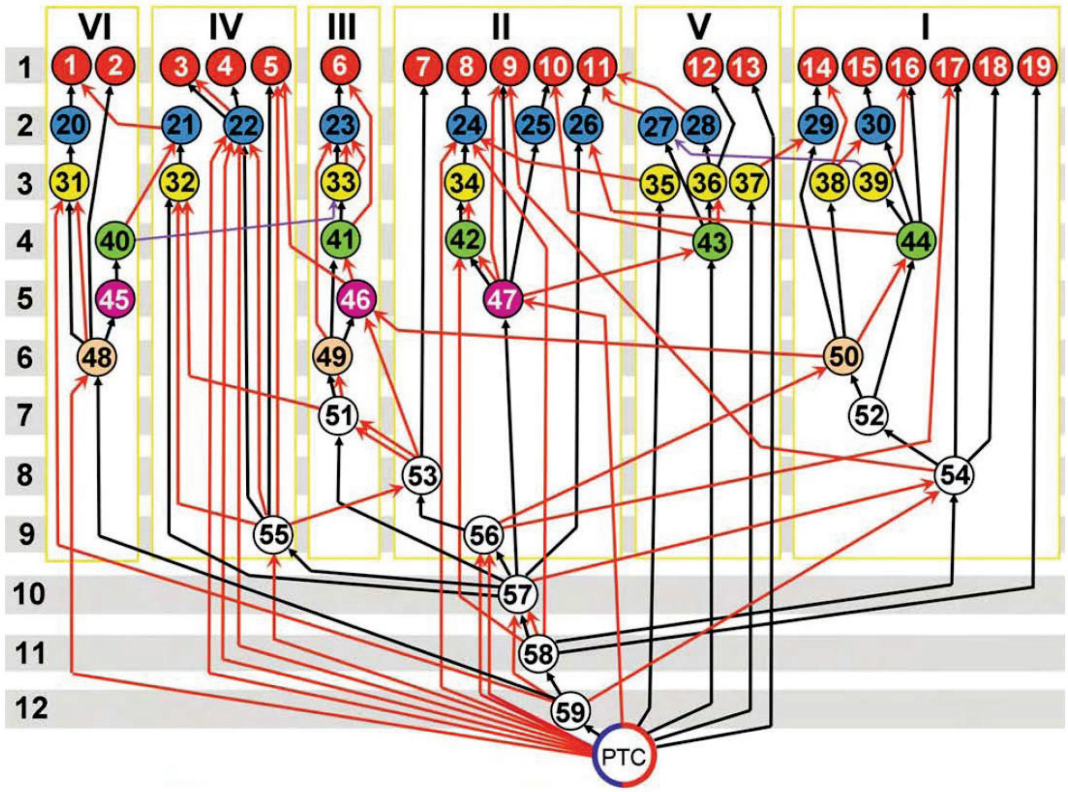

Figure 2

Dismantling the tertiary structure of 23S rRNA.

Removed elements are shown by numbered circles. The position of each element in the 23S rRNA secondary structure is shown in Figure 3A. Elements belonging to the same generation of acquired elements are shown with the same color. Generations are numbered from 1 to 12. Roman numerals indicate secondary structure domains. PTC stands for the symmetric structure in domain V containing the peptidyl-transferase center (the proto-ribosome, see also Figure 3B). An arrow between two elements, e. g. A → B, indicates that the removal of A before B would compromise the integrity of the remaining ribosome. Black arrows denote covalent connections between the respective elements, red arrows A-minor interactions; violet arrows denote a double helix which would correspond to a non-local pseudoknot (Modified from Bokov and Steinberg, Nature 457: 977–980, 2009)

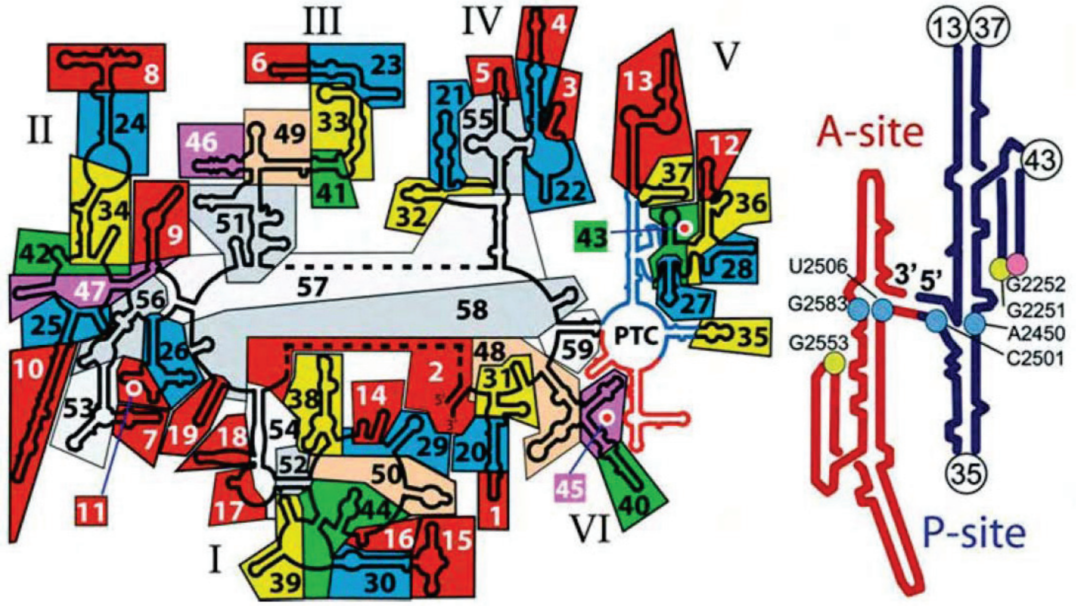

Figure 3

(A) Positions of stepwise removed elements in the secondary structure of 23S rRNA.

Numbers and colors of the elements correspond to those shown in Figure 2. The blue and red structure in domain V (PTC) stands for the proto-ribosome. The red and blue parts of the proto-ribosome correspond to the A and P sites, respectively.

(B) Symmetry in the proto-ribosome.

The three numbered circles stand for the elements of domain V immediately attached to the proto-ribosome. Colored circles show the positions of the nucleotides in the two parts of the proto-ribosome that coordinate nucleotides A76 (blue), C75 (yellow) and C74 (magenta) of tRNAs. The inclination of the lower double helix of the A-site part of the proto-ribosome indicates that in the ribosome structure its position is not completely symmetrical to the corresponding double helix in the P-site part (Modified from Bokov and Steinberg, Nature 457: 977–980, 2009)

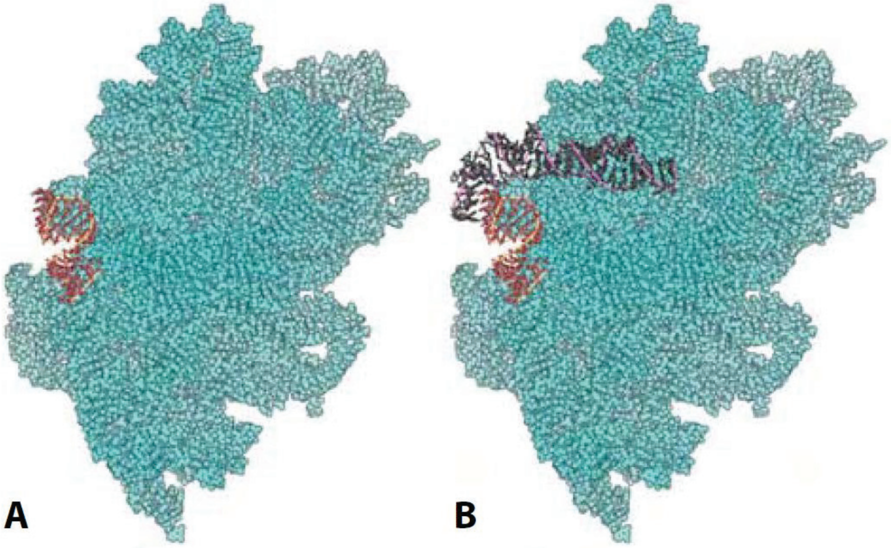

Figure 4

The formation of the non-local pseudoknot 40 → 33.

The blue area depicts the presumed structure of 23S rRNA just before the emergence of element 33. Element 40 (red + yellow) represents a stem capped by a tetraloop. (**A**) The integrity of element 40 does not require the presence of element 33. (**B)** The position of the newly emerged element 33 (black + magenta) can be stabilized by the formation of a double helix with element 40, which constitutes the pseudoknot.

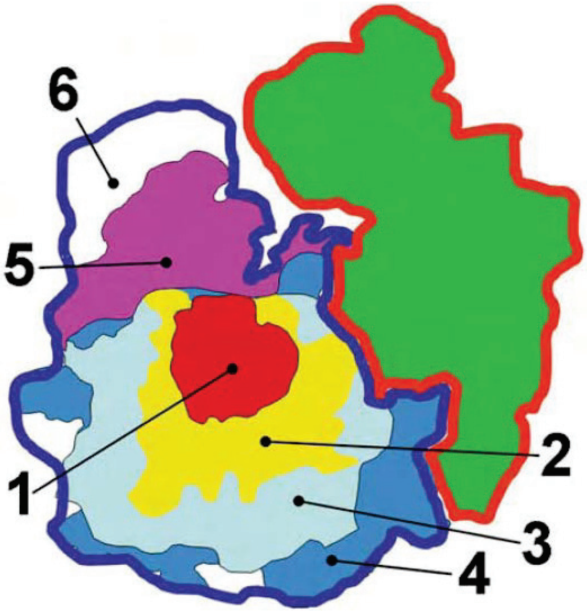

Figure 5

The expansion of the 23S rRNA structure as it evolved.

1: the proto-ribosome, 2 – 5: the size of the 23S rRNA after 8 (2), 20 (3), 50 (4) and all 59 (5) elements have been added to the proto-ribosome. 6: the area of the 50S subunit that does not include rRNA. Green: 30S subunit (Modified from Bokov and Steinberg, Nature 457: 977–980, 2009)

Chapter 4. Article:

Structural elements that control ribosome ratcheting and domain closure

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Contribution of each author :

Konstantin Bokov : participated in preparation of experimental design, did experiments, analyzed results, participated in preparation of manuscript, prepared figures

Sergey V. Steinberg : developed general experimental design, analyzed results, prepared manuscript, participated in preparation of figures

ONE SENTENCE SUMMARY

We elucidated the common mechanism of ratchet-like motion and domain closure in the ribosome structure and the way these rearrangements are regulated.

ABSTRACT

Comparison of the ribosome crystal structures in non-ratcheted and ratcheted conformations has revealed the nature of rearrangements that take place in the ribosome during the ratchet-like motion. Unexpectedly, the rearrangements occur within the small subunit and not between the subunits. Each tertiary domain of 16S rRNA moves along its own trajectory, the scale and direction of which is limited by flexible inter-domain linkers. The rearrangements observed during ribosome ratcheting recapitulate in reverse order those that lead to small subunit domain closure. They can be blocked by the A-minor interaction between adenosines 1492 and 1493 of 16S rRNA and the A-site codon-anticodon double helix, suggesting that these adenosines play the role of the universal switch between the two ribosomal conformations.

INTRODUCTION

The ribosome is a large RNA-protein complex that performs protein synthesis in all living organisms (*Rodnina-2011, Spirin-2009*). It consists of two subunits, small and large, named SSU and LSU respectively. At certain steps of its functional cycle, the ribosome undergoes specific rearrangements known as the ratchet-like motion (*Frank-2000, Frank-2007, Spahn-2004,* Julián -*2008, Horan-2007*) (RLM). In particular, RLM takes place during the translocation, when the peptidyl- and deacylated tRNAs are displaced from the A- and P- to the P- and E-sites, respectively, while the associated mRNA moves three nucleotides in the 5’-direction (*Rodnina-2011*). Originally, RLM was described as a 6˚-counter-clockwise rotation between SSU and LSU (*Frank-2000*). Additional studies suggested that the rearrangement is more complex, as different domains within SSU can also move with respect to each other (*Ogle-2002, Ogle-2003, Ratje-2010, Agirrezabala-2012*). The co-existence of different types of movements between the two subunits and within SSU raises a number of questions, including how can these movements cooperate with each other in providing for a particular function without jeopardizing the integrity of the ribosome structure, and how can they be regulated. Important information to address these questions can be obtained through comparison of the available ratcheted and non-ratcheted ribosome structures.

Here we present results from a systematic comparison of partly ratcheted and non-ratcheted ribosome conformations primarily derived from analysis of three crystal forms of the *E. coli* ribosome in which a 3˚-to-6˚-ratcheted ribosome (termed 'R' here) is co-crystallized with a non-ratcheted ribosome (termed 'N' here) (*Zhang-2009*). The crystal forms (henceforth, named crystals) differed by the number of anticodon stem-loops (ASL) associated with each ribosome, i.e. zero, one or two, (termed '0', '1' and '2', respectively). These crystal structures were selected for analysis specifically because of the presence of a varying number of ASLs, which proved to be an essential element of the results.

The analysis was performed through a series of superpositions of different ratcheted and non-ratcheted ribosome conformations. Each superposition was obtained as the best overlap for a given part of ribosomal RNA, henceforth called the "nucleus". Then, each nucleotide of the ribosome was examined to determine whether it co-superposed with the nucleus. All superpositions were made in parallel within all three pairs of co-crystallized ribosomes: N0-R0, N1-R1 and N2-R2. The results presented here are mainly derived from superpositions in the N2-R2 pair. For the N0-R0 and N1-R1 pairs, the results are discussed only when they were found to deviate significantly from those observed for the N2-R2 pair.

MOBILE DOMAINS IN THE RIBOSOME STRUCTURE

For the first superposition, the entire 23S rRNA was used as the nucleus, which gave a root mean square deviation (RMSD) of 1.5 Å. However, we noted that the average deviation of 1.5 Å was exceeded for 13% of nucleotides, almost all of which were located on the ribosome surface and represented local disturbances (non-colored regions in Fig. 1A, the complete list is provided in Supplemental materials). The RMSD for only these regions was 3.7 Å. We term these flexible regions on the ribosome surface "curls". Since the structural perturbations found in the curls seemed to be unrelated to the displacement between the two subunits, we ignored these regions in subsequent analysis. When the curls were excluded, the RMSD for this superposition dropped more than twofold to 0.69 Å. This allowed us to conclude that the core of 23S rRNA in both R2 and N2 structures has practically the same conformation and thus represents a rigid tertiary domain.

In the same superposition, the RMSD of 16S rRNA was above 7 Å. This result was expected, given that ratcheted (R2) and non-ratcheted (N2) structures of SSU relative to LSU were being superposed. At the same time, the central part of helix 44 (h44) enclosed between base pairs

1415-1485 and 1430-1470 (Fig. 1B), which forms most of inter-subunit bridges (*Yusupov-2001, Gao-2003*), superposed substantially better, with RMSD=1.3 Å (henceforth, we name this region h44c, c=center). The close superposition of the h44c region suggests two possible mechanisms of RLM. In the first mechanism, which is currently generally accepted (*Frank-2000, Dunkle-2011*), the rearrangements leading to RLM take place at the interface between the ribosomal subunits. The good superposition of h44c would thus reflect the proximity of this part of 16S rRNA to the center of rotation. Thus, for a given angle of ratcheting, the absolute displacement of nucleotides of region h44c would be relatively small. In the alternative mechanism, h44c remains immobile with respect to LSU, while the rearrangements leading to subunit rotation occur mostly or exclusively elsewhere in SSU. Such a mechanism would require the existence of flexibility in the 16S rRNA structure outside of h44c that would allow the external parts of SSU to be displaced with respect to the immobile central part.

To distinguish between these two alternative mechanisms, we superposed the 16S rRNA structure in three different ways, using as nuclei, each of the three secondary structure domains D1, D2 and D3 (*Wimberly-2000*). In each superposition, we then determined those areas of the 16S rRNA structure in which the RMSD of each nucleotide in the two superposed structures did not exceed 1.2 Å. This threshold value was chosen empirically, as it resulted in the optimal resolution of mobile and immobile elements. The results of these superpositions are presented in Fig. 1B. They show that the core of each secondary structure domain in the ratcheted ribosome is well superposed with the equivalent region in the non-ratcheted ribosome. None of the three domains co-superposes with the large subunit or with h44c. Thus, we conclude that the cores of all three secondary structure domains represent solid entities that are mobile with respect to each other, to h44c and to the large subunit.

Within 16S rRNA, we found regions that belong to one secondary structure domain yet co-superpose and form a tight tertiary arrangement with another. In particular, a large area at the center of the 16S rRNA secondary structure co-superposes and is tightly attached to D2 (Fig. 1). Also, four local elements, which we call “linkers” (elements L1-L4 in Fig. 1B), co-superpose and form tight interactions with domains that are distant from them in the secondary structure. A similar element was found in LSU (linker L5 in Fig. 1A). Although this element is located in helix 69 of 23S rRNA (H69), it is tightly attached to and moves in unison with domain D2 of 16S rRNA (*Ali-2006*). Based on these findings, we define tertiary domains T1, T2 and T3 as those consisting of the self-superposable parts of the secondary structure domains D1, D2 and D3 together with the linkers that are tightly bound to each of them (Fig. 1B). The RMSD values calculated for the three tertiary domains were 0.71 Å, 0.65 Å and 0.67 Å, respectively. This indicates that each domain represents a solid entity that moves along its own trajectory. The same was also true for h44c, whose RMSD value was determined as 0.62 Å. Other parts of the secondary structure domains, which were not included in any tertiary domain, were labeled as curls and excluded from further analysis (non-colored regions in Fig. 1B and Supplemental material).

TRAJECTORIES OF THE MOBILE DOMAINS

The fact that the three tertiary domains of 16S rRNA and region h44c move as solid bodies allows us to determine their trajectories with respect to 23S rRNA when the ribosome undergoes transition from the non-ratcheted to ratcheted conformation. Each trajectory can be represented as a rotation around and a shift along an axis. For each domain, we determined the position and orientation of the axis, the scale of the rotation and the shift (Supplemental material). Although such representation is not ideal, as the orientation of an axis can depend on the amplitude of the rotation, it provides valuable qualitative information about the character of the domain movements.

As shown in Fig. 2 and in (Supplemental material), the T1, T2 and T3 tertiary domains rotate, respectively, for 8.4˚, 6.1˚ and 6.1˚ roughly in the same direction consistent with RLM. For the three domains, the axes of rotation are different and are notably inclined towards the lower part of the ribosome. The strongest inclination is observed for the axis of the T1 rotation, which deviates from the perpendicular to the subunit interface by 48.5˚. As a consequence of the inclination of the T1 axis, this domain effectively moves towards the large subunit, thus narrowing the inter-subunit gap. While each of the two domains T1 and T2 moves practically along the same trajectory in all three crystals, the trajectory of T3 in crystals N1-R1 and N2-R2 is different from that in R0-N0. The difference originates from the presence of the P-site ASL in crystals N1-R1 and N2-R2, where this ASL interacts with T3, thus affecting its mobility (*Zhang-2009*).

For the h44c region, the parameters of rotation are found to be notably different from those for the T1, T2 and T3 domains. Although in all three of the crystals analyzed, h44c was also found to rotate with respect to 23S rRNA, the angle of rotation was always under 3˚ and 2-4 times smaller than for any of the three tertiary domains (Supplemental material). We thus conclude that the h44c region does not move together with the other domains and, compared to them, remains almost immobile with respect to the LSU. Therefore, most of the rearrangements in the ribosome structure occur within the SSU outside the h44c region and they are not the result of a rotation between the two subunits, as previously thought. This conclusion is consistent with previous observations that in the ratcheted ribosome conformation, all inter-subunit bridges in the h44 region preserve their integrity (*Zhang-2009, Ben-Shem-2010, Jin-2011*).

CONFORMATIONAL CYCLES IN 16S RRNA

Given that the tertiary domains move as solid entities, the conformational rearrangements in 16S rRNA that provide for RLM should occur in the flexible inter-domain regions. Within the 16S rRNA secondary structure, T2 is directly connected to T1 and to h44c (Fig. 1B). Also, linkers L1 and L2 connect T2 to T1 and T2 to h44c, respectively, while linker L4 connects h44c to T1. Through these five connections, the three rigid entities T1, T2 and h44c form the conformational cycle T1-T2-h44c-T1. Within this cycle, the movements of the three domains are limited and mutually dependent. In particular, in the three crystals analyzed, the amplitudes of T1 and T2 rotation are always proportional to each other (Supplemental material), which is consistent with previous studies *(Agirrezabala-2011)*.

Out of the five connector regions, four demonstrate only minor changes: in each case, neighboring nucleotides are only slightly displaced with respect to each other without breaking interactions that they form. However, in the fifth connection between h44c and T2 (Fig. 1B), the rearrangement is more notable. While h44c remains practically immobile, the upper part of h44 together with the whole T2 domain bends in the direction of the E-site by 6.1˚-8.4˚.

The displacement of the T3 domain with respect to T2 is due to conformational rearrangements in two regions. The first region consists of four consecutive WC base pairs formed by nucleotides 929-932 and 1385-1388 (between T2 and T3, Fig. 1B). Previous studies have shown that this region undergoes structural deformations during D3 swivelling (*Schuwirth-2005*). The second region consists of the four WC base pairs formed by nucleotides 1070-1073 and 1102-1105. The conformational flexibility in this region allows the L3 linker to be firmly attached to domain T2 while domain T3 is moving (Fig. 1B). As in the previous cases, all rearrangements in both regions represent only minor changes in positions of individual nucleotides without breaking base pairs. The existence of two covalent links between the T2 and T3 domains creates another conformational cycle T2-T3-T2 in 16S rRNA, which would limit both the direction and the scale of the T3 movement with respect to T2. However, given that the two flexible regions are located in close proximity in the tertiary structure, the ability of this conformational cycle to restrict the T3 movement would be limited. This explains the relatively high amplitude of the T3 movement compared to the other tertiary domains (*Frank-2007, Ratje-2010, Schuwirth-2005*).

Each of the two conformational cycles identified in 16S rRNA is responsible for a particular type of rearrangement in the SSU: the movement within cycle T1-T2-h44c-T1 provides for RLM, while the movement within cycle T2-T3-T2 results in the head swivel. The fact that in the ribosome structures analyzed each of the two movements occurs regardless of the other confirms earlier observations of their independence from each other (*Ratje-2010, Zhang-2009, Guo-2012*).

ADENOSINE 1492 AND 1493-MEDIATED BLOCKAGE OF RLM

Further analysis unexpectedly showed that in the different crystals, the location of the hinge between h44c and T2 is different and depends on the presence of an ASL in the A-site. In crystals N0-R0 and N1-R1, where the A-site is empty, the hinge occurs in the internal loop containing adenosines 1492 and 1493 (A1492/3). In both crystals, this loop displays substantial conformational flexibility (Fig. 3A). However, in crystal N2-R2, where an ASL occupies the A-site, the whole upper part of h44, including A1492/3 and the flanking double helical regions, becomes part of T2. The hinge between h44c and T2 is now located between base pairs 1414-1486 and 1415-1485 (Fig. 3B), where h44 bends towards the minor groove by 3.3˚. The difference in the position of the hinge is due to the A-minor interaction formed in the N2-R2 crystal between A1492/3 and the A-site codon-anticodon double helix (*Ogle-2002*). In the absence of this interaction, the A1492/3 internal loop does not have a distinct conformation and easily adapts to the displacement of domain T2 with respect to h44c. However, the formation of this interaction in crystal N2-R2 organizes the structure of the entire region surrounding A1492/3 and monolithically attaches it to T2.

To validate the biological relevance of the bending observed in crystal N2-R2, we looked for the reasons of its localization between base pairs 1414-1486 and 1415-1485. We found that due to the distinct position of the two base pairs, bending between them in the direction of the minor groove would move the upper part of h44 along the subunit interface without interfering with other parts of the ribosome. This aspect makes the junction between base pairs 1414-1486 and 1415-1485 a potential site for bending. Also, in the tertiary structure of the SSU we found two additional elements which would work to minimize the amplitude of bending between these base pairs. First, in most bacteria, base pairs 1414-1486 and 1415-1485 are U-G and G-U, respectively. As explained in (Supplemental material), two consecutive base pairs U-G and G-U create a serious obstacle for bending of the double helix towards the minor groove. Second, in the tertiary structure of 16S rRNA, the minor groove of h44 between base pairs 1410-1490 and 1415-1985 tightly interacts with linker L2 (helix 27), which solidifies the conformation of h44 (Supplemental material). We thus strongly suggest that the bending of h44 as observed in ribosome R2 is not physiologically relevant and has only been possible because the angle of the ratcheting is relatively small. We therefore expect that in a normally functioning ribosome, the formation of the interaction between A1492/3 and the minor groove of the A-site codon-anticodon double helix will effectively block the ratcheting.

RATCHET-LIKE MOTION AND DOMAIN CLOSURE

The existence of the conformational cycles limits the direction and the amplitude of the movement of the domains, so that they can displace only along the particular trajectories. It is known, however, that during the selection of aminoacyl-tRNA, the SSU undergoes another type of rearrangement, the domain closure, which is accompanied by the formation of interaction between G530 and A1492/3 of 16S rRNA with the A-site codon-anticodon double helix (*Ogle-2002, Ogle-2003*). In view of the restrictions imposed by the conformational cycles on the flexibility of the SSU, it seems probable that RLM and domain closure represent related rearrangements. To determine whether or not the domain closure has any relation to RLM, we analyzed several X-ray conformations of the SSU that had been previously qualified as open or closed (see Figure 4 legend). Comparison of these conformations revealed the same rigid domain movement as observed for RLM (Supplemental material). In other words, the transition between open and closed SSU conformations involves the same rigid tertiary domains and the same flexible regions as during RLM.

We then superposed the available open and closed conformations of 16S rRNA as well as the conformations of 16S rRNA in the ratcheted and non-ratcheted ribosomes, by using h44c as the nucleus, and determined the angle of rotation of T2 with respect to h44c. Using h44c as the nucleus allowed us to focus on rearrangements within the SSU, filtering them from possible minor displacements of h44c with respect to the LSU. Figure 4 demonstrates that closed conformations of the SSU correspond to the non-ratcheted ribosome, while more open SSU conformations correspond to a more ratcheted ribosome. These results lead us to the general conclusion that RLM and the domain closure represent equivalent rearrangements in the SSU going in opposite directions. The two types of rearrangements thus constitute the mirror images of each other. The structural symmetry of the domain closure and the RLM is echoed by functional symmetry: while the domain closure is known to be accompanied by the formation of the interaction between A1492/3 and the A-site codon-anticodon double helix (*Ogle-2002, Ogle-2003*), RLM requires that this interaction be broken.

The mechanism of symmetrical structural rearrangements during domain closure and RLM explains the origin of the open form of the SSU prior to the next elongation cycle. According to the position of the open form in Figure 4, it corresponds to about 60% of the full ratchet. We thus suggest that the open form of the SSU appears at an advanced phase of the translocation as a pause in the transition from the ratcheted to non-ratcheted SSU conformation. Because the open form is known to allow the initial codon-anticodon recognition, by the time the SSU assumes this form the translocation of both mRNA and the anticodon loops should have already finished. At the same time, the open form should be sufficiently distant from the non-ratcheted conformation to disallow the interaction of A1492/3 with the A-site codon-anticodon double helix. Thus, the primary selection of a new aminoacyl-tRNA takes place before the reverse ratcheting is complete, while the completeness is achieved during the subsequent closure of the domains.

CONCLUDING REMARKS

The results of the analysis presented here clearly demonstrate the existence of several levels of restrictions limiting conformational flexibility of the ribosome structure during RLM. First, practically all rearrangements in the ribosome structure associated with RLM take place within the SSU and not between the subunits, as was previously thought. While h44c is tightly attached to the LSU, the rest of the SSU moves with respect to h44c. Even though some minor movements of h44c with respect to the LSU have been detected, they do not change the system of inter-subunit contacts. The tight attachment of h44c to the LSU guarantees the integrity of the subunit association throughout the whole elongation of translation. Although each tertiary domain of SSU can move along a trajectory that is different from those of the other domains, the existence of the two conformational cycles sharply restricts the domains` mobility. Within cycle T1-T2-h44c-T1, the displacement of domains T1 and T2 is virtually reduced to one-dimensional back-and-forth movement. The residual flexibility existing in the upper part of h44c can be diminished through the choice of base pairs 1414-1486 and 1415-1485 and with help of linker L2. On the one hand, such restrictions on domain movement would guarantee the integrity of the SSU tertiary structure. On the other hand, only when the mobility of the tertiary domains is already substantially reduced, the particular interaction of A1492/3 with the A-site codon-anticodon double helix is able to effectively control RLM. The formation of the latter interaction is linked to domain closure and is important for the identification of the cognate aminoacyl-tRNA; its breakage unleashes RLM and leads to translocation.

REFERENCES AND NOTES

X. Agirrezabala. *et al*. Structural characterization of mRNA-tRNA translocation intermediates. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 6094–6099 (2012).

I. K. Ali, L. Lancaster, J. Feinberg, S. Joseph, H. F. Noller, Deletion of a conserved, central ribosomal intersubunit RNA bridge. *Mol. Cell*. **23**, 865–74 (2006).

A. Ben-Shem, L. Jenner, G. Yusupova, M. Yusupov, Crystal Structure of the Eukaryotic Ribosome. *Science* **330**, 1203–1209 (2010).

J. A. Dunkle *et al*., Structures of the bacterial ribosome in classical and hybrid states of tRNA binding. *Science* **332**, 981–984 (2011).

J. Frank, R. K. Agrawal, A ratchet-like inter-subunit reorganization of the ribosome during translocation. *Nature* **406**, 318–22 (2000).

J. Frank, H. Gao, J. Sengupta, N. Gao, D. J. Taylor, The process of mRNA–tRNA translocation. *Proc. Natl. Acad. Sci. U. S. A* **104**, 19671–19678 (2007).

H. Gao *et al.,* Study of the structural dynamics of the E coli 70S ribosome using real-space refinement. *Cell* **113**, 789–801 (2003).

Z. Guo, H. F. Noller, Rotation of the head of the 30S ribosomal subunit during mRNA translocation *Proc. Natl. Acad. Sci. U. S. A.* **109**, 20391-20394 (2012).

L. H. Horan, H. F. Noller, Intersubunit movement is required for ribosomal translocation. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 4881–4885 (2007).

L. B. Jenner, N. Demeshkina, G. Yusupova, M. Yusupov, Structural aspects of messenger RNA reading frame maintenance by the ribosome. *Nat. Struct. Mol. Biol*. **17**, 555-560 (2010).

H. Jin, A. C. Kelley, V. Ramakrishnan, Crystal structure of the hybrid state of ribosome in complex with the guanosine triphosphatase release factor 3. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 15798–15803 (2011).

P. Julián *et al*., Structure of ratcheted ribosomes with tRNAs in hybrid states. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 16924–16927 (2008).

J. M. Ogle, F. V. Murphy, M. J. Tarry, V. Ramakrishnan, Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell* **111**, 721–732 (2002)

J. M. Ogle, A. P. Carter, V. Ramakrishnan, Insights into the decoding mechanism from recent ribosome structures. *Trends Biochem. Sci.* **28**, 259–266 (2003).

A. H. Ratje *et al*., Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. *Nature* **468**, 713–716 (2010).

M.V. Rodnina, W. Wintermeyer, R. Green, R. Eds., *Ribosomes: Structure, Function, and Dynamics* (Springer, Wien - New York, 2011).

T. M. Schmeing, *et al.,* The Crystal Structure of the Ribosome Bound to EF-Tu and Aminoacyl-tRNA. *Science* **326**, 688-693 (2009).

B. S. Schuwirth *et al.,* Structures of the bacterial ribosome at 3.5 Å resolution. *Science* **310**, 827–833 (2005).

C. M. Spahn *et al*., Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation *EMBO J.* **23**, 1008–1019 (2004).

A. S. Spirin, The ribosome as a conveying thermal ratchet machine. *J.Biol. Chem.* **288**, 21103–21119 (2009).

M. M. Yusupov *et al.,* Crystal structure of the ribosome at 5.5 Å resolution. *Science* **292,** 883–896 (2001).

B. T. Wimberly *et al*., Structure of the 30S ribosomal subunit. *Nature* **407**, 327–339 (2000).

W. Zhang, J. A. Dunkle, J. H. D. Cate, Structures of the ribosome in intermediate states of ratcheting. *Science* **325**, 1014-1017 (2009).

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FIGURES

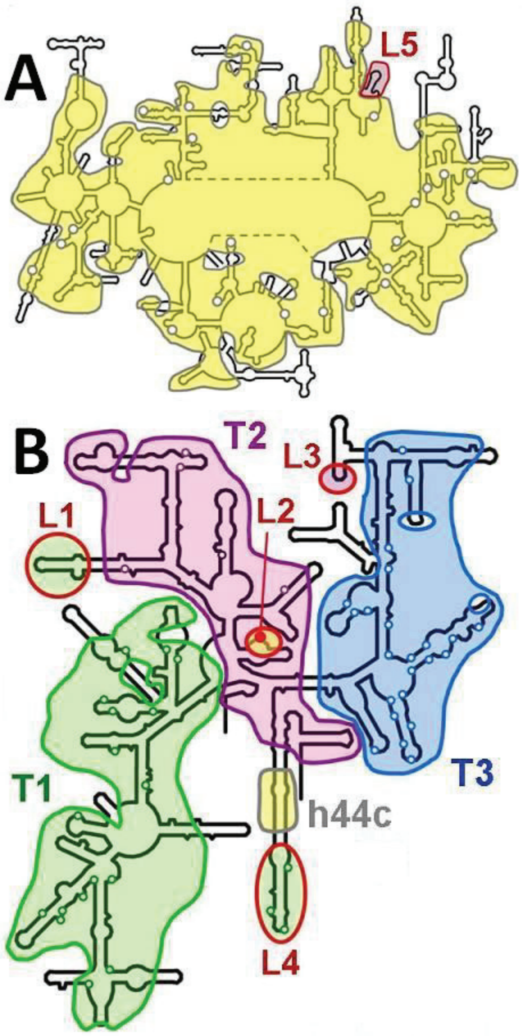


Figure 1

Rigid tertiary domains in 23S (A) and 16S (B) rRNA

Each domain is shown by its own background color: 23S rRNA + h44c (yellow), T1 (green), T2 (magenta) and T3 (blue). Regions outside the colored areas are curls. Within each domain, small circles indicate curls composed of individual nucleotides. The five linkers L1-L5 are contoured by a red line. The complete description of all regions is provided in (*13*).

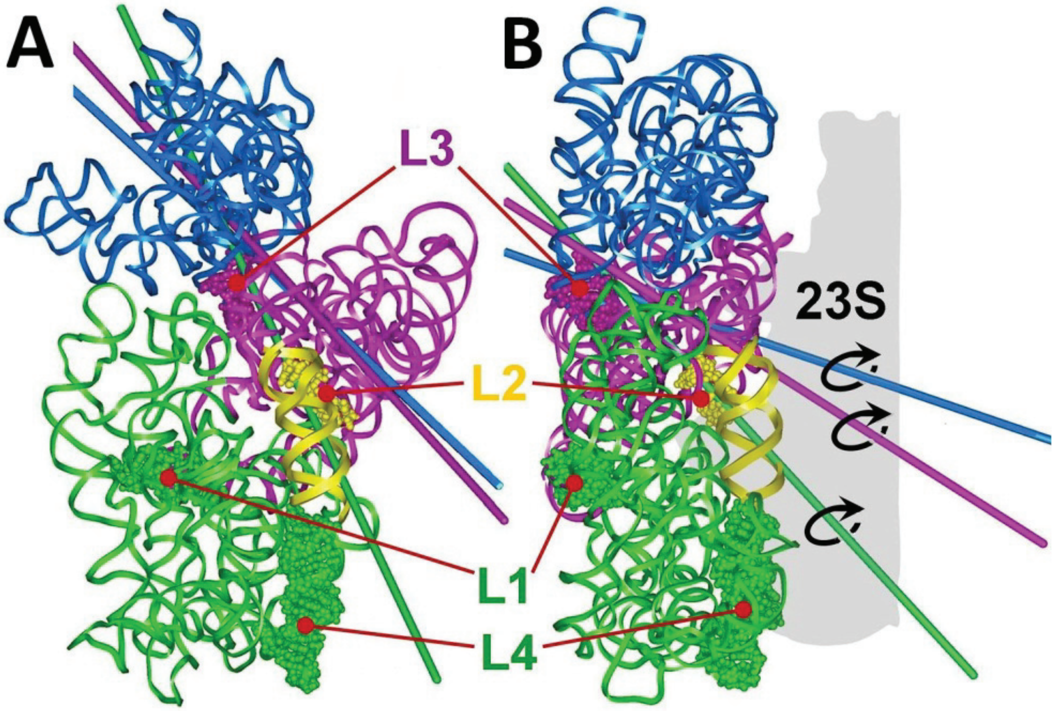


Figure 2

Displacement of tertiary domains T1, T2 and T3 during the ratchet and the swivel

The small subunit is seen from the subunit interface (**A**) and from the A-site (**B**). All domains are colored as in Figure 1. The axes of the T1, T2 and T3 rotation with respect to 23S rRNA are shown for crystal N2-R2. The color of each axis corresponds to the color of the domain. Linkers L1-L4 are shown in CPK representation. The parameters of all movements are provided in Supplemental material).

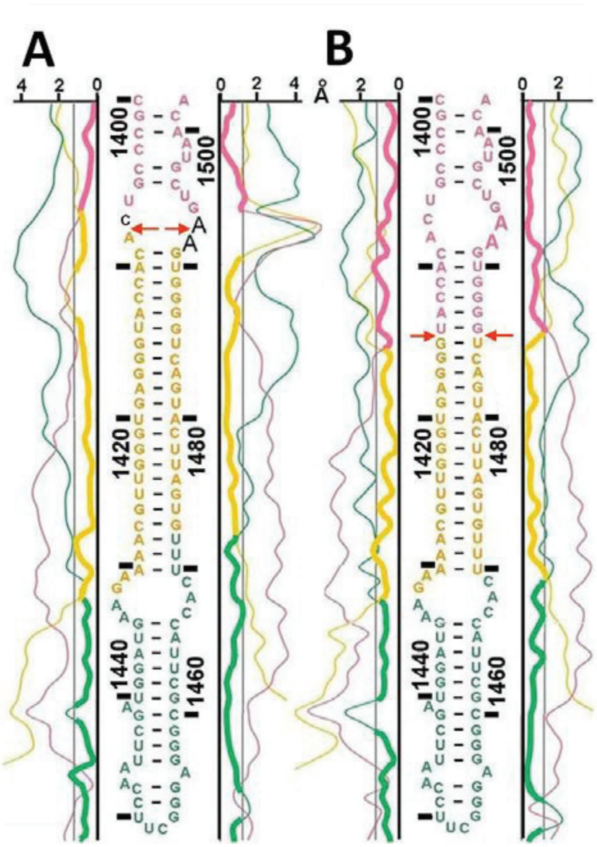


Figure 3

The division of h44 between domains T1 (green), T2 (magenta) and h44c (yellow) in crystals N1-R1 (A) and N2-R2 (B)

The colored lines show the discrepancies in the position of the given nucleotide in the non-ratcheted and ratcheted conformations of 16S rRNA, when the corresponding domains are superposed. The scale in angstroms is shown at the top. The grey vertical lines correspond to the threshold discrepancy of 1.2 Å. Thick curves indicate that the given region is a part of the corresponding tertiary domain. Adenosines 1492-1493 are shown by big letters. The border between T2 and h44c is marked by red arrows.

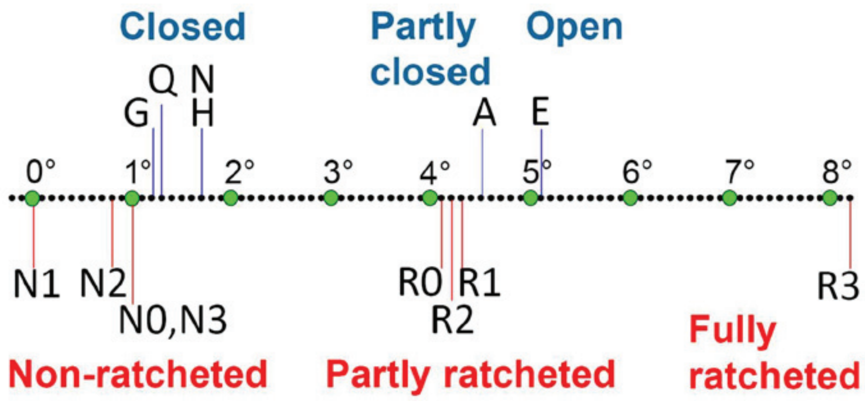


Figure 4

The angle of rotation of domain T2 with respect to h44c in different open and closed conformations of the SSU (above the axis) and in different ratcheted and non-ratcheted ribosome conformations (below the axis)

The position of domain T2 in ribosome N1 is taken as the reference point. The open and closed conformations of the SSU: E (*Wimberly-2000*) (open) pdb 1j5e; A (*Ogle-2002*) (partly closed) pdb 1n32; N (*Schmeing-2009*) pdb 2wrn, Q (*Schmeing-2009*) pdb 2wrq, G (*Jenner-2010*) pdb 3i8g; H (*Jenner-2010*) –pdb 3i8h (all closed). Non-ratcheted N0, N1, N2 and partly ratcheted R0, R1, R2 ribosomes are those analyzed throughout the text (*Zhang-2009*). N3, R3 – non-ratcheted and fully ratcheted ribosomes (*Dunkle-2011*) (pdb 4gd2 and 4gd1, respectively).

SUPPLEMENTARY DATA 1: Identification of tertiary domains

The rigid tertiary domains were identified through systematic comparison of the ribosome structures within pairs N0-R0, N1-R1 and N2-R2. Although in the three cases, the exact locations of the tertiary domains are a little different, in most cases, the differences are not essential. The only essential difference takes place in the upper part of helix h44 (see Fig 3 of the main text)

1.1 The analyzed structures

Supplementary Table 1. The pdb-codes of the analyzed structures

Crystal	N0-R0	N1-R1	N2-R2
Non-ratcheted ribosomes (SSU/LSU)	3i1o / 3i1p	3i1s / 3i1t	3i21 / 3i22
Ratcheted ribosomes (SSU/LSU)	3i1m / 3i1n	3i1q / 3i1r	3i1z / 3i20

1.2 Parts of the ribosomal RNA taken for analysis

Analysis of 23S rRNA was performed based on position of nucleotides 1-878, 898-2110, 2179-2903, which were present in all six analyzed ribosome structures N0, N1, N2, R0, R1, R2).
The same for 16S rRNA: 6-1534

1.3 Tertiary domains in 23S rRNA and in 16S rRNA

23S rRNA:

The whole length of 23S rRNA,
Excluding the above-mentioned regions that do not exist in all analyzed ribosome structures
Excluding linker L5 (nucleotides 1906-1924);
Excluding curls (the list of the nucleotides involved in curls is provided in Supplemental data 5)

16S rRNA:

Domain T1 includes:

Secondary structure domain D1 (nucleotides 27-556);
Including linker S1 (nucleotides 606-634);
Including linker S4 (nucleotides 1431-1469)
Excluding curls (the list of the nucleotides involved in the curls is provided in Supplemental data 5)

Domain T2 includes:

Secondary structure domain D2 (nucleotides 567-912);

Excluding linker S1 (nucleotides 606-634) – goes to domain T1

Excluding linker S2 (nucleotides 888-896) – goes to h44c

Including linker S3 (nucleotides 1074-1083);

Including the upper part of h44 and entire h45 (nucleotides 1397-1408, 1494-1534);

Including the central part of 16S (nucleotides 5-26, 913-930, 1387-1396)

Including linker S5 from 23S rRNA (nucleotides 1906-1924);

Excluding curls (the list of the nucleotides involved in the curls is provided in Supplemental data 5)

Domain T3 includes:

Secondary structure domain D3 (nucleotides 935-1380)

Excluding linker S3 (nucleotides 1074-1083) – goes to domain T2

Excluding curls (the list of the nucleotides involved in the curls is provided in Supplemental data 5)

Element h44c includes:

Nucleotides 1415-1430, 1470-1485 of helix h44

Including linker S2 (nucleotides 888-896 of helix h27)

1.4 Superposition of domains and elimination of curls

1.4.1 Entire 23S rRNA

Supplementary Table 2. 23S rRNA, Superposition by all atoms of phosphorus

Crystal	RMSD
N0-R0	1.21 Å
N1-R1	1.11 Å
N2-R2	1.22 Å

Supplementary Table 3. 23S rRNA, The % of atoms of phosphorus exceeded (curls) and fitted in (parts of the rigid domain) the threshold of 1.2 Å (D = deviation)

Crystal	% of D>1.2 Å (curls)	% of D≤1.2 Å
R0-N0	12.68%; RMSD = 3.07 Å	87.32%; RMSD = 0.55 Å
R1-N1	11.54%; RMSD = 2.91 Å	88.46%; RMSD = 0.55 Å
R2-N2	15.77%; RMSD = 2.7 Å	84.23%; RMSD = 0.64 Å

1.4.2 16S rRNA: Domain D1

Supplementary Table 4. **Domain D1, Superposition by all atoms of phosphorus**

Crystal	RMSD
N0-R0	1.31 Å
N1-R1	1.21 Å
N2-R2	1.28 Å

Supplementary Table 5. **Domain D1, The % of atoms of phosphorus exceeded and fitted in the threshold of 1.2 Å**

Crystal	% of D>1.2 Å (curls)	% of D≤ 1.2 Å
N0-R0	21.0%; RMSD = 2.55 Å	79.0%; RMSD = 0.65 Å
N1-R1	18.2%; RMSD = 2.44 Å	81.8%; RMSD = 0.68 Å
N2-R2	23.0%; RMSD = 2.33 Å	77.0%; RMSD = 0.71 Å

1.4.3 16S rRNA: Domain D2

Supplementary Table 6. **Domain D2, Superposition by all atoms of phosphorus**

Crystal	RMSD
N0-R0	0.77 Å
N1-R1	0.82 Å
N2-R2	0.82 Å

Supplementary Table 7. **Domain D2, The % of atoms of phosphorus exceeded and fitted in the threshold of 1.2 Å**

Crystal	% of D>1.2 Å (curls)	% of D<1.2 Å
N0-R0	9%; RMSD = 1.81 Å	91%; RMSD = 0.57 Å
N1-R1	10%; RMSD = 1.88 Å	90%; RMSD = 0.6 Å
N2-R2	8.3%; RMSD = 2.05 Å	91.7%; RMSD = 0.59 Å

1.4.4 16S rRNA: Domain D3

Supplementary Table 8. **Domain D3, Superposition by all atoms of phosphorus**

Crystal	RMSD
N0-R0	1.15 Å
N1-R1	0.93 Å
N2-R2	1.03 Å

Supplementary Table 9. **Domain D3, The % of atoms of phosphorus exceeded and fitted in the threshold of 1.2 Å**

Crystal	% of D>1.2 Å (curls)	% of D <1.2 Å
N0-R0	25.2%; RMSD = 1.95 Å	74.8%; RMSD = 0.7 Å
N1-R1	13.1%; RMSD = 2.06 Å	86.9%; RMSD = 0.6 Å
N2-R2	17%; RMSD = 2.01 Å	83%; RMSD = 0.68 Å

1.4.5 16S rRNA: Element h44c

Supplementary Table 10. Element h44c, Superposition by all atoms of phosphorus

Crystal	RMSD
N0-R0	0.56 Å
N1-R1	0.64 Å
N2-R2	0.71 Å

Supplementary Table 11. Element h44c, The % of atoms of phosphorus exceeded and fitted in the threshold of 1.2 Å

Crystal	% of D>1.2 Å (curls)	% of D <1.2 Å
N0-R0	2.4%; RMSD = 1.41 Å	97.6%; RMSD = 0.52 Å
N1-R1	2.4%; RMSD = 1.39 Å	97.6%; RMSD = 0.61 Å
N2-R2	5%; RMSD = 1.55 Å	95%; RMSD = 0.64 Å

1.5 Curls

The list of nucleotides presented below was obtained as follows:

The two ribosomes N2 and R2 we superposed using as the nucleus:

a - the whole 23S rRNA

b, c, d - each of the secondary structure domain D1, D2 or D3 of 16S rRNA.

Then, in each superposition, all nucleotides for which the discrepancy in the position of the equivalent P atom was 1.2 Å or higher and which were not parts of linkers were considered as curls and were added to the list.

For crystals N0-R0 and N1-R1 curls were identified in the same way. Although in the three crystals the curls are not completely identical, the difference is not essential. For this reason, only the curls identified in crystal N2-R2 are shown.

Curls consisting of several consecutive nucleotides are colored. For each nucleotide involved in a curl, the identity and the discrepancy in the position of the P-atom, when the corresponding domain was superposed, are provided.

List of curls :

Curls in LSU

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
1-G	3.08	5-A	2.45	42-A	1.32	63-A	1.36	102-U	1.79
2-G	2.65	6-A	1.76	50-U	1.53	72-U	1.27	134-G	1.21
3-U	2.20	7-G	1.67	51-G	1.27	87-U	1.53	136-G	2.65
4-U	2.07	38-A	1.65	60-G	1.43	101-A	5.98	137-U	3.49

Curls in LSU (suit)

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
138-U	4.91	344-A	1.34	846-U	1.90	1076-C	3.20	1345-C	1.90
139-U	5.63	345-A	2.08	847-U	1.90	1077-A	2.91	1366-A	1.22
140-C	2.59	351-C	1.93	854-C	1.48	1078-U	4.68	1379-U	1.31
142-A	3.34	352-A	1.61	855-G	1.36	1080-A	2.79	1382-G	1.39
143-C	3.45	353-C	1.62	875-G	1.30	1081-U	2.78	1411-U	1.50
144-A	2.79	354-A	1.92	877-A	2.44	1082-U	3.12	1414-C	1.28
150-U	1.35	355-U	3.22	878-A	5.30	1083-U	2.21	1419-A	1.61
152-A	1.20	356-G	1.72	898-C	1.34	1084-A	2.64	1458-U	1.57
156-A	1.63	357-C	1.31	900-A	1.29	1085-A	1.56	1459-G	1.23
171-U	1.76	359-G	3.58	902-C	1.72	1086-A	2.00	1460-U	4.86
173-A	1.27	360-U	2.59	928-A	1.84	1087-G	2.01	1487-U	1.25
180-G	1.33	361-G	1.85	929-U	1.49	1088-A	1.90	1490-A	2.24
222-A	1.52	362-A	1.89	930-G	1.51	1089-A	1.26	1491-G	2.46
231-A	1.32	373-U	1.32	931-U	1.53	1090-A	1.22	1492-G	1.44
234-U	1.35	416-U	1.21	932-U	2.51	1091-G	3.04	1493-C	1.41
264-C	2.14	423-A	1.74	1022-G	1.28	1092-C	2.72	1494-A	1.95
265-A	1.25	425-G	1.71	1033-U	1.38	1094-U	2.40	1495-A	1.47
266-G	3.29	426-C	1.63	1042-G	1.49	1095-A	4.53	1496-A	1.63
267-C	1.46	427-U	1.65	1043-C	1.39	1096-A	5.13	1497-U	1.57
269-C	1.47	428-A	1.35	1044-C	1.36	1097-U	3.85	1502-A	2.01
270-A	1.23	484-C	1.65	1045-C	1.42	1098-A	2.92	1503-A	1.34
272-A	1.26	485-C	1.41	1046-A	2.28	1099-G	1.34	1504-A	1.66
275-C	1.28	490-C	1.34	1047-G	2.63	1100-C	3.03	1505-A	1.29
276-U	2.46	500-G	1.24	1048-A	2.19	1101-U	4.04	1506-U	1.26
277-G	1.77	501-A	1.36	1049-C	1.76	1102-C	2.28	1507-C	1.86
278-A	2.28	507-A	1.37	1050-A	2.08	1103-A	2.02	1508-A	1.22
279-A	2.47	529-A	2.01	1053-C	1.27	1104-C	2.65	1509-A	3.67
280-U	2.67	544-C	1.52	1054-A	1.46	1106-G	2.02	1510-G	1.28
281-C	2.38	545-U	2.81	1055-G	1.33	1108-U	1.35	1520-U	1.63
282-A	2.73	546-U	2.74	1056-G	1.93	1109-C	1.75	1523-U	2.00
283-G	2.84	547-A	2.41	1058-U	1.81	1110-G	1.31	1524-G	1.21
284-U	1.42	548-G	1.91	1059-G	2.55	1112-G	1.76	1525-A	1.54
285-G	1.69	549-G	1.29	1060-U	2.52	1120-G	1.23	1527-G	1.44
286-U	1.94	550-C	1.32	1061-U	3.05	1158-C	1.22	1531-C	1.32
287-G	1.30	647-G	1.94	1062-G	2.23	1168-G	1.64	1533-C	2.92
288-U	1.48	654-A	1.38	1063-G	5.40	1170-C	1.54	1534-U	3.43
289-G	1.52	655-A	1.35	1064-C	5.13	1171-G	1.75	1535-A	2.89
290-U	1.59	709-U	1.65	1065-U	4.80	1172-C	1.76	1536-C	3.58
291-G	2.11	710-U	1.52	1066-U	5.82	1174-U	2.53	1537-G	5.22
295-G	1.54	711-G	2.00	1067-A	3.35	1175-A	2.00	1538-G	3.35
304-U	1.35	712-G	3.21	1068-G	2.76	1176-U	1.29	1544-A	1.24
305-C	1.37	713-G	3.59	1069-A	6.03	1179-G	2.92	1577-C	1.37
311-A	1.48	714-U	3.66	1070-A	3.45	1180-U	2.46	1581-G	1.71
313-G	1.42	715-A	3.50	1071-G	1.50	1181-U	3.09	1582-C	1.53
314-C	1.20	716-A	4.00	1072-C	3.50	1182-G	1.64	1584-U	1.90
315-G	1.47	717-C	3.86	1073-A	2.57	1206-G	1.24	1585-C	1.96
326-G	1.38	843-G	1.24	1074-G	3.74	1236-G	1.34	1588-G	1.38
334-C	1.35	845-A	1.35	1075-C	4.58	1307-A	1.29	1594-U	1.22

Curls in LSU (suit)

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
1630-A	1.45	1920-C	1.97	2183-A	8.39	2314-A	3.53	2793-C	5.53
1702-G	1.40	1921-G	2.04	2184-A	5.53	2315-G	2.66	2794-C	3.79
1722-A	1.52	1922-G	2.30	2185-U	4.95	2316-G	1.84	2795-C	3.74
1724-G	1.96	1923-U	2.26	2186-G	3.33	2317-A	1.62	2796-U	3.99
1728-C	1.86	1924-C	1.78	2187-U	4.30	2318-G	1.55	2797-U	4.48
1730-C	1.27	1925-C	1.87	2188-U	4.56	2319-G	1.46	2798-U	4.89
1732-C	2.00	1926-U	1.96	2189-U	4.84	2323-G	1.53	2799-A	3.86
1734-G	1.26	1927-A	1.26	2190-G	5.34	2327-A	3.09	2800-A	1.81
1839-G	1.24	1931-U	1.33	2191-A	2.68	2335-A	1.26	2801-G	2.23
1847-A	2.19	2033-A	1.40	2192-U	1.59	2338-C	1.52	2802-G	2.63
1848-A	1.25	2056-G	1.63	2193-G	1.84	2371-G	1.27	2803-G	2.99
1849-G	1.55	1931-U	1.33	2194-U	1.20	2402-U	3.07	2804-U	3.88
1850-G	1.68	2033-A	1.40	2205-A	1.22	2406-A	1.35	2805-C	3.78
1856-U	1.44	2056-G	1.63	2217-G	1.33	2407-A	1.22	2806-C	3.53
1867-G	1.26	2085-U	1.55	2227-A	1.47	2409-G	1.42	2807-U	3.79
1869-G	2.24	2086-U	1.28	2230-G	1.30	2410-G	1.31	2808-G	2.74
1870-C	2.19	2089-C	1.24	2240-U	1.23	2475-C	1.32	2809-A	1.40
1871-A	2.34	2091-C	1.26	2268-A	1.34	2494-G	1.27	2811-G	1.34
1872-A	1.73	2096-C	1.31	2286-G	1.35	2502-G	1.24	2833-U	1.31
1873-G	1.58	2097-A	1.57	2288-A	1.98	2506-U	1.21	2837-A	1.48
1875-G	1.42	2098-U	2.01	2294-G	1.22	2525-G	1.33	2877-G	1.44
1876-A	1.96	2099-U	1.59	2297-A	1.86	2602-A	2.77	2885-G	1.22
1884-G	1.78	2100-G	2.57	2299-U	1.43	2632-A	2.05	2886-A	1.35
1890-A	1.40	2101-A	3.69	2300-C	2.69	2663-G	1.54	2887-A	2.20
1907-G	1.58	2102-G	5.34	2301-C	3.15	2667-C	1.71	2888-C	2.34
1908-C	1.99	2103-C	7.71	2302-U	1.80	2668-G	1.30	2889-C	2.14
1909-C	1.43	2104-C	10.2	2303-G	1.45	2696-U	1.32	2890-G	1.79
1910-G	1.41	2105-U	9.36	2304-G	2.48	2746-U	1.79	2891-U	1.80
1911-U	2.49	2106-U	8.84	2305-U	3.35	2749-A	1.33	2892-G	1.53
1912-A	2.49	2107-G	11.1	2306-C	2.55	2750-A	1.21	2893-A	1.64
1913-A	3.38	2108-A	7.65	2307-G	3.12	2753-A	1.39	2899-A	1.30
1914-C	3.28	2109-U	5.84	2308-G	3.46	2759-G	1.27	2900-A	2.56
1915-U	3.03	2110-G	4.80	2309-A	2.52	2760-C	1.35	2901-C	3.26
1916-A	3.05	2179-C	3.50	2310-C	2.96	2762-C	1.26	2902-C	3.41
1917-U	2.99	2180-U	4.16	2311-A	5.35	2765-A	1.35	2903-U	5.04
1918-A	2.10	2181-U	5.55	2312-U	2.05	2791-G	4.04		
1919-A	1.98	2182-U	5.92	2313-C	3.62	2792-A	3.65		

Curls in D1

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
54-C	1.28	79-G	6.70	86-G	3.35	93-U	1.70	157-U	1.87
71-A	2.26	80-A	5.64	87-C	4.29	95-C	1.34	158-G	2.43
74-A	1.37	81-A	5.94	88-U	3.98	96-U	2.03	159-G	2.48
75-G	2.42	82-G	3.27	89-U	2.25	121-U	1.39	160-A	1.71
76-G	2.02	83-C	3.76	90-C	3.89	143-A	1.38	161-A	2.11
77-A	1.51	84-U	2.17	91-U	4.29	155-A	1.57	162-A	1.62
78-A	5.21	85-U	3.12	92-U	2.10	156-C	1.58	163-C	1.69

Curls in D1 (suit)

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
164-G	1.79	213-G	1.78	341-C	1.84	446-G	1.24	478-A	1.71
165-G	1.54	214-C	1.94	342-C	1.88	457-G	3.02	479-U	1.28
166-U	1.91	215-C	2.82	343-U	2.05	458-U	1.57	486-U	1.51
167-A	1.89	216-U	2.43	344-A	2.28	459-A	1.31	489-C	1.20
169-C	1.74	217-C	2.45	345-C	3.25	460-A	1.79	491-G	1.29
188-C	1.54	218-U	1.75	346-G	2.80	461-A	1.53	492-C	1.46
189-A	1.31	239-U	1.71	347-G	2.68	462-G	1.45	493-A	1.58
198-G	1.25	242-G	1.45	348-G	1.70	463-U	2.03	494-G	1.43
202-G	1.92	244-U	1.22	349-A	1.37	467-U	1.71	497-G	1.23
203-G	3.36	248-C	1.77	373-A	1.50	468-A	1.97	515-G	1.32
204-G	3.53	249-U	1.41	405-U	1.23	469-C	2.21	518-C	1.49
205-A	2.92	250-A	1.31	417-G	1.37	470-C	2.36	519-C	1.23
206-C	4.85	271-C	1.29	424-G	1.37	471-U	1.50	530-G	1.59
207-C	3.63	273-U	1.38	432-A	1.33	472-U	2.52	531-U	1.26
208-U	3.55	330-C	1.50	436-C	1.28	473-U	2.25	532-A	1.39
209-U	2.03	337-G	1.59	437-U	1.34	474-G	3.14	533-A	1.48
210-C	1.43	338-A	2.03	438-U	1.55	475-C	1.93	538-G	1.22
211-G	1.20	339-C	2.36	440-C	1.52	476-U	2.30		
212-G	4.77	340-U	1.83	445-G	1.33	477-C	2.07		

Curls in D2

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
597-G	1.28	618-C	2.09	630-A	1.47	748-G	1.42	846-G	1.57
602-A	1.37	619-U	2.43	632-U	1.33	774-G	1.73	847-G	2.58
603-U	1.47	620-C	2.12	636-U	1.54	814-A	1.22	889-A	1.80
604-G	1.32	621-A	1.58	683-G	1.29	833-G	1.27	892-A	1.25
605-U	1.54	622-A	1.27	684-U	1.65	840-C	1.30	893-C	1.89
606-G	1.22	623-C	1.22	709-U	1.30	841-C	3.26	894-G	1.82
613-C	1.55	626-G	1.54	710-G	1.44	842-U	3.91	895-G	1.25
615-G	1.56	627-G	1.45	723-U	1.30	843-U	3.23	896-C	1.34
616-G	1.85	628-G	1.60	724-G	1.44	844-G	4.31	912-C	1.20
617-G	1.49	629-A	1.78	734-G	1.54	845-A	2.07		

Curls in D3

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
989-U	1.61	1007-U	2.03	1021-A	1.80	1034-G	2.64	1136-C	2.07
991-U	1.46	1008-U	2.95	1022-A	2.53	1035-A	1.91	1137-C	2.99
994-A	1.55	1009-U	1.70	1024-G	2.22	1037-C	2.09	1138-G	4.37
998-C	1.46	1011-C	1.75	1025-U	2.51	1038-C	1.27	1141-C	3.09
1001-C	2.02	1012-A	1.29	1027-C	3.10	1042-A	1.37	1142-G	1.78
1002-G	1.33	1013-G	1.78	1029-U	2.43	1074-G	1.24	1143-G	2.54
1003-G	1.35	1015-G	1.62	1030-U	2.54	1100-C	1.38	1166-G	1.34
1004-A	1.32	1016-A	2.02	1031-C	4.00	1104-G	1.23	1167-A	2.39
1005-A	1.47	1019-A	1.38	1032-G	3.25	1131-G	1.82	1168-U	2.79
1006-G	2.54	1020-G	1.56	1033-G	3.62	1132-C	1.98	1169-A	1.59

Curls in D3 (suit)

nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD	nucleotide	RMSD
1211-U	1.93	1220-G	1.57	1242-G	1.46	1274-A	1.37	1310-G	1.74
1213-A	1.40	1221-G	1.40	1258-G	1.51	1286-U	1.43	1312-G	1.26
1217-C	1.29	1225-A	1.47	1263-C	1.41	1297-G	1.79	1322-C	1.73
1218-C	1.26	1228-C	1.59	1266-G	1.29	1302-C	1.66	1323-G	1.64
1219-A	1.47	1241-G	1.36	1267-C	1.24	1304-G	1.53	1334-G	1.39

SUPPLEMENTARY DATA 2: Parameters of movement for d1, d2, d3 h44c

Supplementary Table 12. Inclination of the axes of rotation of tertiary domains T1, T2 and T3 with respect to 23S rRNA to the perpendicular to the subunit interface

Crystal	**T1**	**T2**	**T3**
N0-R0	49°	37.5°	47.5°
N1-R1	46°	32.5°	13.5°
N2-R2	48.5°	34 °	19°

Supplementary Figure 1.

Positions of the axes of rotation for different domains of the 30S subunit in crystals N0-R0 (left), N1-R1 (center) and N2-R2 (right).

The small subunit is oriented as in Fig 2b of the main text (i.e. the large subunit is located on the right from the small one. Domains T1, T2 and T3 are green, brown and blue, respectively. The colors of the axes correspond to those of the domains. The axes of the T1 and T2 rotations have about the same position in all three crystals. The axis of the T3 rotation has a higher inclination in crystal N0-R0 than in the other two crystals.

<u>Supplementary Table 13.</u> The angle of rotation and the shift of the tertiary domains with respect to the LSU (when the LSU is superposed)

Crystal	**T1**	**T2**	**T3**	**h44c**
R0-N0	Rotates: 8.09 ° Shifts: 0.79 Å	Rotates: 5.12 ° Shifts: 0.64 Å	Rotates: 9 ° Shifts: 0.34 Å	Rotates: 2.09 ° Shifts: 0.42 Å
R1-N1	Rotates: 9.47 ° Shifts: 0.99 Å	Rotates: 6.67 ° Shifts: 0.51 Å	Rotates: 6.07 ° Shifts: 0.35 Å	Rotates: 2.47 ° Shifts: 0.46 Å
R2-N2	Rotates: 8.41 ° Shifts: 1.06 Å	Rotates: 6.1 ° Shifts: 0.51 Å	Rotates: 6.1 ° Shifts: 0.46 Å	Rotates: 2.86 ° Shifts: 0.54 Å

In all cases, the domain in the ratcheted ribosome is shifted with respect to its position in the non-ratcheted ribosome along the axis of rotation described in Supplemental table 12. The whole domain rotates around this axis by the angle seen in Supplemental table 13. In all cases, the direction of the shift is such that it displaces the corresponding domain farther from the large subunit.

SUPPLEMENTARY DATA 3: The bending of helix h44 between base pairs 1414-1486 and 1415-1485

Here we explain why the bend in helix h44 of ribosome R2 occurs between base pairs 1414-1486 and 1415-1485. We also describe the aspects of the 16S rRNA structure that resist bending of h44 at this very place by an angle larger than that observed in ribosome R2 (3.2˚). These aspects are attributed to helix h44 (base pairs U1414-G1486 and G1415-U1485) and to helix h27 (linker L2, which interacts with h44).

3.1. Theoretical introduction into the bendability of double helices

Our analysis of the bendability of h44 (to our best knowledge, term 'bendability' as the ability of a double helix to bend at particular places in a particular direction was coined by Edward N Trifonov) is mainly based on the pioneering works that described the mechanism of local perturbations in the B-DNA WC double helices (Zhurkin VB, Lysov YP, Ivanov VI: Anisotropic flexibility of DNA and the nucleosomal structure. *Nucleic Acids Res.* **6**, 1081-96 (1979), Calladine CR: Mechanics of sequence-dependent stacking of bases in *B*-DNA. *J Mol Biol.* **161**, 343-352, (1982)). The major results of these papers were that WC double helices bend towards the grooves and not towards the backbones and also that pyrimidine-purine steps favor bending towards the major groove and disfavor bending toward the minor groove, while purine-pyrimidine steps behave in the exact opposite way.

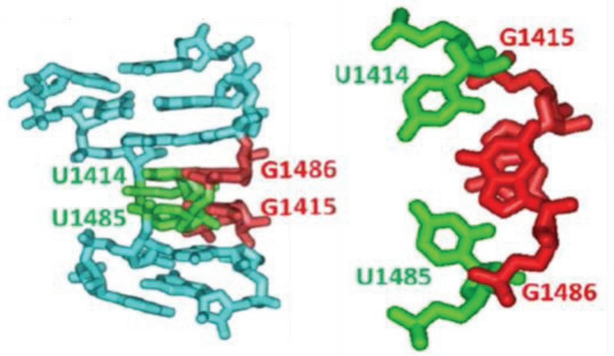
For the A-RNA, such a theory does not yet exist. However, given that helix h44, like B-DNA, is a right-handed double helix in which neighboring base pairs stack on each other, some aspects of this theory are applicable to h44 as well. One aspect that distinguishes RNA from DNA is that RNA predominantly exists in the A-form, in which the major groove is buried inside while the minor groove is exposed to the surface. Due to this feature, the bending of RNA in the direction of the major groove would face immediate steric problems, while bending towards the minor groove does not face such problems and therefore, can be achieved much easier. Of course, the latter is only true if there are no additional aspects that can interfere with such bending.

3.2. The bend in helix h44 in the context of the whole ribosome

Analysis of the part of helix h44 enclosed between base pairs 1410-1490 and 1420-1480 shows that in most places of this region, bending of h44 in the direction of the minor groove is impossible due to interference with either the large or small subunit. The only place in the whole region where bending of h44 does not lead to an immediate steric clash with one of the two subunits is between base pairs 1414-1486 and 1415-1485. At this place, the bended part of h44 will be displaced in the inter-subunit space parallel to the subunit interface without collision with any ribosome subunit. We thus can conclude that, due to the particular position of h44 at the interface between the two subunits, the junction between base pairs 1414-1486 and 1415-1485 of h44 constitutes the only bendable section in the whole above-mentioned region of h44. This explains the fact that in ribosome R2, the bend towards the minor groove occurs exactly at this location.

3.3. In the structure of 16S rRNA, there are two aspects that are able to minimize the scale of the bending in helix h44 between base pairs 1414-1486 and 1415-1485 towards the minor groove.

3.3.1. The first aspect consists in the particular choice of the nucleotides composing these two base pairs. In the overwhelming majority of available nucleotide sequences of 16S rRNA, base pairs 1414-1486 and 1415-1485 are, respectively, pyrimidine-purine and purine-pyrimidine, and in most prokaryotes, they are, respectively, U-G and G-U (see the statistics below). The contact between these base pairs thus represents a pyrimidine-purine step, which causes the two purines to interfere with each other in the minor groove. This interference strongly disfavors bending of the double helix in the direction of the minor groove. Due to the presence of non-Watson-Crick base pairs U-G and G-U, the two purines become additionally displaced towards the minor groove, which only aggravates the interference between them if the helix bends in this direction (see Supplementary Figure 2).



Supplementary Figure 2. The fragment of helix h44 encompassing base pairs U1414-G1486 and G1415-U1485 (left).

Due to the particular geometry of the GU base pairs, guanosines G1486 and G1415 are notably displaced towards the minor groove (right). Due to their interference with each other, the two guanosines prevent bending of the double helix towards the minor groove. The interference is maximal when both base pairs are GU. A replacement of one of the two GU base pairs by a Watson-Crick purine-pyrimidine base pair will make the interference a little less effective. If one of the two GU base pair is replaced by a pyrimidine-purine base pair, the interference between the two base pairs will no longer exist.

<u>Comment:</u> Statistics on the identity of base pairs 1414-1486 and 1415-1485 in 16s rRNA of bacterial ribosomes

The data on identity of base pairs 1414-1486 and 1415-1485 in 16S rRNA were taken from the database of Cannone JJ, Subramanian S, Schnare MN, Collett JR, D'Souza LM, Du Y, Feng B, Lin N, Madabusi LV, Muller KM, Pande N, Shang Z, Yu N, Gutell RR. 2002. The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC bioinformatics 3*:2.

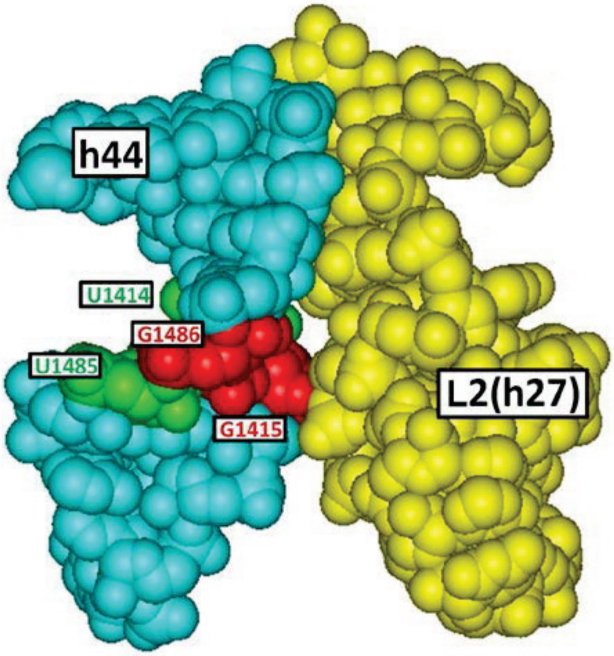
Total number of sequences of 16S rRNA that have been analyzed: 8386 100%

Of these sequences, 1414-1486=UG and 1415-1485=GU 5339 63.7%

1414-1486=(CG or UA) and 1415-1485=GU 2632 31.4%

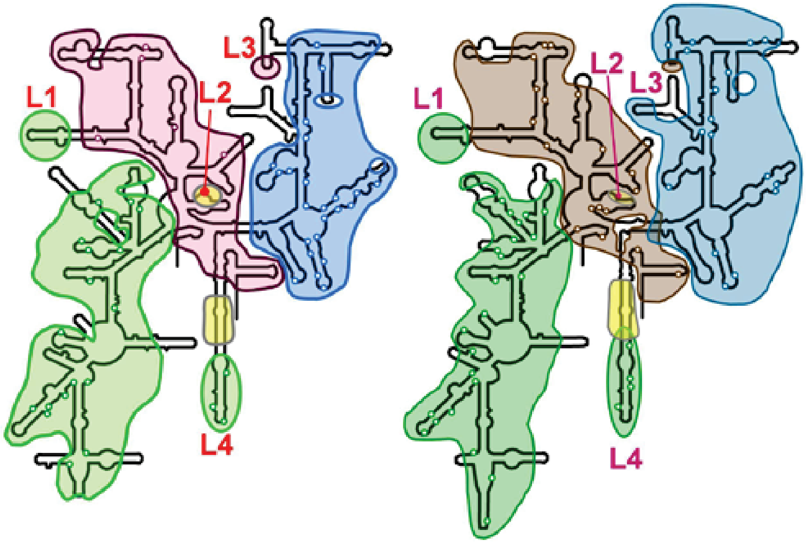
Therefore, 16S rRNA sequences in which 1415-1485 is GU, AU or GC while 1414-1486 is UG, CG or UA constitute together >95% of all 16S rRNA sequences. This result clearly demonstrates that the nucleotide identities of the two base pairs 1415-1485 and 1414-1486 have been naturally selected in a way to prevent bending between them towards the minor groove.

3.3.2 The second aspect, that makes helix h44 more resistant to the bending between base pairs 1414-1486 and 1415-1485 towards the minor groove, is the tight interaction of this region of h44 with linker L2 (helix h27). More specifically, eight nucleotides of h27 (nucleotides 887-888, 892-893 and 909-912) pack tightly with the minor groove of h44, forming contacts with six nucleotides of h44 on both sides of the bending (nucleotides 1413, 1415-1416 and 1488-1490) (see Supplementary Figure 3). Together, the particular choice of the nucleotide identities of base pairs 1414-1486 and 1415-1485 and the tight interaction of the minor groove of h44 with linker L2 (h27) will minimize the ability of h44 to bend towards the minor groove.



Supplementary Figure 3. The tight interaction between the upper part of helix h44 and linker L2.
Nucleotides forming base pairs 1414-1486 and 1415-1485 are indicated and are marked in colors as in Figure 1. h27 tightly interacts with h44 both above and below the bending between base pairs 1414-1486 and 1415-1485. Also, nucleotide G1415 directly interacts with helix h27.

SUPPLEMENTARY DATA 4 : THE RIGID TERTIARY DOMAINS AND FLEXIBLE CONNECTOR REGIONS IN 16S RRNA (IN RATCHETING AND DOMAIN CLOSURE)



Supplementary Figure 4. The rigid tertiary domains and flexible connector regions in 16S rRNA observed during ratcheting (left) and domain closure (right)

The rigid tertiary domains in 16S rRNA identified through comparison of:

Left: the *E.coli* ribosome structures N2 (non-ratcheted) and R2 (partly ratcheted). In the main text, the same figure is shown as Figure 1b. **Right:** the *T. thermophilus* SSU structures E (open conformation, the apo-subunit, pdb 1j5e, Wimberly B.T. *et al*. *Nature* **407**, 327-339 (2000).) and A (closed conformation associated with mRNA and ASL soaked into the crystal in the presence of paromomycin, pdb 1n32, Ogle J.M. *et al*. *Cell* **111**, 721–732, (2002)).

While ribosome structures N2 and R2 are notably different from each other (angle of the T2 rotation between the two structures is 4.4˚ (see Figure 4 of the main text), the difference between structures E and A is substantially less (the equivalent angle is only 0.6˚). Due to the closeness of structures E and A, the identification of rigid tertiary domains in structures E and A became a more delicate procedure compared to that used for analysis of structures N2 and R2. As mentioned in the main text, the threshold value used for the N2-R2 analysis was 1.2 Å. For the E-A analysis, the corresponding value was chosen to be only 0.45 Å. A threshold value larger than that would have made the identified domains substantially overlapped with each other. Even with the chosen threshold value of 0.45 Å, some minor overlaps are still present. In spite of some minor differences, the tertiary domains that were rigid during the ratcheting and those that were rigid during the domain closure are practically identical. This aspect has allowed us to state that two phenomena of the ribosome ratcheting and domain closure are closely related to each other.

Chapter 5. Discussion

The results presented in this thesis were obtained based on systematic *in-silico* analysis of the available ribosome conformations.

Such analysis has become possible due to the substantial progress in crystallography of the ribosome that happened in the first decade of this century. The high resolution crystallographic data provided the details of the ribosome structure that would have been impossible to obtain based on the cryo-electron microscopy data, due to the substantially lower resolution of the latter. The detailed analysis of the ribosome structure has led us to a new level of understanding of how this RNA-protein complex functions. In particular, systematic comparison of the known ribosome conformations existing in the form of PDB files has allowed us to reveal the principal characteristics of ribosomal dynamic.

The ratchet-like motion (RLM) was originally described as a reciprocal rotation of the two subunits (*Frank-2000*). However, as described below, certain mobility was also found within the SSU (*Ogle-2002, Ogle-2003*). We also found that during the ratcheting, the central bridges of the subunit interface remain intact despite the 9° amplitude of the RLM (*Dunkle-2011*). Thus, the concept of ratcheting as a simple intersubunit rotation required reconsideration. This is of special importance, because ratcheting is linked to translocation (*Horan-2007*). Therefore, without understanding the mechanisms of ribosomal dynamic, it seems impossible to understand the mechanism of translocation.

We started the analysis of RLM soon after the publication of the first crystal structures of the ribosome in the ratcheted state (*Zhang-2009*). Systematic comparison of these structures allowed us to elucidate the pattern of internal mobility in the ribosome. Contrary to the LSU, whose structure is almost monolithic, the SSU was shown to be composed of several rigid tertiary domains, each moving along its own unique trajectory. This finding is supported by other studies, which suggested that different domains within the SSU can move with respect to each other (*Agirrezabala-2012*).

We discovered that during the ratcheting, one of the SSU domains is tightly attached to the LSU and remains immobile relative to the latter. This immobile domain forms the central inter-subunit bridges, which explains their integrity during the ratcheting. At the same time, this finding suggests a new concept of RLM as a movement within the SSU and not between the subunits.

We determined the borders between tertiary domains and the trajectories of the rigid domains in the SSU. We also determined how the domain motions in RLM can be regulated. An essential role in this regulation is played by the linkers. We described linkers as elements with dual nature. A linker remains covalently attached to one domain and, at the same time, forms a tight association with another domain. This covalent attachment works as a hinge between two connected domains. Together, the linkers guarantee the integrity of the SSU in RLM.

We found that RLM may be blocked in the presence of the A-site tRNA. This understanding allowed us to relate the RLM and the SSU domain closure (=SSC). Comparison of the SSU in the open and closed conformations revealed a pattern of domain reorganization that we previously observed in RLM.

We concluded that RLM and SSC represent equivalent rearrangements going in opposite directions. The interactions formed as a result of SSC must be broken in order to allow RLM. Further analysis is required to link these rearrangements to particular functional events during codon-anticodon recognition and ribosomal translocation.

The RLM project was part of my work in the laboratory of Dr. Steinberg. Another project pertained to systematic analysis of the ribosome structure in order to model early ribosome evolution. This research constitutes the other part of my thesis.

At the beginning of the project, we were not the only research group using crystallographic data for evolutionary studies. There was an attempt to link the evolutionary conservation of a given rRNA component (loop, double-helix, etc.) with its distance from the PTC (*Smit-2007*). According to other data, the PTC represents a single self-folding RNA segment and thus its emergence did not require any prerequisites. On the contrary, the decoding region of the SSU is formed by several such segments. Therefore, the LSU was suggested to be more ancient than the SSU (*Smith-2008*). In another model, two LSU conformations from different prokaryotic species were superposed and the 23S rRNA was enclosed in a set of nested spheres centered at the PTC. Each layer corresponded to the space limited by two neighboring spheres. The structure of each such layer was analyzed to evaluate how similar it is in both rRNA structures. The highest similarity was observed at the PTC and gradually decreased when the layers approached the ribosome surface. The authors considered this observation as confirmation that the PTC is the most ancient part of the LSU (*Hsiao-2009*).

The approach that we proposed was purely structural in nature. It was based on the presumption that the early ribosome evolution proceeded through a stepwise expansion of the rRNA. We tried to remodel these steps in reverse order. For that, we identified an rRNA element that was the most recent acquisition for a given moment of the rRNA evolution. Deletion of this element provided access to preceding evolutionary acquisitions, etc.

We suggested a set of rules to find the recent evolutionary acquisitions of rRNA. One of the rules concerned the participation of recently acquired element in the A-minor interaction also known as the A-minor motif. The motif is formed by two components. One of the components, the stack of unpaired adenosines, forms tight interaction to the regular double helix with WC base pairing, the other component of A-minor. The A-minor allows for the formation of a stable structure from two interacting elements, one of which is structurally flexible. With a stack of adenosines, such flexible elements can be attached to a double-helix that is structurally self-sufficient. In evolution, the A-minor interaction would be an easy way to accommodate newly emerged elements of the rRNA.

We worked out an algorithm for elimination of rRNA elements without jeopardizing the structural integrity of the remaining part. The deletion procedure was systematically applied to the tertiary structure of the 23S rRNA. It led to the particular region of the 23S that is known to play a critical functional role. We named this region the “proto-ribosome”. The order of deletions allowed us to build the evolutionary model for the 23S rRNA starting from the proto-ribosome.

After the publication of our model, we raised the question concerning the evolutionary pathway for the 16S rRNA. According to our unpublished results, the 16S rRNA appeared to follow the same evolutionary principles as the 23S rRNA.

One may ask, whether there are structural motifs, different from A-minor, that can serve as evolutionary markers. Our preliminary results suggest that such a role can be attributed to the Along-Groove packing Motif (=AGPM) (*Gagnon-2002*). This motif consists of two double-helices tightly packed by their minor grooves. One of the two helices has regular WC base-pairing. The other one has a GU base-pair located at the point of interaction of the helices. This GU-pair can be considered as a structural adaptor of the evolutionary more recent helix with GU-pair to the more ancient helix with WC base-pairs. In 23S rRNA, the AGPM as evolutionary markers are in agreement with A-minors.

We can deduce a general rule concerning the relative age of two interacting structural elements, one of which is the WC double-helix. The rule is based on the fact that the WC double-helix is a self-sufficient entity whose formation does not require any other prerequisites. Thus, when any element specifically interacts with a double-helix, this interaction is a result of adaptation of a more recent element to a more ancient double-helix.

We expect that the arguments presented above would be helpful for understanding of the rationale that is behind our concept of the ribosome evolution.

The major result of the work presented in this thesis consists in finding answers to two fundamental problems of ribosome biology. The first problem pertains to the mechanism of rearrangements in the ribosome structure that take place during the elongation cycle of translation, while the second problem concerns the mechanism of evolution of the ribosome from the RNA world. As one can see below, the solutions for both problems were found solely through thorough analysis of the ribosome tertiary structure. The latter aspect makes this research in some sense, unique in the field.

Although the current thesis includes only three papers, the lab of Dr. Steinberg continues studies on different aspects related to the ribosome structure and the mechanisms of protein synthesis. Among the aspects closely related to this thesis are the evolution of the small ribosomal subunit and the co-evolution of 23S and 16S rRNA, as well as the evolution of ribosomal proteins and the role played by these proteins in the assembly of the ribosome tertiary structure. Solving these problems will make a major impact on our knowledge of how the ribosome has emerged, how it assembles and how it functions.

CONCLUSION

1. The initial element of the 23S rRNA, the proto-ribosome, emerged as a result of duplication of a 110-nucleotide RNA fragment, providing for the symmetrically arranged peptidyl transferase center. Further evolution of the 23S rRNA proceeded through insertion of relatively short fragments of RNA at different parts of the polynucleotide chain. If a newly emerged fragment forms an A-minor interaction with more ancient elements of the ribosome, it must donate to this interaction the adenosine stack, and not the double helix. These simple suggestions have been very powerful for understanding the details of the ancient evolution of the ribosome. It also shows that systematic in-silico analysis of the ribosome tertiary structure is a promising method for studying ribosome evolution and function.
2. The RLM, originally described as a motion between subunits, occurs mainly within the SSU. Contrary to the LSU, whose structure is almost monolithic, the SSU is composed of several rigid domains. During RLM, each rigid domain moves along its own trajectory. The central element of the SSU remains immobile relatively the LSU, which explains the integrity of the inter-subunit bridges existing between this element and the LSU.
3. The existence of the linkers restricts and co-ordinates the movements of the rigid domains. The linkers arrange the rigid domains of the SSU in two conformational cycles. Within one of the two conformational cycles, which includes Domains I and II of the secondary structure of 16S rRNA, the movement of the domains in one direction corresponds to the transition from a non-ratcheted to a ratcheted state, while the opposite movement constitutes SSC.
4. The RLM can be blocked by the A-minor interaction of adenosines A1492 and A1493 of 16S rRNA with the cognate codon-anticodon double helix in the A-site.

REFERENCES

Adams, J. M., and Capecchi, M. R. (1966) N-Formylmethionyl-sRNA as the initiator of protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **55 :** 147-55.

AEvarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., al-Karadaghi, S., Svensson, L. A. and Liljas, A. (1994) Three-dimensional structure of the ribosomal translocase: elongation factor G from Thermus thermophilus. *EMBO J.* **13 :** 3669-77.

Agirrezabala, X., Liao, H.Y., Schreiner, E., Fu, J., Ortiz-Meoz, R.F., Schulten, K., Green, R. and Frank, J. (2012) Structural characterization of mRNA-tRNA translocation intermediates. *Proc. Natl. Acad. Sci. U. S. A.* **109 :** 6094-9

Agmon, I., Bashan, A., Zarivach, R. and Yonath, A. (2005) Symmetry at the active site of the ribosome: structural and functional implications. *Biol. Chem.* **386** : 833–844.

Agrawal, R.K., Penczek, P., Grassucci, R.A., Li, Y., Leith, A., Nierhaus, K.H. and Frank, J. (1996) Direct visualization of A-, P-, and E-site transfer RNAs in the Escherichia coli ribosome. *Science*, **271 :** 1000-2.

Agrawal, R.K., Penczek, P., Grassucci, R.A. and Frank, J. (1998) Visualization of elongation factor G on the Escherichia coli 70S ribosome: the mechanism of translocation. *Proc. Natl.Acad. Sci. U. S. A.* **95 :** 6134-8.

Ali, I.K., Lancaster, L., Feinberg, J., Joseph, S. Noller, H.F. (2006) Deletion of a conserved, central ribosomal intersubunit RNA bridge. *Mol. Cell*. **23** : 865–74.

Allen, D.W. and Zamecnik, P.C. (1962) The effect of puromycin on rabbit reticulocyte ribosomes. *Biochim. Biophys. Acta*. **55 :** 865-74

Allende, J. E., Monro, R. and F. Lipmann, F. (1964) Resolution of the E. coli amino acyl sRNA transfer factor into two complementary fractions. *Proc. Natl. Acad. Sci. U. S. A.* **51 :** 1211-6.

Allende, J.E. and Weissbach, H. (1967) GTP interaction with a protein synthesis initiation factor preparation from Escherichia coli. *Biochem. Biophys. Res. Commun.* **28 :** 82-88.

Allfrey, V., Daly, M.M and Mirsky, A.E. (1953) Synthesis of protein in the pancreas II. The role of ribonucleoprotein in protein synthesis. *J. Gen. Physiol.* **37:** 157–75.

An, G., Click, B.R., Friesen, J.D. and Ganoza, M.C. (1980) Identification and quantification of elongation factor EF-P in Escherichia coli cell-free extracts. *Can. J. Biochem.* **58 :** 1312-4.

Aoki, H., Adams, S.L., Chung, D.G., Yaguchi, M., Chuang, S.E. and Ganoza, M.C. (1991) Cloning, sequencing and overexpression of the gene for prokaryotic factor **EF-P** involved in peptide bond synthesis. *Nucleic Acids Res*. **19 :** 6215-20.

Aoki, H., Dekany, K., Adams, S.L. and Ganoza, M.C. (1997) The gene encoding the elongation factor P protein is essential for viability and is required for protein synthesis. *J. Biol. Chem*. **272 :** 32254-9

Aoki, H., Xu, J., Emili, A., Chosay, J.G., Golshani, A. and Ganoza, M.C. (2008) Interactions of elongation factor EF-P with the Escherichia coli ribosome. *FEBS J.* **275 :** 671-81

Arlinghaus, R., Favelukes, G. and Schweet, R. (1963) A ribosome bound intermediate in protein synthesis. *Biochem. Biophys. Res. Commun*. **11 :** 92-6.

Arlinghaus, R., Schaeffer, J. and Schweet, R. (1964) Mechanism of peptide bond formation in polypeptide synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **51 :** 1291-9

Armache, J.P. et al. (2010) Cryo-EM structure and rRNA model of a translating eukaryotic 80S ribosome at 5.5-A resolution. *Proc. Natl. Acad. Sci. U. S. A.* **107 :** 19748-53

Atherly, A.G. (1978) Peptidyl-transfer RNA hydrolase prevents inhibition of protein synthesis initiation. *Nature* **275** : 769.

Attardi, G. and Amaldi, F. (1970) Structure and synthesis of ribosomal RNA. *Annu Rev Biochem*. **39 :** 183-226.

Avery, O.T., MacLeod, C.M. and McCarty, M. (1944) Studies on the chemical nature of the substance inducing transformation of Pneumococcal types. *J. Exp. Med.* **79** : 137-59.

Bada, J.L. (2001) State-of-the-art instruments for detecting extraterrestrial life. *Proc. Natl. Acad. Sci. U. S. A.* **98** : 797–800

Ballestd, J.P.G. and Vazquez, D. (1974) Activities of ribosomal cores deprived of proteins L7, L10, L11 and L12. *FEES Lett.* **48:** 266-70.

Ban, N., Nissen, P., Hansen, J., Moore, P.B. and Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 A resolution. *Science* **289** : 905-20.

Barta, A., Steiner, G., Brosius, J., Noller, H.F. and Kuechler, E. (1984) Identification of a site on 23S ribosomal RNA located at the peptidyl transferase center. *Proc. Natl. Acad. Sci. U. S. A*. **81** : 3607-11.

Becker, H.D., Giegé, R. and Kern, D. (1996) Identity of prokaryotic and eukaryotic tRNA-Asp for aminoacylation by aspartyl-tRNA synthetase from Thermus thermophiles. *Biochemistry,* **35 :** 7447–58.

Beadle, G.W. (1945) Biochemical genetics. *Chem. Rev.* **37** : 15-96

Belbruno, E., Moro-Martin, A., Malhotra, R. and Savransky, D. (2012) Chaotic Exchange of Solid Material between Planetary Systems: Implications for Lithopanspermia. *Astrobiology* (on-line)

Belitsina, N.V., Tnalina, G.Z. and Spirin, A.S. (1981) Template-free ribosomal synthesis of polylysine from lysyl-tRNA. *FEBS Lett.* **131 :** 289–92.

Belitsina, N.V., Tnalina, G.Z. and Spirin, A.S. (1982) Template-free ribosomal synthesis of polypeptides from aminoacyl-tRNAs. *Biosystems* **15 :** 233–41.

Beljanski, M. and Ochoa, S. (1958) Protein biosynthesis by a cell-free bacterial system. *Proc. Natl. Acad. Sci. U. S. A*. **44 :** 494-501.

Belozersky, A.N and Spirin, A.S. (1958) A correlation between the compositions of deoxyribonucleic and ribonucleic acids. *Nature* **182 :** 111-2

Benner, S.A. and Ellington, A.D. (1991) RNA world. *Science* **252 :** 1232

Ben-Shem, A., Jenner, L., Yusupova, G. and Yusupov, M. (2010) Crystal Structure of the Eukaryotic Ribosome. *Science* **330** : 1203–9.

Berchtold, H., Reshetnikova, L., Reiser, C.O., Schirmer, N.K., Sprinzl, M. and Hilgenfeld, R. (1993) Crystal structure of active elongation factor Tu reveals major domain rearrangements. *Nature* **365 :** 126-32.

Berg, P. (1956), Acyl adenylates; the interaction of adenosine triphosphate and L-methionine. *J. BioI. Chem,* **222 :** 1025-34.

Berg, P. (1961) Specificity in protein synthesis. *Ann. Rev. Biochem.* **30 :** 293–324.

Bergemann, K. and Nierhaus, K.H. (1983) Spontaneous, elongation factor G independent translocation of Escherichia coli ribosomes. *J. Biol. Chem.* **258 :** 15105-13.

Berisd, H., Groner, Y. and Revei, M. (1971) Effect of a purified initiation factor F3 (B) on the selection of ribosomal binding sites on phage MS2 RNA. *Nature New Biol.* **234 :** 44-7.

Berisio, R., Schluenzen, F., Harms, J., Bashan, A., Auerbach, T., Baram, D. and Yonath, A. (2003) Structural insight into the role of the ribosomal tunnel in cellular regulation. *Nat. Struct. Biol.* **10 :** 366-70

Bishop, J., and Schweet, R. (1961) Participation of two enzyme fractions in the transfer of amino acids from amino acyl-RNA to reticulocyte ribosomes. *Biochim. Biophys. Acta*, **54 :** 617-9.

Bishop, J.O. (1966) Initiation of haemoglobin polypeptide chains. *Biochim Biophys Acta.* **119** : 130-45.

Blaha, G., Stanley, R.E. and Steitz, T.A. (2009) Formation of the first peptide bond: the structure of EF-P bound to the 70S ribosome. *Science* **325 :** 966-70.

Bohman, K., Ruusala, T., Jelanc, P.C., and Kurland, C.G. (1984) Kinetic impairment of restrictive streptomycin-resistant ribosomes. *Mol. Gen. Genet.* **198 :** 90-9.

Bokov K, Steinberg, S.V. (2009) A hierarchical model for evolution of 23S ribosomal RNA. *Nature* **457**: 977– 980

Borsook, H., Deasy, C.L., Haagen-Smit, A.J., Keighley, G., and Lowy, P. (1950) Metabolism of C^{14}-labeled glycine, L-histidine, L-Ieucine, and L-lysine. *J. Biol. Chem*. **187:** 839-48.

Boutorine, Y.I., Steinberg, S.V. (2012) Twist-joints and double twist-joints in RNA structure. *RNA.*, **18** : 2287-98

Brachet, J. (1941) La détection histochimique et le microdosage des acides pentosenucléique (tissus animaux- développement embryonnaire des amphibiens). *Enzymologia* **10 :** 87–96

Brachet, J. (1947) Nucleic acids in the cell and in the embryo. *Symp. Soc. Exp. Biol. Cambridge University press.* **1**: 207

Brachet, J., (1955) The biological role of pentose nucleic acid. (In Chargaff, E. and Davidson, J. N. (eds.), The Nucleic Acids – chemistry and biology, **II** : 475-519**,** *Academic Press Inc., New York*)

Branlant, C., Krol, A., Machatt, M.A., Pouyet, J., Ebel, J.P., Edwards, K. and Kössel, H. (1981) Primary and secondary structures of Escherichia coli MRE 600 23S ribosomal RNA. Comparison with models of secondary structure for maize chloroplast 23S rRNA and for large portions of mouse and human 16S mitochondrial rRNAs. *Nucleic Acids Res*. **9 :** 4303-24.

Brenner, S., Jacob, F. and Meselson, M. (1961) An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **190 :** 576-81.

Brenner, S., Beckwith, J.R. (1965) *Ochre* mutants, a new class of suppressible nonsense mutants. *J. Mol. Biol.* **13 :** 629-37.

Brenner, S., Barnett, L., Katz, E.R. and Crick, F.H. (1967) UGA: a third nonsense triplet in the genetic code. *Nature* **213 :** 449-50.

Bretscher, M.S. and Marcker, K.A. (1966) Polypeptidyl-sigma-ribonucleic acid and amino-acyl-sigma-ribonucleic acid binding sites on ribosomes. *Nature* **211 :** 380-4.

Bretscher, M.S. (1968) Translocation in protein synthesis: a hybrid structure model. *Nature* **218:** 675-7.

Bretscher, M.S. (1968b) Polypeptide chain termination: an active process. *J. Mol. Biol.* **34 :** 131-6.

Brimacombe, R., Stöffler, G. and Wittmann, H.G. (1978) Ribosome structure. *Annu Rev Biochem*. **47 :** 217-49.

Brosius, J., Palmer, M.L., Kennedy, P.J. and Noller, H.F. (1978) Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* **75 :** 4801-5.

Brosius, J, Dull, T.J. and Noller, H.F. (1980) Complete nucleotide sequence of a 23S ribosomal RNA gene from Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* **77 :** 201-4.

Brown, J.C. and Smith, A.E. (1970) Initiator codons in eukaryotes. *Nature* **226 :** 610-2.

Brownlee, G.G, Sanger, F. and Barrell, B.G. (1967) Nucleotide sequence of 5S-ribosomal RNA from Escherichia coli. *Nature* **215 :** 735-6.

Burkard, G., Eclancher, B. and Weil, J.H. (1969) Presence of N-formyl-methionyl-transfer RNA in bean chloroplasts. *FEBS Lett.* **4 :** 285-7.

Cabañas, M.J. and Modolell, J. (1980) Nonenzymic translocation and spontaneous release of noncognate peptidyl transfer ribonucleic acid from Escherichia coli ribosomes. *Biochemistry* **19 :** 5411-6.

Caldwell, P.C. and Hinshelwood, C. (1950). Some considerations on autosynthesis in bacteria**.** *J. Chem. Soc.* **1950 :** 3156-9.

Cammarano, P., Londei, P., Biagini, R., DeRosa, M. and Gambacorta, A. (1982) Characterization of the secondary structure features of *Escherichia coli, Caldariella acidophila* and mammalian ribosomal RNA species by chemical modification of sterically exposed bases. *Eur. J. Biochem.* **128 :** 297-307.

Cannon, M., Krug, R. and Gilbert, W. (1963) The binding of s-RNA by *Escherichia coli* ribosomes. *J. Mol Biol.* **7 :** 360-78.

Cannon, M. (1967) The ribosomal binding site for peptidyl-transfer-ribonucleic acid. *Biochem. J.* **104 :** 934-46.

Cannone, J.J. et al. (2002) The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. *BMC Bioinformatics* **3** : 2.

Capecchi, M.R. (1967) Polypeptide chain termination in vitro: isolation of a release factor. *Proc. Natl. Acad. Sci. U. S. A.* **58 :** 1144-51.

Carbon, P., Ehresmann, C., Ehresmann, B. and Ebel, J,P. (1979) The complete nucleotide sequence of the ribosomal 16-S RNA from Excherichia coli. Experimental details and cistron heterogeneities. *Eur. J. Biochem.* **100 :** 399-410.

Caskey, C.T., Redfield, B. and Weissbach, H. (1967) Formylation of guinea pig liver methionyl-sRNA. *Arch. Biochem Biophys.* **120 :** 119-23.

Caspersson, T. and Schultz, J. (1939) Pentose nucleotides in the cytoplasm of growing tissues, *Nature* **143 :** 602-3.

Caspersson, T. (1947) The relations between nucleic acid and protein synthesis*.* *Symp. Soc. Exp. Biol.* **1 :** 127-151

Cavalli-Sforza., L.L. and Edwards, A.W.F. (1967) Phylogenetic analysis. Models and estimation procedures. *Am. J. Hum. Genet*. **19 :** 233-257.

Chae, Y.B., Mazumder, R.and Ochoa, S. (1969) Polypeptide chain initiation in E. coli: isolation of homogeneous initiation factor E2 and its relation to ribosomal proteins. *Proc. Natl. Acad. Sci. U. S. A.* **62 :** 1181-8.

Chan, Y,L., Gutell, R., Noller, H.F. and Wool, I.G. (1984) The nucleotide sequence of a rat 18 S ribosomal ribonucleic acid gene and a proposal for the secondary structure of 18 S ribosomal ribonucleic acid. *J. Biol. Chem.* **259 :** 224-30.

Chantrenne, H. (1943) Association de la cytochrome-oxidase à des complexes sédimentables dans le cytoplasme vivante. *Acta Biologica Belgica* **III :** 99-102.

Chapeville, F., Lipmann, F., von Ehrenstein, G., Weisblum, B., Ray, W.J.Jr. and S. Benzer, S. (1962) On the role of soluble ribonucleic acid in coding for amino acids. *Proc. Natl. Acad. Sci. U. S. A*. **48** : 1086-92.

Cech, T.R. (2000) Structural biology. The ribosome is a ribozyme. *Science* **289 :** 878-9.

Chin, W., Kronenberg, H., Dee, P., Maloof. F. and Habener J. (1981) Nucleotide sequence of the mRNA encoding the pre-ɑ-subunit of mouse thyrotropin. *Proc. Natl. Acad. Sci. U. S. A.* **78 :** 5329-33.

Chuang, D. and Simpson, M.V. (1971) A translocation-associated ribosomal conformational change detected by hydrogen exchange and sedimentation velocity. *Proc. Natl. Acad. Sci. U. S. A.* **68 :** 1474-8

Clark, B.F.C. and Marcker, K.A. (1966) The role of N-formyl-methionyl-sRNA in protein biosynthesis. *J. Mol. Biol.* **17:** 394-406.

Claude, A. (1941) Particulate components of cytoplasm. (in: Genes and Chromosomes: Structure and Organization. *Cold Spring Harbor Symp. Quant. Biol.* **9 :** 263-71).

Claude, A. (1943) The constitution of protoplasm. *Science,* **97 :** 451-6.

Cohen, S.S. (1970) Are/were mitochondria and chloroplasts microorganisms? *Am. Sci.* **58 :** 281-9.

Collatz, E., Ulbrich, N., Tsurugi, K., Lightfoot, H.N., MacKinlay, W., Lin, A. and Wool, I.G. (1977) Isolation of eukaryotic ribosomal proteins. Purification and characterization of the 40 S ribosomal subunit proteins Sa, Sc, S3a, S3b, S5', S9, S10, S11, S12, S14, S15, S15', S16, S17, S18, S19, S20, S21, S26, S27' and S29. *J. Biol. Chem*. **252 :** 9071-80.

Colombo, B., Vesco, C. and Baglioni, C. (1968) Role of ribosomal subunits in protein synthesis in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* **61 :** 651-8.

Comb, D.G., Sarkar, N., Devallet, J. and Pinzino, C.J. (1965) Properties of transfer-like RNA associated with ribosomes. *J. Mol. Biol.* **12 :** 509-13.

Conway, T.W. and Lipmann, F. (1964) Characterization of a ribosome-linked guanosine triphosphatase in *Escherichia coli* extracts. *Proc. Natl. Acad. Sci. U. S. A.* **52** : 1462-9.

Cooper, D. and Gordon, J. (1969) Effect of aminoacyl transfer ribonucleic acid on competition between guanosine 5'-triphosphate and guanosine 5'-diphosphate for binding to a polypeptide chain elongation factor from Escherichia coli. *Biochemistry.* **8 :** 4289-92.

Crick, F.H., Griffith, J.S. and Orgel, L.E. (1957) Codes without commas. *Proc. Natl. Acad. Sci. U. S. A.* **43** : 416-21.

Crick, F.H. (1958) On protein synthesis. (in: The Biological Replication of Macromolecules. *Symposia of the Society for Experimental Biology,* (London) **XII** : 138–63)

Crick, F.H. (1966) Codon--anticodon pairing: the wobble hypothesis. *J. Mol. Biol*. **19 :** 548-55.

Crick, F. H. (1968) The origin of the genetic code. *J. Mol. Biol*. **38** : 367–369 .

Crick, F.H. (1970) Central dogma of molecular biology. *Nature,* **227** : 561-3.

Cukras, A.R., Southworth, D.R., Brunelle, J.L., Culver, G.M. and Green R. (2003) Ribosomal proteins S12 and S13 function as control elements for translocation of the mRNA:tRNA complex. *Mol. Cell* **12 :** 321-8.

Cuzin, F., Kretchmer, N., Greenberg, R.E., Hurwitz, R. and Chapeville, F. (1967) Enzymatic hydrolysis of N-substituted aminoacyl-tRNA. *Proc. Natl. Acad. Sci. U. S. A.* **58 :** 2079-86.

Czworkowski, J., Wang, J., Steitz, T.A. and Moore, P.B. (1994) The crystal structure of elongation factor G complexed with GDP, at 2.7 A resolution. *EMBO J.* **13 :** 3661-8.

De Duve, C. (1988) Transfer RNAs: the second genetic code. *Nature* **333 :** 117-8.

De Groot, N., Panet, A. and Lapidot, Y. (1968) Enzymatic hydrolysis of peptidyl-tRNA. *Biochem. Biophys. Res. Commun.* **31 :** 37-42.

De Groot, N., Groner, Y. and Lapidot, Y. (1969) Peptidyl-tRNA. VII. Substrate specificity of peptidyl-tRNA hydrolase. *Biochim. Biophys. Acta* **186 :** 286-96.

Demeshkina, N., Jenner, L., Westhof, E., Yusupov, M. and Yusupova, G. (2012) A new understanding of the decoding principle on the ribosome. *Nature* **484 :** 456-9.

De Moss, J.A. and Novelli, G.D. (1956) An amino acid dependent exchange between 32P-labeled inorganic pyrophosphate and ATP in microbial extracts. *Biochim. Biophys. Acta* **22** : 49-61.

Doherty, E.A., Batey, R.T., Masquida, B. and Doudna, J.A. (2001) A universal mode of helix packing in RNA. *Nat. Struct. Biol.* **8** : 339–343.

Doolittle, W.F. and Brown, J.R. (1994) Tempo, mode, the progenote, and the universal root. *Proc. Natl. Acad. Sci. U. S. A.* **91 :** 6721-8.

Doudna, J.A. and Rath, V.L. (2002) Structure and function of the eukaryotic ribosome: the next frontier. *Cell* **109** : 153–156.

Dounce, A.L. (1952) Duplicating mechanisms for peptide chain and nucleic acid synthesis. *Enzymologia* **15 :** 251–8.

Dounce, A.L. (1953) Nucleic acid template hypothesis. *Nature*, **172**: 541.

Dunkle, J.A. et al. (2011) Structures of the bacterial ribosome in classical and hybrid states of tRNA binding. *Science* **332 :** 981–4.

Dzionara, M., Kaltschmidt, E. and Wittmann, H.G. (1970) Ribosomal proteins. 8. Molecular weights of isolated ribosomal proteins of *Escherichia coli. Proc. Natl. Acad. Sci. U. S. A.* **67 :** 1909-13.

Ehrich, W.E., Drabkin, D.L. and Forman, C. (1949) Nucleic acids and the production of antibody by plasma cells. *J. Exp. Med*., **90** : 157-68.

Eperon, I.C., Anderson, S. and Nierlich, D.P. (1980) Distinctive sequence of human mitochondrial ribosomal RNA genes. *Nature,* **286 :** 460-7.

Esakova, O. and Krasilnikov, A.S. (2010) Of proteins and RNA: the RNase P/MRP family. *RNA* **16 :** 1725-47.

Falaschi, A., Adler, J. and Khorana, H.G. (1963) Chemically synthesized deoxypolynucleotides as templates for ribonucleic acid polymerase. *J. Biol. Chem*. **238** : 3080-5.

Fessenden, J. and Moldave, K. (1961) Evidence for two protein factors in the transfer of amino acids from soluble-RNA to ribonucleoprotein particles *Biochem. Biophys. Res. Commun*. **6 :** 232-5.

Firtel, R.A., Timm, R., Kimmel, A.R. and McKeown, M. (1979) Unusual nucleotide sequences at the 5' end of actin genes in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U. S. A* **76 :** 6206-10.

Forget, B.G. and Weissman, S.M. (1967) Nucleotide sequence of KB cell 5S RNA. *Science* **158 :** 1695-9.

Fox, J.L. and Ganoza, M.C. (1968) Chain termination in vitro. Studies on the specificity of amber and ochre triplets. *Biochem. Biophys. Res. Commun*. **32 :** 1064-70.

Frank, J., Penczek, P., Grassucci, R. and Srivastava, S. (1991) Three-dimensional reconstruction of the 70S Escherichia coli ribosome in ice: the distribution of ribosomal RNA. *J. Cell Biol.* **115 :** 597-605.

Frank, J. et al. (1995) A model of protein synthesis based on cryo-electron microscopy of the *E.coli* ribosome. *Nature* **376 :** 441-4.

Frank, J., Agrawal, R.K. (2000) A ratchet-like inter-subunit reorganization of the ribosome during translocation. *Nature* **406 :** 318-22.

Frank, J., Gao, H., Sengupta, J., Gao, N. and Taylor, D.J. (2007) The process of mRNA–tRNA translocation. *Proc. Natl. Acad. Sci. U. S. A* **104** : 19671–19678.

Frank, J. (2009) Single-particle reconstruction of biological macromolecules in electron microscopy-30 years. *Q. Rev. Biophys.* **42 :** 139-58.

Gagnon, M.G. and Steinberg, S.V. (2002) GU receptors of double helices mediate tRNA movement in the ribosome. *RNA*, **8 :** 873–7.

Galibert, F., Larsen, C.J., LeLong, J.C. and Boiron, M. (1965) RNA of low molecular weight in ribosomes of mammalian cells. *Nature*, **207 :** 1039-41.

Gamow, G., (1954) Possible relation between deoxyribonucleic acid and protein structures *Nature*, **173** : 318

Ganem, B. (1987) RNA world. *Nature* **328 :** 676.

Ganoza, M.C. and Nakamoto, T. (1966) Studies on the mechanism of polypeptide chain termination in cell-free extracts of *E. coli. Proc. Natl. Acad. Sci. U. S. A.* **55 :** 162-9.

Ganoza, M.C. and Aoki, H. (2000) Peptide bond synthesis: function of the efp gene product. *Biol Chem.* **381 :** 553-9.

Gao, H. *et al.* (2003).vStudy of the structural dynamics of the E coli 70S ribosome using real-space refinement. *Cell* **113** : 789–801.

Gao, Y.G., Selmer ,M., Dunham, C.M., Weixlbaumer, A., Kelley, A.C. and Ramakrishnan, V. (2009) The structure of the ribosome with elongation factor G trapped in the post-translocational state. *Science* **326 :** 694-9.

Gavrilova, L.P., Ivanov, D.A. and Spirin, A.S. (1966) Studies on the structure of ribosomes. 3. Stepwise unfolding of the 50 s particles without loss of ribosomal protein. *J Mol Biol.* **16 :** 473-89.

Gavrilova, L.P. and Smolyaninov, V.V. (1971) Study of the mechanism of translocation in ribosomes. 1. Poly- phenylalanine synthesis in Escherichia coli ribosomes without participation of guanosine-5'-triphosphate and protein translation factors. *Mol. Biol.* (Rusian) **5 :** 710-7.

Gavrilova, L.P. and Spirin, A.S. (1971b) Stimulation of "non-enzymic" translocation in ribosomes by p-chloromercuribenzoate. *FEBS Lett.* **17 :** 324-6.

Gavrilova, L.P. and Spirin, A.S. (1972) Mechanism of translocation in ribosomes. II. Activation of spontaneous (nonenzymic) translocation in ribosomes of Escherichia coli by p-chloromercuribenzoate. *Mol. Biol.* (Russian) **6 :** 248-54.

Gavrilova, L.P., Koteliansky, V.E. and Spirin, A.S. (1974) Ribosomal protein S12 and 'non-enzymatic' translocation. *FEBS Lett.* **45 :** 324-8.

Gavrilova LP, Spirin AS. (1974b) Interaction of SH-reagents with the ribosomal 30 S subparticle and 'non-enzymatic' translocation. *FEBS Lett.* **39 :** 13-6.

Gavrilova, L.P., Kostiashkina, O.E., Koteliansky, V.E., Rutkevich, N.M. and Spirin, A.S. (1976) Factor-free ("non-enzymic") and factor-dependent systems of translation of polyuridylic acid by Escherichia coli ribosomes. *J. Mol. Biol.* **101** : 537-52.

Geigenmüller, U. and Nierhaus, K.H. (1990) Significance of the third tRNA binding site, the E site, on E. coli ribosomes for the accuracy of translation: an occupied E site prevents the binding of non-cognate aminoacyl-tRNA to the A site. *EMBO J.* **9 :** 4527-33.

Ghosh, H.P. and Khorana, H.G. (1967) Studies on polynucleotides, LXXXIV. On the role of ribosomal subunits in protein synthesis. *Proc. Nat. Acad. Sci. U. S. A,* **58 :** 2455-61.

Gilbert, W. (1963) Polypeptide synthesis in Escherichia coli. II The polypeptide chain and sRNA., *J. Mol. Biol.* **6:** 389-403.

Gilbert, W. (1986) Origin of life: The RNA world. *Nature,* **319:** 618.

Glick, B.R. and Ganoza, M.C. (1975) Identification of a soluble protein that stimulates peptide bond synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **72 :** 4251-60.

Glick, B.R. and Ganoza, M.C. (1976) Characterization and site of action of a soluble protein that stimulates peptide-bond synthesis. *Eur. J. Biochem.* **71 :** 483-91.

Glick, B.R., Chladek, S. and Ganoza, M.C. (1979) Peptide bond formation stimulated by protein synthesis factor EF-P depends on the aminoacyl moiety of the acceptor. *Eur. J. Biochem.,* **97 :** 23–8.

Glotz C, Zwieb C, Brimacombe R, Edwards K, Kössel H. (1981) Secondary structure of the large subunit ribosomal RNA from Escherichia coli, Zea mays chloroplast, and human and mouse mitochondrial ribosomes. *Nucleic Acids Res.* **9 :** 3287-306.

Glukhova, M.A., Belitsina, N.V. and Spirin, A.S. (1975) A study of codon-dependent binding of aminoacyl-tRNA with the ribosomal 30-S subparticle of *Escherichia coli*. Determination of the active-particle fraction and binding constants in different media. *Eur J Biochem.,* **52 :** 197-202.

Gnirke, A., Geigenmüller, U., Rheinberger, H.J. and Nierhaus, L.H. (1989) The allosteric three-site model for the ribosomal elongation cycle. Analysis with a heteropolymeric mRNA. *J. Biol. Chem.* **264 :** 7291-301.

Goldstein, J.L., Beaudet, A.L. and Caskey, C.T. (1970) Peptide chain termination with mammalian release factor. *Proc. Natl. Acad. Sci. U. S. A.* **67 :** 99-106.

Goldstein, J., Milman, G., Scolnick, E. and Caskey, T. (1970b) Peptide chain termination. VI. Purification and site of action of S. *Proc. Natl. Acad. Sci. U. S. A.* **65 :** 430-7.

Gordon, J. (1967) Interaction of guanosine 5'-triphosphate with a supernatant fraction from *E. coli* and aminoacyl-sRNA. *Proc. Natl. Acad. Sci. U. S. A*. **58 :** 1574-8.

Gordon, J. (1968) A stepwise reaction yielding a complex between a supernatant fraction from *E. coli*, guanosine 5'-triphosphate, and aminoacyl-sRNA. *Proc. Natl. Acad. Sci. U. S. A.* **59 :** 179-83.

Gordon, J. (1969) Hydrolysis of guanosine 5'-triphosphate associated with binding of aminoacyl transfer ribonucleic acid to ribosomes. *J. Biol. Chem.* **244 :** 5680-6.

Gottesman, M.E. (1967) Reaction of ribosome-bound peptidyl transfer ribonucleic acid with aminoacyl transfer ribonucleic acid or puromycin. *J. Biol. Chem.* **242 :** 5564-71.

Grajevskaja, R.A, Ivanov, Y.V. and Saminsky, E.M. (1982) 70-S ribosomes of *Escherichia coli* have an additional site for deacylated tRNA binding. *Eur. J. Biochem.* **128 :** 47-52.

Gros, F., Hiatt, H., Gilbert, W., Kurland, C.G., Risebrough, R.W. and Watson, J.D. (1961) Unstable ribonucleic acid revealed by pulse labelling of Escherichia coli. *Nature* **190**: 581-5.

Grossi, L.G. and Moldave, K.(1959) Transfer of RNA-bound amino acids to microsomal proteins. *Biochim. Biophys. Acta* **35 :** 275-7.

Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N. and Altman, S. (1983) The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell* **35 :** 849-57.

Z. Guo, Z., Noller, H.F. (2012) Rotation of the head of the 30S ribosomal subunit during mRNA translocation *Proc. Natl. Acad. Sci. U. S. A.* **109** : 20391-4.

Gupta, R., Lanter, J.M. and Woese, C.R. (1983) Sequence of the 16S Ribosomal RNA from *Halobacterium volcanii,* an *Archaebacterium*. *Science.* **221 :** 656-9.

Gutell, R.R., Weiser, B., Woese, C.R. and Noller, H.F. (1985) Comparative anatomy of 16-S-like ribosomal RNA. *Prog. Nucleic. Acid. Res. Mol. Biol.* **32 :** 155-216.

Gutell, R.R., Larsen, N. and Woese, C.R. (1994) Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. *Microbiol. Rev.* **58 :** 10–26.

Hadjiolov, A.A., Georgiev, O.I., Nosikov, V.V. and Yavachev, L.P. (1984) Primary and secondary structure of rat 28S ribosomal RNA. *Nucleic Acids Res.* **12 :** 3677-93.

Haenni, A.L., Lucas-Lenard, J. (1968) Stepwise synthesis of a tripeptide. *Proc. Natl. Acad. Sci.*

U. S. A. **61 :** 1363-9.

Hall, B.D. and Doty, P. (1959) The preparation and physical chemical properties of ribonucleic acid from microsomal particles. *J. Mol. Biol.* **1**: 111-126

Hanawa-Suetsugu, K., Sekine, S., Sakai, H., Takaho, T., Unzai, S., Tame, J.R.H., Shirouzu, M. and Yokoyama, S. (2004) Crystal structure of the elongation factor P from *Thermus thermophilus. Proc. Natl. Acad. Sci. U. S. A.* **101 :** 9595–600.

Hansen, J.L., Schmeing, T.M., Moore, P.B. and Steitz, T.A. (2002) Structural insights into peptide bond formation. *Proc. Natl Acad. Sci. U. S. A.* **99** : 11670–11675.

Hardesty, B., Arlinghaus, R., Shaeffer, J. and Schweet, R. (1963) Hemoglobin and polyphenylalanine synthesis with reticulocyte ribosomes. (in : Synthesis and Structure of Macromolecules: *Cold Spring Harbor Symp. Quant. Biol.* **28 :** 215-223).

Harms, J. et al. (2001) High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell* **107** : 679–688.

Hausner, T.P., Geigenmüller, U. and Nierhaus, K.H. (1988) The allosteric three-site model for the ribosomal elongation cycle. New insights into the inhibition mechanisms of aminoglycosides, thiostrepton, and viomycin. *J. Biol. Chem.* **263 :** 13103-11.

Hawtrey, A.O. (1964) A possible mechanism for peptide bond formation during amino-acid transfer from aminoacyl-*s*RNA to protein. *Nature* **202 :** 1179-81.

Hayashi, H. and Miura, K.I. (1966**)** Anticodon sequence as a possible site for the activity of Transfer RNA. (in : The Genetic Code. *Cold Spring Harb Symp Quant Biol* **31 :** 63-70).

Held, W.A., Ballou, B., Mizushima, S. and Nomura, M. (1974) Assembly mapping of 30 S ribosomal proteins from *Escherichia coli.* Further studies. *J. Biol. Chem.* **249 :** 3103-11.

Hershey, J.W., Dewey, K.F. and Thach, R.E. (1969) Purification and properties of initiation factor f-1. *Nature.* **222 :** 944-7.

Heywood, S.M. (1970) Formation of the initiation complex using muscle messenger RNAs. *Nature.* **225 :** 696-8.

Hill, T.L. (1969) A proposed common allosteric mechanism for active transport, muscle contraction, and ribosomal translocation. *Proc. Natl. Acad. Sci. U. S. A.* **64 :** 267-74.

Hirashima, A. and Kaji, A. (1972) Purification and properties of ribosome-releasing factor. *Biochemistry* **11 :** 4037-44.

Hirashima, A., and Kaji, A. (1973) Role of elongation factor G and a protein factor on the release of ribosomes from messenger ribonucleic acid. *J. Biol. Chem.* **248 :** 7580-7.

Hoagland, M.B. (1955) An enzymic mechanism for amino acid activation in animal tissues. *Biochim. Biophys. Acta* **16** : 288-9.

Hoagland, M.B., Keller, E.B. and Zamecnik, P.C. (1956) Enzymatic carboxyl activation of amino acids. *J. Biol. Chem*., **218 :** 345-58.

Hoagland, M.B., Zamecnik, P.C. and M. L. Stephenson, M.L. (1957) Intermediate reactions in protein biosynthesis., *Biochim. et Biophys. Acta*, **24** : 215-6.

Hoagland, M.B., Stephenson, M.L., Scott, J.F., Hecht, L.I. and Zamecnik, P.C. (1958) A soluble ribonucleic acid intermediate in protein synthesis. *J. Biol. Chem.* **231** : 241-57.

Hoagland, M.B. (1958b) Amino-acid activation by individual enzymes. *Proc. R. Soc. Lond. B. Biol. Sci.* **149** : 401-2.

Hoagland, M.B., Stephenson, M.L., Scott, J.F., Hecht, L.I. and Zamecnik, P.C. (1958c) A soluble ribonucleic acid intermediate in protein synthesis. *J. Biol. Chem.* **231 :** 241-57.

Hoagland, M.B., Zamecnik, P.C. and Stephenson, M.L. (1959) A hypothesis concerning the roles of particulate and soluble ribonucleic acids in protein synthesis. (in : Symposium on Molecular Biology. ed. by E.R. Zirkle. *Chicago: University of Chicago Press* : 105-14).

Hoagland, M.B., and Comly, L.T. (1960) Interaction of soluble ribonucleic acid and microsomes. *Proc. Nail. Acad. Sci. U. S. A.* **46** : 1554-63.

Holley, R.W., Apgar, J., Everett, G.A., Madison, J.T., Marquisee, M., Merrill, S.H, Penswick, J.R. and Zamir, A. (1965) Structure of a ribonucleic acid. *Science* **147** : 1462-5**.**

Hopfield, J.J. (1974) Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. *Proc. Natl. Acad. Sei. U. S.* A. 71 : 4135-9.

Horan, L.H. and Noller, H.F. (2007) Intersubunit movement is required for ribosomal translocation. *Proc. Natl. Acad. Sci. U. S. A.* **104** : 4881–5.

Hou, Y.M. and Schimmel, P. (1988). A simple structural feature is a major determinant of the identity of a transfer RNA. *Nature* **333 :** 140-5.

Housman, D., Jacobs-Lorena, M., Rajbhandary, U.L. and Lodish, H.F. (1970) Initiation of haemoglobin synthesis by methionyl-tRNA. *Nature* **227 :** 913-8.

Howard, G.A. and Gordon, J. (1974) Peptidyl transferase activity of ribosomal particles lacking protein L11. *FEBS Lett.* **48 :** 271-4.

Howard, M.J., Lim, W.H., Fierke, C.A. and Koutmos, M. (2012) Mitochondrial ribonuclease P structure provides insight into the evolution of catalytic strategies for precursor-tRNA 5' processing. *Proc. Natl. Acad. Sci. U. S A.* **109 :** 16149-54.

Hsiao, C., Mohan, S., Kalahar, B.K. and Williams, L.D. (2009) Peeling the onion: ribosomes are ancient molecular fossils. *Mol. Biol. Evol.* **26 :** 2415-25.

Hultin, T. (1950) Incorporation in vivo of N^{15}-labeled glycine into liver fractions of newly hatched chicks. *Exp. Cell Research* **1** : 376-81.

Hury, J., Nagaswami, U., Larios-Sanz, M., Fox, G.E. (2006) Ribosome origins: The relative age of 23S rRNA Domains. *Orig. Life Evol. Biosph.* **36 :** 421–9.

Huxley, H.E. and Zubay, G. (1960). Electron microscope observations on the structure of microsomal particles from *Escherichia coli. J. Mol. Biol.* **2 :** 10–8.

Ibuki, F., Gasior, E. and Moldave, K. (1966) The interaction of aminoacyl soluble ribonucleic acid and aminoacyl transferase I. *J. Biol. Chem.* **241 :** 2188-93.

Jacob, F. and Monod, J. (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol*. **3** : 318-56.

Jacobson, K.B., Nishimura, S., Barnett, W.E., Mans, R.J., Cammarano, P. and Novelli, G.D. (1964) On the lack of a uniform specificity of aminoacyl ribonucleic acid synthetases from different organisms. *Biochim. Biophys. Acta* **91 :** 305-12.

Jackson, R. and Hunter, T. (1970) Role of methionine in the Initiation of haemoglobin synthesis. *Nature*, **227 :** 672-6

Janosi, L., Shimizu, I., and Kaji, A. (1994). Ribosome recycling factor (ribosome releasing factor) is essential for bacterial growth. *Proc. Natl. Acad. Sci. U. S. A.* **91 :** 4249–53.

Jeener, R. and Brachet, J. (1941) Association, dans un même granule, de ferments et des pentosenucléoprotéides cytoplasmiques, *Acta Biologica Belgica* **I** : 476-81

Jeener, R. and Brachet, J. (1942) Sur la présence d'hormones protéiques et d'hémoglobine dans Les granules a pentosenucléoprotéides. *Acta Biologica Belgica* **II :** 447-50.

Jenner, L.B., Demeshkina, N., Yusupova, G. and Yusupov, M. (2010) Structural aspects of messenger RNA reading frame maintenance by the ribosome. *Nat. Struct. Mol. Biol*. **17** : 555-60.

Jerez, C., Sandoval, A., Allende. J., Henes, C. and Ofengand, J. (1969) Specificity of the interaction of aminoacyl ribonucleic acid with a protein-guanosine triphosphate complex wheat embryo. *Biochemistry* **8 :** 3006-3014

Jin, H., Kelley, A.C., Loakes, D. and Ramakrishnan, V. (2010) Structure of the 70S ribosome bound to release factor 2 and a substrate analog provides insights into catalysis of peptide release. *Proc. Natl. Acad. Sci. U. S. A.* **107 :** 8593-8.

Jin, H., Kelley, A.C. and Ramakrishnan, V. (2011) Crystal structure of the hybrid state of ribosome in complex with the guanosine triphosphatase release factor 3. *Proc. Natl. Acad. Sci. U. S. A.* **108 :** 15798-803.

Joyce, G.F. (1989) RNA evolution and the origins of life. *Nature* **338 :** 217-24.

Joyce, G.F. (2002) The antiquity of RNA-based evolution. *Nature* **418 :** 214-21.

Julián, P., Konevega, A.L., Scheres, S.H., Lázaro, M., Gil, D., Wintermeyer, W., Rodnina. M,V. and Valle, M. (2008) Structure of ratcheted ribosomes with tRNAs in hybrid states. *Proc. Natl. Acad. Sci. U. S. A.* **105 :** 16924-7.

Kaempfer, R. (1968) Ribosomal subunit exchange during protein synthesis. *Proc. Natl. Acad. Sci. U. S. A*. **61 :** 106-13.

Kaempfer, R. (1969) Ribosomal subunit exchange in the cytoplasm of a eukaryote. *Nature*. **222 :** 950-3.

Kaltschmidt, E., Dzionara, M. and Wittmann, H.G. (1970a) Ribosomal proteins. XV. Amino acid compositions of isolated ribosomal proteins from 30S and 50S subunits of *Escherichia coli. Mol. Gen. Genet.* **109 :** 292-7.

Kaltschmidt, E. and Wittmann, H.G. (1970b) Ribosomal proteins. XII. Number of proteins in small and large ribosomal subunits of Escherichia coli as determined by two-dimensional gel electrophoresis. *Proc. Natl. Acad. Sci. U. S. A.* **67 :** 1276-82.

Karimi, R., Pavlov, M., Buckingham, R. and Ehrenberg, M. (1999) Novel roles for classical factors at the interface between translation termination and initiation. *Mol. Cell* **3 :** 601-9.

Kavran, J.M. and Steitz, T.A. (2007) Structure of the base of the L7/L12 stalk of the Haloarcula marismortui large ribosomal subunit: analysis of L11 movements. *J. Mol. Biol*. **371 :** 1047–1059.

Keller, E.B., Zamecnik, P.C. and Loftfield, R.B. (1954) The role of microsomes in the incorporation of amino acids into proteins. *J. Histochem. Cytochem.* **2:** 378-86.

Kemkhadze, K.S., Odintsov, V.B., Semenkov, Y.P. and Kirillov, S.V. (1981) Quantitative study of the interaction of aminoacyl-tRNA with the a site of Escherichia coli ribosomes:

equilibrium and kinetic parameters of binding in the absence of EF-Tu factor and GTP. *FEBS Lett.* **125 :** 10-4.

Khorana, H.G., et al. (1966) Polynucletide Synthesis and the Genetic Code. (in : The Genetic Code. *Cold Spring Harbor Symp . Quant. Biol.* **31** : 39-49).

Kim, D.F. and Green, R. (1999) Base-pairing between 23S rRNA and tRNA in the ribosomal A site. *Mol. Cell* **4** : 859–864.

Kirillov, S.V., Makarov, E.M. and Semenkov, Y.P. (1983) Quantitative study of interaction of deacylated tRNA with Escherichia coli ribosomes. Role of 50 S subunits in formation of the E site. *FEBS Lett.* **157 :** 91-4.

Kiselev, L.L. and Frolova, L.Y. (1964) "Contact areas" of transport RNA in specific interaction with aminoacyl-rna-synthetases. *Biokhimiia.* (Russian) **29 :** 1177-89.

Klein, D.J., Schmeing, T.M., Moore, P.B. and Steitz, T.A. (2001) The kink-turn: a new RNA secondary structure motif. *EMBO J.* **20 :** 4214-21.

Klein, D.J., Moore, P.B. and Steitz, T.A. (2004) The contribution of metal ions to the structural stability of the large ribosomal subunit. *RNA* **10 :** 1366-79.

Klein, D.J., Moore, P.B. and Steitz, T.A. (2004b) The roles of ribosomal proteins in the structure assembly, and evolution of the large ribosomal subunit. *J Mol Biol.* **340 :** 141-77.

Köchel, H.G. and Küntzel, H. (1982) Mitochondrial L-rRNA from Aspergillus nidulans: potential secondary structure and evolution. *Nucleic Acids Res.* **10 :** 4795-801.

Kolakofsky, D., Dewey, K.F., Hershey, J.W. and Thach, R.E. (1968) Guanosine 5'-triphosphatase activity of initiation factor f2. *Proc. Natl. Acad. Sci. U. S. A.* **61 :** 1066-70.

Korostelev, A., Zhu, J., Asahara, H. and Noller, H.F. (2010) Recognition of the amber UAG stop codon by release factor RF1. *EMBO J.* **29 :** 2577-85.

Kössel, H. and RajBhandary, U.L. (1968) Studies on polynucleotides. LXXXVI. Enzymic hydrolysis of *N*-acylaminoacyl-transfer RNA. *J. Mol. Biol.* **35** : 539–60.

Kössel, H. (1969) Purification and properties of peptidyl-tRNA **hydrolase** from *Escherichia coli*. *Biochim. Biophys. Acta* **204** : 191–202.

Kozak, M. (1981) Possible role of flanking nucleotides in recognition of the AUG initiator codon by eukaryotic ribosomes. *Nucleic Acids Res.* **9 :** 5233-52.

Kozak, M. (1981b) Mechanism of mRNA recognition by eukaryotic ribosomes during initiation of protein synthesis. *Curr. Top. Microbiol. Immunol.* **93 :** 81-123.

Krayevsky, A.A., Kukhanova, M.K. and Gottikh, B.P. (1975) Peptidyl transferase centre of bacterial ribosomes: substrate specificity and binding sites. *Nucleic Acids Res*. **2 :** 2223-36.

Krisko, I., Gordon, J. and Lipmann, F. (1969) Studies on the interchangeability of one of the mammalian and bacterial supernatant factors in protein biosynthesis. *J Biol Chem.* **244 :** 6117-23.

Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E. and Cech, T.R. (1982) Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of *Tetrahymena*. *Cell* **31 :** 147-57.

Kuriki, Y. and Kaji, A. (1967) Specific binding of sRNA to ribosomes during polypeptide synthesis and the nature of peptidyl sRNA. *J. Mol. Biol.* **25 :** 407-23.

Kurland, C.G., Voynow, P., Hardy, S.J., Randall, L. and Lutter, L. (1969) Physical and functional heterogeneity of E. coli ribosomes. (in : The Mechanism of Protein Synthesis. *Cold Spring Harb Symp Quant Biol.* **34 :** 17-24).

Kurland, C.G. and Ehrenberg, M. (1984) Optimization of translation accuracy. *Prog. Nucleic Acid Res. Mol. Biol.* **31 :** 191-219.

Lamborg, M.R. and Zamecnik, P.C. (1960) Amino acid incorporation into protein by extracts of *E. coli. Biochim. Biophys. Acta*. **42** : 206-11.

Leder, P. and Nau, M.M. (1967) Initiation of protein synthesis. 3. Factor-GTP-codon-dependent binding of F-met-tRNA to ribosomes. *Proc. Natl. Acad. Sci. U. S. A*. **58 :** 774-81.

Leijonmarck, M., Eriksson, S. and Liljas, A. (1980) Crystal structure of a ribosomal component at 2.6A resolution. *Nature* **286:** 824-6.

Lelong, J.C., Grunberg-Manago, M., Dondon, J., Gros, D. and Gros, F. (1970) Interaction between guanosine derivatives and factors involved in the initiation of protein synthesis. *Nature* **226 :** 505-10.

Lengyel, P., Speyer, J.F. and Ochoa, S. (1961) Synthetic polynucleotides and the amino acid code. *Proc. Natl. Acad. Sci. U. S. A*. **47 :** 1936-42.

Lengyel, P. (1966) Problems in protein biosynthesis. *J. Gen. Physiol*. **49 :** 305-30.

Lerman, M.I., Spirin, A.S., Gavrilova, L.P. and Golov, V.F. (1966) Studies on the structure of ribosomes. II. Stepwise dissociation of protein from ribosomes by caesium chloride and the re-assembly of ribosome-like particles. *J. Mol. Biol.* **15 :** 268-81.

Lill, R., Robertson, J.M. and Wintermeyer, W. (1984) tRNA binding sites of ribosomes from *Escherichia coli. Biochemistry* **23 :** 6710-7.

Lipmann, F. et al. (1958) Symposium on amino acid activation. Chairman's introduction: some facts and problems. *Proc. Natl. Acad. Sci. U. S. A.* **44** : 67-73.

Littauer, U.Z., Kornberg, A. (1957) Reversible synthesis of polyribonucleotides with an enzyme from *Escherichia coli*. *J Biol Chem*., **226 :** 1077-92.

Littauer, U.Z. and Eisenberg, H. (1959) Ribonucleic acid from *Escherichia coli* preparation, characterization and physical properties. *Biochim. Biophys. Acta* **32:** 320–37.

Littauer, U.Z. (1961) Biochemical activity and structural aspects of ribonucleic acids. (in : Harris, R.J.C. ed., Symposium on Protein Biosynthesis. *Academic Press Inc. London, Wassenaar* : 143–62)

Littlefield, J.W, Keller, E.B and Gross, J. (1955) Studies on cytoplasmic ribonucleoprotein particles from the liver of the rat. *J. Biol. Chem.* **217** : 111-24.

Lockingen, L.S and DeBusk, A.G. (1955) A model for intracellular transfer of DNA (gene) specificity. *Proc. Natl. Acad. Sci. U. S A.* **41** : 925-934.

Loftfield, R.B. (1957) The biosynthesis of protein. (in : Butler, J.A.V. and Katz, B., eds., Progress in Biophysics and Biophysical Chemistry. Vol.**8**. *Pergamon Press, London*)

Loftfield, R.B. (1972) The mechanism of aminoacylation of transfer RNA. *Prog. Nucleic Acid Res. Mol.* Biol. **12 :** 87-128.

Lucas-Lenard, J. and Lipmann, F. (1966) Separation of three microbial amino acid polymerization factors. *Proc. Natl. Acad. Sci. U. S. A*. **55 :** 1562-6.

Lucas-Lenard, J., Tao, P. and Haenni, A.L. (1969) Further studies on bacterial polypeptide elongation. (in : The Mechanism of Protein Synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34 :** 455-62.)

Lucas-Lenard, J. (1971) Protein biosynthesis. *Annu. Rev. Biochem*. **40 :** 409-48.

Maden, B.E., Traut, R.R. and Monro, R.E. (1968) Ribosome-catalysed peptidyl transfer: the polyphenylalanine system. *J. Mol. Biol.* **35 :** 333-45.

Maeda, A., (1961) Some properties of ribonucleic acid from yeast 80S particle; effect of magnesium ions. *J. Biochem.* **60 :** 377-85.

Marcker, K.A. and Sanger, F. (1964) N-formyl-methionyl-S-RNA. *J. Mol. Biol.* **8 :** 835-40.

Man'kin, A.S., Kopylov, A.M, Rubtsov, P.M. and Skriabin, K.G. (1981) Model of the secondary structure of eukaryotic ribosomal 18S rRNA. *Dokl. Akad. Nauk SSSR.* (Russian) **256** : 1006-10.

Marcot-Queiroz, J., Julien, J., Rosset, R. and Monier, R. (1965) Les acides ribonucléiques des ribosomes de la levure. *Bull. Soc. Chim. Biol.* **47 :** 183-94.

McClain, W.H. and Foss, K. (1988) Changing the identity of a tRNA by introducing a G-U wobble pair near the 39 acceptor end. *Science* **240 :** 793–6.

McKeown, M., and Firtel R.A. (1981) Differential expression and 5' end mapping of actin genes in *Dictyostelium*. *Cell* **24 :** 799-807.

Mears, J.A., Cannone, J.J., Stagg, S.M., Gutell, R.R., Agrawal, R.K. and Harvey, S.C. (2002) Modeling a minimal ribosome based on comparative sequence analysis. *J. Mol. Biol.* **321 :** 215-34.

Mears, J.A., Sharma, M.R., Gutell, R.R., McCook, A.S., Richardson, P.E., Caulfield, T.R., Agrawal, R.K. and Harvey, S.C. (2006) A structural model for the large subunit of the mammalian mitochondrial ribosome. *J. Mol. Biol.* **358 :** 193-212.

Menninger, J.R., Mulholland, M.C. and Stirewalt, W.S. (1970) Peptidyl-tRNA hydrolase and protein chain termination. *Biochim Biophys Acta.* **217 :** 496-511.

Menninger, J.R. (1976) Peptidyl-transfer RNA dissociates during protein synthesis from ribosomes of *Escherichia coli*. *J. Biol. Chem.* **251** : 3392-8.

Menninger, J.R. (1978) The accumulation as peptidyl-transfer RNA of isoaccepting transfer RNA families in *E.coli* with temperature-sensitive peptidyl-tRNA hydrolase. *J. Biol. Chem.* **253** : 6808-13.

Menninger, J.R. (1979) Accumulation of peptidyl-tRNA is lethal to *Escherichia coli*. *J. Bacteriol.* **137** : 694–6.

Miall, S.H., Kato, T. and Tamaoki, T. (1970) A factor promoting dissociation of Escherichia coli ribosomes. *Nature* **226 :** 1050-2.

Michot, B., Bachellerie, J.P., Raynal, F. and Renalier, M.H. (1982) Homology of the 5'-terminal sequence of 28 S rRNA of mouse with yeast and Xenopus. Implication for the secondary structure of the 5.8S - 28S RNA complex. *FEBS Lett.* **140 :** 193-7.

Miller, R.L. and Schweet, R. (1968) Isolation of a protein fraction from reticulocyte ribosomes required for de novo synthesis of hemoglobin. *Arch. Biochem. Biophys*. **125 :** 632-46.

Miller, D.L. and Weissbach H. (1969) An interaction between the transfer factors required for protein synthesis. *Arch. Biochem. Biophys.* **132 :** 146-50.

Miller, D.L. and Weissbach, H. (1970) Interactions between the elongation factors: the displacement of GPD from the TU-GDP complex by factor Ts. *Biochem. Biophys. Res. Commun.* **38 :** 1016-22.

Milman, G., Goldstein, J., Scolnick, E. and Caskey, T. (1969) Peptide chain termination. 3. Stimulation of in vitro termination. *Proc. Natl. Acad. Sci. U. S. A.* **63 :** 183-90.

Mizushima, S. and Nomura, M. (1970) Assembly mapping of 30S ribosomal proteins from *E. coli. Nature.* **226 :** 1214-8.

Moazed, D. and Noller, H.F. (1989) Intermediate states in the movement of transfer RNA in the ribosome. *Nature* **342** : 142-8.

Moazed, D. and Noller, H.F. (1989b) Interaction of tRNA with 23S rRNA in the ribosomal A, P, and E sites. *Cell* **57 :** 585-97.

Monro, R.E. and Marcker, K.A. (1967) Ribosome- catalysed reaction of puromycin with a formylmethionine-containing oligonucleotide. *J. Mol. Biol.* **25 :** 347-50.

Monro, R.E. (1967b) Catalysis of peptide bond formation by 50 S ribosomal subunits from *Escherichia coli. J. Mol. Biol.* **26 :** 147-51.

Monro, R.E., Cerná. J. and Marcker, K.A. (1968) Ribosome-catalyzed peptidyl transfer: substrate specificity at the P-site. *Proc. Natl. Acad. Sci. U. S. A.* **61 :** 1042-9.

Montgomery, D.L., Leung, D.W., Smith, M., Shalit, P., Faye, G. and Hall, B.D. (1980) Isolation and sequence of the gene for iso-2-cytochrome c in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. USA* **77 :** 541-5.

Moore, V.G., Atchison, R.E., Thomas, G., Moran, M. and Noller, H.F. (1975) Identification of a ribosomal protein essential for peptidyl transferase activity. Proc. Natl. Acad. Sci. U. S. A. **72 :** 844-8.

Moore, P. (1988) The ribosome returns. *Nature*, **331 :** 223-7.

Moore, P.B. (1993) Ribosomes and the RNA World. (in : R.F.Gesteland and J.F.Atkins, eds., The RNA World. *Cold Spring Harbor Laboratory Press, Cold Spring Harbor*).

Moras, D. (1993) Structural aspects and evolutionary implications of the recognition between tRNAs and aminoacyl-tRNA synthetases. *Biochimie*. **75 :** 651-7.

Nakamoto, T., Conway, T., Allende, J., Spyrides, G. and Lipmann, F. (1963) Formation of peptide bonds-I Peptide formation from aminoacyl-S-RNA. (in : Synthesis and Structure of Macromolecules. *Cold Spring Harbor Symposia on Quantitative Biology* **28 :** 227-31).

Nakatogawa, H. and Ito, K. (2002) The ribosomal exit tunnel functions as a discriminating gate. *Cell.* **108 :** 629-36.

Nathans, D. and Lipmann, F. (1961) Amino acid transfer from aminoacyl-ribonucleic acids to protein on ribosomes of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **47 :** 497-504.

Nazar, R.N., Sitz, T.O. and Busch, H. (1975) Homologies in eukaryotic **5.8S** ribosomal **RNA**. *Biochem. Biophys. Re.s Commun.* **62 :** 736-43.

Nazar, R.N., Sitz, T.O. and Busch, H. (1975b) Structural analyses of mammalian ribosomal ribonucleic acid and its precursors. Nucleotide sequence of ribosomal **5.8S** ribonucleic acid. *J. Biol. Chem.* **250 :** 8591-7.

Nazar, R.N. and Roy, K.L. (1976) The nucleotide sequence of turtle **5.8S** rRNA. *FEBS Lett.* **72 :** 111-6.

Nikulin, A. et al. (2003) Structure of the L1 protuberance in the ribosome. *Nat. Struct. Biol.* **10** : 104–108.

Nierhaus, K.H. and Montejo, V. (1973) A protein involved in the peptidyltransferase activity of Escherichia coli ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* **70 :** 1931-5.

Nierhaus, K.H., Montejo, V., Nierhaus, D., Diertich, S. and Schrandt, I. (1974) Proteins at the peptidyltransferase center from *E. coli* ribosomes. *Acta. Biol. Med. Ger.* **33 :** 613-9.

Nierhaus, K.H. (1990) The allosteric three-site model for the ribosomal elongation cycle: features and future. *Biochemistry,* **29 :** 4997-5008.

Ninio, J. (1974) A semi-quantitative treatment of missense and nonsense suppression in the strA and ram ribosomal mutants of *Escherichia coli*. Evaluation of some molecular parameters of translation in vivo. *J. Mol. Biol.* **84 :** 297-313.

Ninio, J. (1975) Kinetic amplification of enzyme discrimination. *Biochimie,* **57 :** 587–95.

Nirenberg, M.W. and Matthei, J.A. (1961) The dependence of cell- free protein synthesis in *E.coli* upon naturally occurring or synthetic polyribonucleotides. *Proc. Nail. Acad. Sci. U. S. A*. **47** :1588-1602.

Nirenberg, M.W., Matthaei, J.H., Jones, O.W., Martin, R.G. and Barondes, S.H. (1963) Approximation of genetic code via cell-free protein synthesis directed by template RNA. *Fed. Proc*. **22**: 55-61

Nirenberg, M.W., Leder, P., Bernfield, M.R., Brimacombe, R.L., Trupin, J.S., Rottman, F.M.

and O'Neal, C. (1965) RNA codewords and protein synthesis, VII. On the general nature of the RNA code. *Proc. Natl. Acad. Sci. U. S. A*. **53** : 1161-8.

Nishimura, S., Jacob, T.M. and Khorana, H.G. (1964) Synthetic deoxyribopolynucleotides as templates for ribonucleic acid polymerase: the formation and characterization of a ribopolynucleotide with a repeating trinucleotide sequence. *Proc. Natl. Acad. Sci. U. S. A*. **52 :** 1494-501.

Nishizuka, Y. and Lipmann, F. (1966a) The interrelationship between guanosine triphosphatase and amino acid polymerization. *Arch. Biochem. Biophys*. **116** : 344-51.

Nishizuka, Y. and Lipmann, F. (1966b) Comparison of guanosine triphosphate split and polypeptide synthesis with a purified E. coli system. *Proc. Natl. Acad. Sci. U. S. A.* **55 :** 212-219

Nismann, B., Bergmann, F.H. and Berg, P. (1957) Observations on amino acid-dependent exchanges of inorganic pyrophosphate and ATP. *Biochim. Biophys. Acta*, **26** : 639-40.

Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F., Nyborg, J. (1995) Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-Tu, and a GTP analog. *Science* **270 :** 1464-72.

Nissen, P., Ban, N., Hansen, J., Moore, P.B., and Steitz, T.A. (2000) The structural basis of ribosome activity in peptide bond synthesis. *Science* **289 :** 920–30.

Nissen, P., Ippolito, J.A., Ban, N., Moore, P.B. and Steitz, T.A. (2001) RNA tertiary interactions in the large ribosomal subunit: the **A**-minor motif. *Proc. Natl. Acad. Sci. U. S. A.* **98 :** 4899-903.

Noller, H.F. et al. (1981) Secondary structure model for 23S ribosomal RNA. *Nucleic Acids Res*. **9 :** 6167-89.

Noller, H.F., Hoffarth, V. and Zimniak, L. (1992) Unusual resistance of peptidyl transferase to protein extraction procedures. *Science* **256** : 1416–1419.

Noller, H.F. (2004) The driving force for molecular evolution of translation. *RNA* **10 :** 1833-7.

Nomura, M. and Hosokawa, K. (1965) Biosynthesis of ribosomes: fate of chloramphenicol particles and of pulse-labeled RNA in *Escherichia coli*. *J. Mol. Biol.* **12 :** 242-65.

Nomura, M. and Lowry, C.V. (1967a) Phage F2 RNA-directed binding of formylmethionyl-tRNA to ribosomes and the role of 30S ribosomal subunits in initiation of protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **58 :** 946-53.

Nomura, M., Lowry, C.V. and Guthrie, C. (1967b) The initiation of protein synthesis: joining of the 50S ribosomal subunit to the initiation complex. *Proc. Natl. Acad. Sci. U. S. A.* **58 :** 1487-93.

Nomura, M., Mizushima, S., Ozaki, M., Traub, P. and Lowry, C.V. (1969) Structure and function of ribosomes and their molecular components. (in : The Mechanism of Protein Synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34 :** 49-61).

Normanly, J., Ogden, R.C., Horvath, S.J. and Abelson, J. (1986) Changing the identity of a transfer RNA. *Nature* **321 :** 213-9.

O' Brien TW (2002) Evolution of a protein-rich mitochondrial ribosome: implications for human genetic disease. *Gene* **286**: 73–79

Ochoa, S. (1963) Synthetic polynucleotides and the genetic code. *Fed. Proc*. **22 :** 62-74.

Ogawa, K. and Kaji, A. (1975) Requirement for ribosome-releasing factor for the release of ribosomes at the termination codon. *Eur. J. Biohem.* **58 :** 411-9.

Ogle, J. M. et al. (2001) Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* **292 :** 897–902.

Ogle, J.M., Murphy, F.V., Tarry, M.J. and Ramakrishnan, V. (2002) Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell* **111 :** 721-32.

Ogle, J.M., Carter, A.P. and Ramakrishnan, V. (2003) Insights into the decoding mechanism from recent ribosome structures. *Trends Biochem. Sci.* **28** : 259–66.

Ohta, T., Sarkar, S. and Thach, R.E. (1967) The role of guanosine 5'-triphosphate in the initiation of peptide synthesis. 3. Binding of formylmethionyl-tRNA to ribosomes. *Proc. Natl. Acad. Sci. U. S. A*. **58 :** 1638-44.

Okamoto, T. and Takanami, M. (1963) Interaction of ribosomes and some synthetic polyribonucleotides. *Biochim. Biophys. Acta* **68 :** 325-7.

Olsen, G.J., McCarroll, R. and Sogin, M.L. (1983) Secondary structure of the Dictyostelium discoideum small subunit ribosomal RNA. *Nucleic Acids Res.* **11 :** 8037-49.

Olsen, G.J. and Woese, C.R. (1997) Archaeal genomics: an overview. *Cell* **89 :** 991-4.

Ono, Y., Skoultchi, A., Klein, A. and Lengyel, P. (1968) Peptide chain elongation: discrimination against the initiator transfer RNA by microbial amino-acid polymerization factors. *Nature* **220 :** 1304-7.

Pai, R.D., Zhang, W., Schuwirth, B.S., Hirokawa, G., Kaji, H., Kaji, A. and Cate, J.H. (2008) Structural Insights into ribosome **recycling factor** interactions with the 70S ribosome. *J. Mol. Biol.* **376 :** 1334-47.

Palade, G.E. (1955) A slmal particulate component of the cytoplasm. *J. Biophys. Biochem. Cytol.* **1** : 59-68.

Pardee, A.B. (1954) Nucleic acid precursors and protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **40** : 263-70.

Pardee, A.B. (1958) Experiments on the transfer of information from DNA to enzymes. *Exp. Cell Res. Suppl*. **6** : 142-151

Parfenov, D.V. and Saminskiĭ, EM. (1985) Deacylated tRNA binds with 50S subunits of *Escherichia coli* ribosomes at a special site not corresponding to the P'-site. *Mol. Biol.* (Russian) **19 :** 1378-85.

Payne, P.I. and Dyer, T.A. (1971) The occurrence of **5.8S** ribonucleic acid in plants. *Biochem. J.* **125 :** 100.

Pene, J., Knight, E. and Darnell, J. E. (1968) Characterization of a new low molecular weight RNA in *HeLa* cell ribosomes. *J. Mol. Biol.* **33 :** 609-23.

Pestka, S. (1967) Studies on the formation of transfer ribonucleic acid-ribosome complexes. II. A possible site on the 50 S subunit protecting aminoacyl transfer ribonucleic acid from deacylation. *J. Biol. Chem.* **242 :** 4939-47.

Pestka, S. (1969) Studies on the formation of transfer ribonucleic acid-ribosome complexes. VI. Oligopeptide synthesis and translocation on ribosomes in the presence and absence of soluble transfer factors. *J. Biol. Chem*. **244** : 1533-9.

Pestka, S. (1974) Assay for nonenzymatic and enzymatic translocation with *Escherichia coli* ribosomes. *Methods Enzymol*. **30 :** 462-70.

Petropoulos, A.D. and Green, R. (2012) Further in vitro exploration fails to support the allosteric three-site model*. J. Biol. Chem.* **287** : 11642-8

Polacek, N. and Mankin, A.S. (2005) The ribosomal peptidyl transferase center: structure, function, evolution, inhibition. *Crit. Rev. Biochem. Mol. Biol*. **40 :** 285-311.

Ramakrishnan, V. and White, S.W. (1992) The structure of ribosomal protein S5 reveals sites of interaction with 16S rRNA. *Nature* **358 :** 768-71.

Rao, P. and Moldave, K. (1967) The binding of aminoacyl sRNA and GTP to transferase I.

Biochem. Biophys. Res. Commun. **28 :** 909-13.

Ratje. A.H. *et al*. (2010) Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. *Nature* **468** : 713–16.

Ravel, J.M., Shorey, R.L. and Shive, W. (1967) Evidence for a guanine nucleotide-aminoacyl-RNA complex as an intermediate in the enzymatic transfer of aminoacyl-RNA to ribosomes. *Biochem. Biophys. Res. Commun.* **29 :** 68-73.

Ravel, J.M., Shorey, R.L., Froehner, S. and Shive, W. (1968) A study of the enzymic transfer of aminoacyl-RNA to Escherichia coli ribosomes. *Arch. Biochem. Biophys*. **125 :** 514-26.

Ravel, J.M., Shorey, R.L. and Shive, W. (1968b) The composition of the active intermediate in the transfer of aminoacyl-RNA to ribosomes. *Biochem. Biophys. Res. Commun***. 32 :** 9-14.

Ravel, J.M., Shorey, R.L., Garner, C.W., Dawkins, R.C. and Shive, W. (1969) The role of an aminoacyl-tRNA-GTP-protein complex in polypeptide synthesis. (in : The mechanism of protein synthesis. *Cold Spring Harb Symp Quant Biol*. **34 :** 321-30).

Remme, J., Margus, T., Villems, R. and Nierhaus, K.H. (1989) The third ribosomal **tRNA-**binding site, the E site, is occupied in native polysomes. *Eur. J. Biochem.* **183 :** 281-4.

Revel, M. and Gros, F. (1966) A factor from *E. coli* required for the translation of natural messenger RNA. *Biochem. Biophys. Res. Commun*., **25 :** 124-32.

Revel, M., Lelong, J.C., Brawerman, G. and Gros, F. (1968) Function of three protein factors and ribosomal subunits in the initiation of protein synthesis in *E. coli. Nature* **219 :** 1016-21.

Rheinberger, H.J., Sternbach, H. and Nierhaus, K.H. (1981) Three tRNA binding sites on *Escherichia coli* ribosomes. Proc. Natl. Acad. Sci. U. S. A. **78 :** 5310-4.

Rheinberger, H.J. and Nierhaus, K.H. (1983) Testing an alternative model for the ribosomal peptide elongation cycle. *Proc. Natl. Acad. Sci. U. S. A.* **80 :** 4213-7.

Rheinberger, H.J., Sternbach, H. and Nierhaus, K.H. (1986) Codon-anticodon interaction at the ribosomal E site. *J. Biol. Chem.* **261 :** 9140-3.

Rheinberger, H.J, Nierhaus, K.H. (1986b) Allosteric interactions between the ribosomal transfer RNA-binding sites A and E. *J. Biol. Chem*. **261 :** 9133-9.

Rich. A. and Watson, J. (1954) Some relations between DNA and RNA., *Proc. Natl. Acad. Sci. U. S. A.* **40 :** 759-64

Riley, M., B. Pardee, A.B., Jacob, F. and Monod, J. (1960) On the expression of a structural gene. *J. Mol. Biol.* **2** : 216-25.

Roberts, R.B. ed. (1958) Microsomal Particles and Protein Synthesis. (The First Symposium of the Biophysical Society at the Massachusetts Institute of Technology, Cambridge), *Pergamon Press Inc*.

Rodnina, M.V., El'skaya, A.V., Semenkov, Yu.P, Kirillov, S.V. (1988) Number of tRNA binding sites on 80 S ribosomes and their subunits. *FEBS Lett.* **231 :** 71-4.

M.V. Rodnina, M.V., Wintermeyer, W. and Green, R. Eds. (2011) Ribosomes: Structure, Function, and Dynamics (*Springer Inc., Wien - New York*).

Rosset, R., and Monier, R. (1963) A propos de la présence d'acide ribonucléique de faible poids moléculaire dans les ribosomes d*'Escherichia coli*. Biochim. Biophys. Acta **68 :** 653-6.

Rubtsov, P.M., Musakhanov, M.M., Zakharyev, V.M., Krayev, A.S., Skryabin, K.G. and Bayev, A.A. (1980) The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **8 :** 5779-94.

Ruusala, T., Andersson D., Ehrenberg, M. and Kurland, C.G. (1984) Hyper-accurate ribosomes inhibit growth. *EMBO J.* **3 :** 2575-80.

Salas, M., Hille, M.B., Last, J.A., Wahba, A.J and Ochoa. S. (1967) Translation of the genetic message, II. Effect of initiation factors on the binding of formyl-methionyl-trna to ribosomes. *Proc. Natl Acad. Sci. U. S. A.* **57** : 387-94.

Samaha, R.R., Green, R. and Noller, H.F. (1995) A base pair between tRNA and 23S rRNA in the peptidyl transferase centre of the ribosome. *Nature* **377** : 309–314.

Sarabhai, A.S., Stretton, A.O., Brenner, S. and Bolle, A. (1964) Co-linearity of the gene with the polypeptide chain. *Nature* **201 :** 13-7.

Savelsbergh, A. et al. (2000) Stimulation of the GTPase activity of translation elongation factor G by ribosomal protein L7/12. *J. Biol. Chem*. **275** : 890–894.

Schaup, H.W., Green, M. and Kurland, C.G. (1970) Molecular interactions of ribosomal components. I. Identification of RNA binding sites for individual 30S ribosomal proteins. *Mol. Gen. Genet.* **109 :** 193-205.

Schimmel, P.R. and Söll, D. (1979) Aminoacyl-tRNA synthetases: general features and recognition of transfer RNAs. *Annu. Rev. Biochem.* **48 :** 601-48.

Schimmel, P. (1987) Aminoacyl tRNA synthetases: general scheme of structure-function relationships in the polypeptides and recognition of transfer RNAs. *Ann. Rev. Biochem.*

56 : 125-58.

Schimmel, P. and Alexander, R.W. (1998) All you need is RNA. *Science* **281 :** 658–9.

Schmeing, T.M., Voorhees, R.M., Kelley, A.C., Gao, Y.G., Murphy, F.V.4th, Weir, J.R. and Ramakrishnan, V. (2009) The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* **326 :** 688-94.

Schmitt, M., Neugebauer, U., Bergmann, C., Gassen, H.G. and Riesner, D. (1982) Binding of tRNA in different functional states to Escherichia coli ribosomes as measured by velocity sedimentation. *Eur. J. Biochem.* **127 :** 525-9.

Schreier, M.H. and Noll, H. (1971) Conformational changes in ribosomes during protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **68 :** 805-9

Schulman, L. and Pelka, H. (1975) The structural basis for the resistance of *Escherichia coli* formylmethionyl transfer ribonucleic acid to cleavage by *Escherichia coli* peptidyl transfer ribonucleic acid hydrolase. *J. Biol. Chem.* **250** : 542-7.

Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M. and Cate, J.H. (2005) Structures of the bacterial ribosome at 3.5 A resolution. *Science*, **310 :** 827-34. and

Schwarz, Z.S. and Kössel, H. (1980) The primary structure of 16S rDNA from *Zea mays* chloroplast is homologous to *E. coli* 16S rRNA. *Nature* **283 :** 739-42.

Scolnick, E., Tompkins, R., Caskey, T. and Nirenberg, M. (1968) Release factors differing in specificity for terminator codons. *Proc. Natl. Acad. Sci. U. S. A.* **61 :** 768-74.

Scolnick, E.M. and Caskey, C.T. (1969) Peptide chain termination. V. The role of release factors in mRNA terminator codon recognition. *Proc. Natl. Acad. Sci. U. S. A.* **64 :** 1235-41.

Selmer, M., Dunham, C.M., Murphy, F.V.4th, Weixlbaumer, A., Petry, S., Kelley, A.C., Weir, J.R. and Ramakrishnan V. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* **313 :** 1935-42

Semenkov, Y.P., Rodnina, M.V. and Wintermeyer, W. (1996) The "allosteric three-site model" of elongation cannot be confirmed in a well-defined ribosome system from *Escherichia coli. Proc. Natl. Acad. Sci. U. S. A.* **93 :** 12183-8.

Sharma, M.R., et al. (2007) Cryo-EM study of the spinach chloroplast ribosome reveals the structural and functional roles of plastid-specific ribosomal proteins. *Proc. Natl. Acad. Sci. U. S. A,* **104 :** 19315–20.

Sharma, M.R., Booth, T.M., Simpson, L., Maslov, D.A. and Agrawal, R.K. (2009) Structure of a mitochondrial ribosome with minimal RNA. *Proc. Natl. Acad. Sci. U. S .A.* **106 :** 9637-42.

Shine, J. and Dalgarno, L. (1974) The 3'-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. *Proc. Natl. Acad. Sci. U. S. A.* **71 :** 1342-6.

Shine, J. and Dalgarno, L. (1975) Determinant of cistron specificity in bacterial ribosomes. *Nature* **254 :** 34–8.

Siekevitz, P., Zamecnik, P. C. (1951) In vitro incorporation of l-C14-DL-alanine into proteins of rat-liver granular fractions. *Fed. Proc*. **10** : 246-7.

Siekevitz, P. (1952) Uptake of radioactive alanine *in vitro* into the proteins of rat liver fractions. *J. Biol. Chem*. **195** :5 49–65.

Skogerson, L. and Moldave, K. (1968) Characterization of the interaction of aminoacyl-transferase II with ribosomes. Binding of transferase II and translocation of peptidyl transfer ribonucleic acid. *J. Biol. Chem.* **243 :** 5354-60.

Skoultchi, A., Ono, Y., Waterson, J. and Lengyel, P. (1969) Peptide chain elongation. (in : The mechanism of protein synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34 :** 437-54).

Smit, S., Widmann, J., Knight, R. (2007) Evolutionary rates vary among rRNA structural elements. *Nucleic Acids Res.* **35 :** 3339-54.

Smith, K.C., Cordes, E. and Schweet, R.S. (1959) Fractionation of transfer ribonucleic acid. *Biochim. Biophys. Acta.* **33 :** 286-7.

Smith, A.E. and Marcker, K.A.(1968) N-formylmethionyl transfer RNA in mitochondria from yeast and rat liver. *J. Mol. Biol.* **38 :** 241-3.

Smith, T.F., Lee, J.C., Gutell, R.R. and Hartman, H. (2008) The origin and evolution of the ribosome. *Biol. Direct.* **3 :** 16 (13 pages).

Sokolff, L. and Rappaport, H.P. (1972) Isolation of isoaccepting tRNAs. *Arch. Biochem. Biophys.* **153 :** 788-96.

Sonenberg, N., Wilchek, M. and Zamir, A. (1973) Mapping of Escherichia coli ribosomal components involved in peptidyl transferase activity. *Proc. Natl. Acad. Sci. U. S. A.* **70 :** 1423-6.

Southworth, D.R., Brunelle, J.L. and Green, R. (2002) EFG-independent translocation of the mRNA:tRNA complex is promoted by modification of the ribosome with thiol-specific reagents. *J. Mol. Biol.* **324 :** 611-23.

Spahn, C.M. *et al*., (2004) Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation *EMBO J.* **23** : 1008–19.

Spirin, A.S. (1961) The "temperature effect" and macromolecular structure of high-polymer ribonucleic acids of various origin. *Biokhimiia* (Russian) **26 :** 454-63.

Spirin, A.S. (1969) A model of the functioning ribosome: locking and unlocking of the ribosome subparticles. (in : The mechanism of protein synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34** : 197-207).

Spirin, A.S. (1973) "Enzymatic" and "nonenzymatic" translation. *Basic Life Sci.* **1 :** 311-25.

Spirin, A.S. (1999) Ribosomes, *Kluwer Academic/Plenum Publishers, New York*.

A. S. Spirin, (2009) The ribosome as a conveying thermal ratchet machine. *J. Biol. Chem.* **288** : 21103–19.

Spitnik-Elson P. (1963) Fractionation of ribosomal protein from *Escherichia coli* by ammonium sulfate precipitation. *Biochim Biophys Acta*. **74** : 105-12.

Spitnik-Elson, P. (1964) Fractionation of the ribosomal protein of escherichia coli by ion-exchange chromatography on carboxymethyl cellulose. *Biochim. Biophys. Acta*. **80** : 594-600.

Staehelin, T., Maglott, D.M. and Monro, R.E. (1969) On the catalytic center of peptidyl transfer: a part of the 50S ribosome structure. (in : The mechanism of protein synthesis. *Cold Spring Harb Symp Quant Biol.* **34 :** 39-48).

Stanley, W.M.Jr., Salas, M., Wahba, A.J. and Ochoa, S. (1966) Translation of the genetic message: factors involved in the initiation of protein synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **56 :** 290-5.

Stark, H., Mueller, F., Orlova, E.V., Schatz, M., Dube, P., Erdemir, T., Zemlin F., Brimacombe, R. and van Heel, M. (1995) The 70S *Escherichia coli* ribosome at 23 A resolution: fitting the ribosomal RNA. *Structure* **3 :** 815-21

Steinberg, S.V., Boutorine, Y.I. (2007a) G-ribo: a new structural motif in ribosomal RNA. *RNA*, **13** : 549-54.

Steinberg, S.V., Boutorine, Y.I. (2007b) G-ribo motif favors the formation of pseudoknots in ribosomal RNA. *RNA*, **13** : 1036-42.

Steitz, T.A. and Moore, P.B. (2003) RNA, the first macromolecular catalyst: the ribosome is a ribozyme. *Trends Biochem. Sci.* **28 :** 411-8.

Stiegler, P., Carbon, P., Zuker, M., Ebel, J.P. and Ehresmann, C. (1981) Structural organization of the 16S ribosomal RNA from *E. coli.* Topography and secondary structure. *Nucleic Acids Res*. **9 :** 2153-72.

Stillman, B. (ed.) (2001) The Ribosome. (*Cold Spring Harbor Symp. Quant. Biol.* **66**).

Stöffler, G. and Wittmann, H.G. (1971) Sequence differences of *Escherichia coli* 30S ribosomal proteins as determined by immunochemical methods. *Proc. Natl. Acad. Sci. U. S. A.* **68 :** 2283-7.

Stretton, A.O. and Brenner, S. (1965) Molecular consequences of the ***amber*** mutation and its suppression. *J. Mol. Biol.* **12 :** 456-65.

Subramanian, A.R., Ron, E.Z. and Davis, B.D. (1968) A factor required for ribosome dissociation in *Escherichia coli. Proc. Natl. Acad. Sci. U. S. A*. **61 :** 761-7.

Subramanian, A.R. and Davis, B.D. (1970) Activity of the initiation factor F3 in dissociating *Escherichia coli* ribosomes. *Nature* **228** : 1273-5

Subramanian, A.R. and Davis, B.D. (1973) Release of 70 S ribosomes from polysomes in *Escherichia coli. J. Mol. Biol.* **74 :** 45-56.

Suzuka, I., Kaji, H. and Kaji, A. (1965) Binding of specific sRNA to 30S subunits-comparison with the binding to 70S ribosomes. *Biochem. Biophys. Res. Commun.* **21 :** 187-93.

Suzuki, T., Terasaki, M., Takemoto-Hori, C., Hanada, T., Ueda, T., Wada, A. and Watanabe, K. (2001). Structural compensation for the deficit of rRNA with proteins in the mammalian mitochondrial ribosome. Systematic analysis of protein components of the large ribosomal subunit from mammalian mitochondria. *J. Biol. Chem.* **276 :** 21724–36.

Sy, J., and McCarty, K. S. (1970) Characterization of 5.8S RNA from a complex with 26S ribosomal RNA from *Arbacia punctulia. Biochem. Biophys. Acta* **199 :** 86-94.

Szathmáry, E. and Maynard Smith, J. (1997) From replicators to reproducers: The first major transitions leading to life. *J. Theor. Biol.* **187 :** 555–71.

Szép, S., Wang, J. and Moore, P.B. (2003) The crystal structure of a 26-nucleotide RNA containing a hook-turn. *RNA* **9 :** 44-51.

Szewczak, A.A., Moore, P.B,, Chang. Y,L. and Wool, I.G. (1993) The conformation of the sarcin/ricin loop from 28S ribosomal RNA. Proc. Natl. Acad. Sci. U. S. A. **90 :** 9581-5.

Takanami. M. (1962) Transfer of amino acids from soluble ribonucleic aci to ribosome. III.

Further studies on the interaction between ribosome and soluble ribonucleic acid. *Biochim Biophys Acta*. **61 :** 432-44.

Takanami**,** M. and Okamoto, T. (1963) Interaction of ribosomes and synthetic polyribonucleotides. *J. Mol. Biol*. **7 :** 323-33.

Takanami, M. and Yan, Y. (1965) The release of polypeptide chains from ribosomes in cell-free amino acid-incorporating systems by specific combinations of bases in synthetic polyribonucleotides. *Proc. Natl. Acad. Sci. U. S. A.* **54 :** 1450-8.

Tanaka, N., Kinoshita, T. and Masukawa, H. (1968) Mechanism of protein synthesis inhibition by fusidic acid and related antibiotics. *Biochem. Biophys. Res. Commun.* **30 :** 278-83.

Thach, R.E., Dewey, K.F., Brown, J.C. and Doty, P. (1966) Formylmethionine codon AUG as an initiator of polypeptide synthesis. *Science* **153 :** 416-8.

Thompson, R.C. and Stone, P.M. (1977) Proofreading of the codon-anticodon interaction on ribosomes. *Proc. Natl. Acad. Sci*. *U. S. A.* **74 :** 198-202.

Thompson, R.C., Dix, D.B., Gerson, R.B. and Karim, A.M. (1981) A GTPase reaction accompanying the rejection of Leu-tRNA2 by UUU-programmed ribosomes. Proofreading of the codon-anticodon interaction by ribosomes. *J. Biol. Chem.* **256 :** 81-6.

Thompson, R.C., and Dix, D.B. (1982) Accuracy of protein biosynthesis. A kinetic study of the reaction of poly (U)-programmed ribosomes with a leucyl-tRNA2-elongation factor Tu-GTP complex. *J. Biol. Chem.* **257 :** 6677-82.

Thompson, R.C. and Karim. A.M. (1982b) The accuracy of protein biosynthesis is limited by its speed: high fidelity selection by ribosomes of aminoacyl-tRNA ternary complexes containing GTP [gamma S]. *Proc. Natl. Acad. Sci. U. S. A.* **79 :** 4922-6.

Thompson, R.C., Dix, D.B. and Karim, A.M. (1986) The reaction of ribosomes with elongation factor Tu.GTP complexes. Aminoacyl-tRNA-independent reactions in the elongation cycle determine the accuracy of protein synthesis. *J. Biol. Chem.* **261 :** 4868-74.

Tissieres, A., and Watson, J.D. (1958) Ribonucleoprotein particles from *Escherichia coli*. *Nature* **182** : 778-780.

Tissieres, A., Schlessinger, D. and Gros, F. (1960) Amino acid incorporation into proteins by *Escherichia coli* ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* **46 :** 1450-63.

Tompkins, R.K., Scolnick, E.M. and Caskey, C.T. (1970) Peptide chain termination. VII. The ribosomal and release factor requirements for peptide release. *Proc. Natl. Acad. Sci.*

U. S. A. **65 :** 702-8.

Traut, R.R. and Monro, R.E. (1964) The puromycin reaction and its relation to protein synthesis. *J. Mol. Biol.* **20 :** 63-77.

Traut, R.R. (1966) Acrylamide gel electrophoresis of radioactive ribosomal protein. *J. Mol. Biol.* **21 :** 571-6.

Traut, R.R., Delius, H., Ahmad-Zadeh, C., Bickle, T.A., Pearson, P. and Tissières, A. (1969) Ribosomal proteins of *E. coli:* stoichiometry and implications for ribosome structure. (in : The mechanism of protein synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34 :** 25-38).

Tritton, T,R. and Armitage, I.M. (1978) Phosphorus-31 NMR studies of *E. coli* ribosomes. *Nucleic Acids Res*. **5 :** 3855-69.

Tsurugi, K., Collatz, E., Todokoro, K., Ulbrich, N., Lightfoot, H.N. and Wool, I.G. (1978) Isolation of eukaryotic ribosomal proteins. Purification and characterization of the 60 S ribosomal subunit proteins La, Lb, Lf, P1, P2, L13', L14, L18', L20, and L38. *J. Biol. Chem.* **253 :** 946-55.

Udem, S.A., Kaufman, K. and Warner, J.R. (1971) Small ribosomal ribonucleic acid species of *Saccharomyces cerevisiae*. *J Bacteriol.* **105 :** 101-6.

Veldman, G.M., Klootwijk, J., de Regt, V.C., Planta, R.J., Branlant, C., Krol, A. and Ebel, J.P. (1981) The primary and secondary structure of yeast 26S rRNA. *Nucleic Acids Res.* **9 :** 6935-52.

Wahba, A.J., Iwasaki, K., Miller, M.J., Sabol, S., Sillero, M.A. and Vasquez, C. (1969) Initiation of protein synthesis in *Escherichia coli*. II. Role of the initiation factors in polypeptide synthesis. (in : The mechanism of protein synthesis. *Cold Spring Harbor Symp. Quant. Biol.* **34 :** 291-299).

Warner, J.R., Rich, A. and Hall, C.E. (1962) Electron microscope studies of ribosomal cluster synthetizing hemoglobin. *Science* **138 :** 1399-1403.

Warner, J. R. and Rich, A. (1964) The number of soluble RNA molecules on reticulocyte polyribosomes. *Proc. Natl. Acad. Sci. U. S. A.* **51 :** 1134-41.

Watson, J.D., and Crick, F.H. (1953a) Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* **171 :** 737-8.

Watson, J.D., and Crick, F.H. (1953b) Genetical implications of the structure of deoxyribonucleic acid. *Nature* **171** : 964–7.

Watson, J. D. (1963) Involvement of RNA in the synthesis of proteins. *Science*, **140 :** 17-26.

Watson, J. D. (1964) The synthesis of proteins upon ribosomes. *Bull. Soc. Chim. Biol.,* **46 :** 1399-1425.

Webster, G.C. (1957) Factors required for amino acid incorporation by disrupted ribonucleoprotein particles. *Arch. Biochem. Biophys*. **70** : 622-4.

Webster, R.E., Engelhardt, D.L. and Zinder, N.D. (1966) In vitro protein synthesis: chain initiation. *Proc. Natl. Acad. Sci. U. S. A.* **55 :** 155-61.

Weisblum, B. and Davies, J. (1968) Antibiotic inhibitors of the bacterial ribosome. *Bacteriol Rev*. **32 :** 493-528.

Wettstein, F.O., Staehelin, T. and Noll, A. (1963) Ribosomal aggregate engaged in protein synthesis: characterization of the ergosome. *Nature* **197** : 430-5.

Wettstein, F.O. and Noll, H. (1965) Binding of transfer ribonucleic acid to ribosomes engaged in protein synthesis: number and properties of ribosomal binding sites. *J. Mol. Biol*. **11 :** 35-53.

White, S.A., Nilges, M., Huang, A., Brünger, A.T. and Moore, P.B. (1992) NMR analysis of helix I from the 5S RNA of *Escherichia coli. Biochemistry* **31 :** 1610-21.

Wigle, D.T. and Dixon, G.H. (1970) transient incorporation of methionine at the n-terminus of protamine newly synthesized in trout testis cells. *Nature* **227 :** 676-80.

Williamson, R. and Brownlee, G.G. (1969) The sequence of 5S ribosomal RNA from two mouse cell lines. *FEBS Lett.* **3 :** 306-8.

Wilson, D.B. and Dintzis, H.M. (1970) Protein chain initiation in rabbit reticulocytes. *Proc. Natl. Acad. Sci. U. S. A.* **66 :** 1282-9.

Wilson, K.S., Appelt, K., Badger, J., Tanaka, I. and White, S.W. (1986) Crystal structure of a prokaryotic ribosomal protein. *Proc. Natl. Acad. Sci. U. S. A.* **83 :** 7251-5.

Wimberly, B., Varani, G. and Tinoco, I.Jr. (1993) The conformation of loop E of eukaryotic 5S ribosomal RNA. *Biochemistry* **32 :** 1078-87.

Wimberly, B.T., Brodersen, D.E., Clemons, W.M. Jr., Morgan-Warren, R.J., Carter, A.P., Vonrhein. C., Hartsch, T. and Ramakrishnan, V. (2000) Structure of the 30S ribosomal subunit. *Nature* **407 :** 327-39.

Wittmann, H.G. (1983) Architecture of prokaryotic ribosomes. *Annu. Rev. Biochem.* **52 :** 35-65.

Woese, C. (1970) Molecular mechanics of translation: a reciprocating ratchet mechanism. *Nature* **226 :** 817-20.

Woese, C.R. et al. (1980) Secondary structure model for bacterial 16S ribosomal RNA: phylogenetic, enzymatic and chemical evidence. *Nucleic Acids Res*. **8 :** 2275-93.

Woese, C.R. (1998) The universal ancestor. *Proc. Natl. Acad. Sci. U. S. A.* **95 :** 6854-9.

Woese, C.R. (2001) Translation: In Retrospect and Prospect. *RNA* **7 :** 1055-67.

Woledge, J., Corry, M.J., Payne, P.I. (1974) Ribosomal RNA homologies in flowering plants: comparison of the nucleotide sequences in 5.8-S rRNA from broad bean, dwarf bean, tomato, sunflower and rye. *Biochim. Biophys. Acta* **349 :** 339-50.

Wuyts, J., Van de Peer. I. and De Wachter, R. (2001) Distribution of substitution rates and location of insertion sites in the tertiary structure of ribosomal RNA. *Nucleic Acid Res.* **29:** 5017–28.

Yarmolinsky, M.B. and Haba, G.L. (1959) Inhibition by puromycin of amino acid incorporation into protein. *Proc. Natl. Acad. Sci. U. S. A.* **45 :** 1721-9.

Yarus, M., Berg, P. (1967) Recognition of tRNA by aminoacyl tRNA synthetases. *J. Mol. Biol.* **28 :** 479-90.

Yonath, A., Leonard, K.R., Weinstein, S. and Wittmann, H.G. (1987) Approaches to the determination of the three-dimensional architecture of ribosomal particles. (in : Evolution of Catalytic Function. *Cold Spring Harbor Symp. Quant. Biol.* **52 :** 729-41).

Yonath, A. (2010) Polar bears, antibiotics, and the evolving ribosome (Nobel Lecture). *Angew. Chem. Int. Ed. Engl.* **49 :** 4341-54.

Yusupov, M.M., Yusupova, G.Z., Baucom, A., Lieberman, K., Earnest, T.N., Cate, J.H. and Noller, H.F. (2001) Crystal structure of the ribosome at 5.5A resolution. *Science* **292 :** 883-96.

Zaher, H.S. and Green, R. (2009) Quality control by the ribosome following peptide bond formation. *Nature,* **457 :** 161–6.

Zamecnik, P.C. (1960) Historical and current aspects of the problem of protein synthesis. *Harvey Lect*. **54 :** 256-81.

Zamecnik, P.C. (1969) An historical account of protein synthesis, with current overtones - A personalized view. (in : The mechanism of protein synthesis.*Cold Spring Harbor Symp. Quant. Biol.* **34** : 1-16).

Zamir, A., Leder, P. and Elson, D. (1966) A ribosome-catalyzed reaction between N-formylmethionyl-trna and puromycin. *Proc. Natl. Acad. Sci. U. S. A.* **56 :** 1794-801.

Zamir, A., Miskin, R. and Elson, D. (1971) Inactivation and reactivation of ribosomal subunits: amino acyl-transfer RNA binding activity of the 30 s subunit of *Escherichia coli. J. Mol. Biol.* **60 :** 347-64.

Zhang, B. and Cech, T.R. (1997) Peptide bond formation by in vitro selected ribozymes. *Nature* **390** : 96–100.

Zhang, B, Cech, T.R. (1998) Peptidyl-transferase ribozymes: transreactions, structural characterization and ribosomal RNAlike features. *Chem. Biol.* **5**: 539 – 553

Zhang, W., Dunkle, J.A. and Cate, J.H. (2009) Structures of the ribosome in intermediate states of ratcheting. *Science* **325 :** 1014-7.

Zhou, J., Lancaster, L., Trakhanov, S. and Noller, H.F. (2012) Crystal structure of release factor RF3 trapped in the GTP state on a rotated conformation of the ribosome. *RNA* **18** : 230-40.

Zwieb, C., Glotz, C. and Brimacombe, R.(1981) Secondary structure comparisons between small subunit ribosomal RNA molecules from six different species. *Nucleic Acids Res*. **9** : 3621-40.