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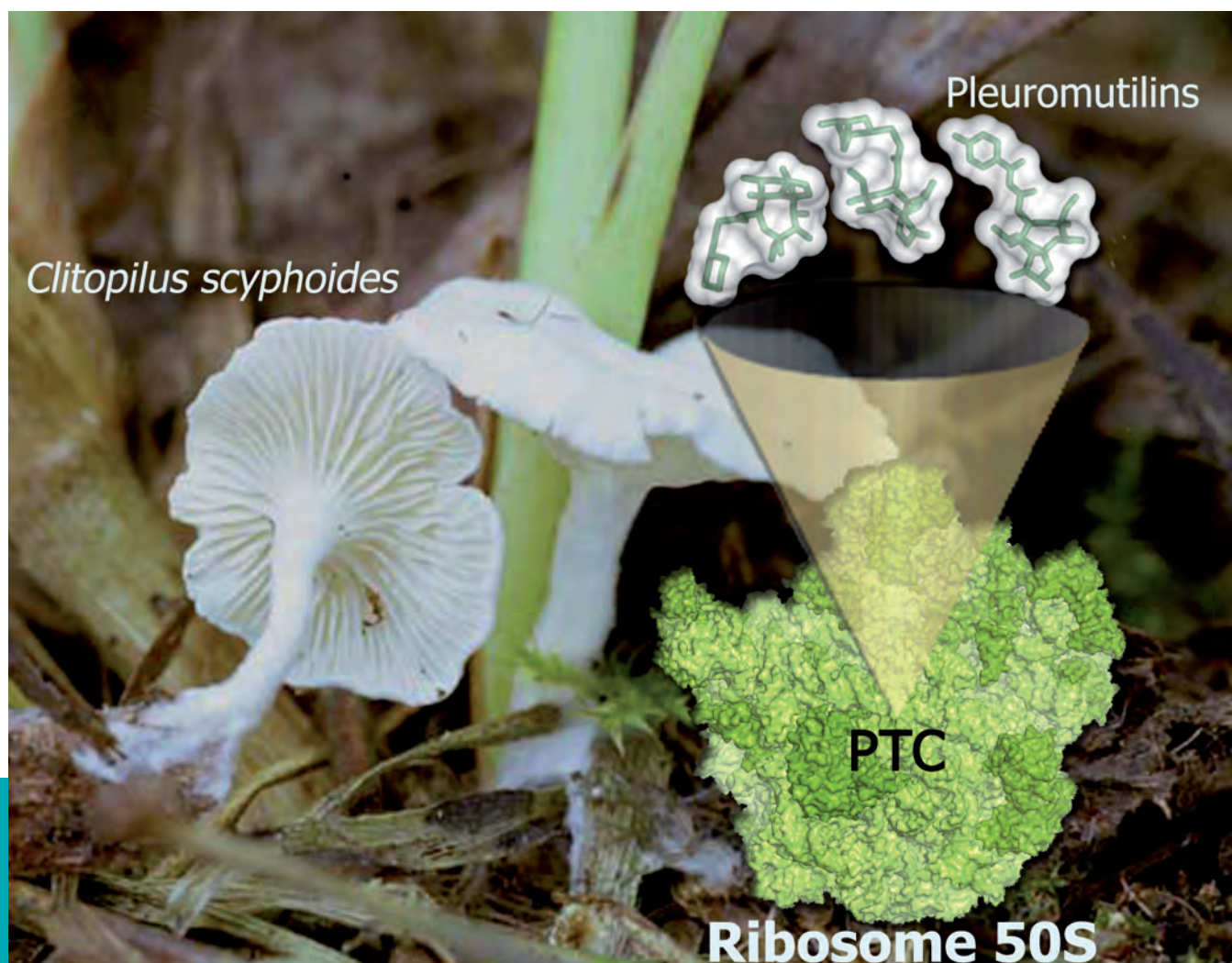
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Ribosome Chemistry

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Cover Picture

Chen Davidovich, Matthew Belousoff, Itai Wekselman, Tal Shapira, Miri Krupkin, Ella Zimmerman, Anat Bashan*, and Ada Yonath

The cover picture shows the ribosome 50S subunit on a fungal background. Represented fungi, *Clitopilus scyphoides*, produce antibiotics of the pleuromutilin family. Pleuromutilins “moving” into the ribosome Peptidyl Binding Centre (PTC) are also shown. The figure was contributed by Anat Bashan and Ada Yonath (see The Proto-Ribosome: An Ancient Nano-machine for Peptide Bond Formation, Davidovich et al., p. 29 in this issue).



The Proto-Ribosome: An Ancient Nano-machine for Peptide Bond Formation

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Abstract: The ribosome is a ribozyme whose active site, the peptidyl transferase center (PTC), is situated within a highly conserved universal symmetrical region that connects all ribosomal functional centers involved in amino acid polymerization. The linkage between this elaborate architecture and A-site tRNA position revealed that the A- to P-site passage of the tRNA 3' terminus during protein synthesis is performed by a rotary motion, synchronized with the overall tRNA/mRNA sideways movement, and guided by the PTC. This rotary motion leads to suitable stereochemistry for peptide bond formation as well as for substrate-mediated catalysis.

Analysis of the substrate binding modes to ribosomes led to the hypothesis that the ancient ribosome produced single

peptide bonds and non-coded chains, potentially in a similar manner to the modern PTC. Later in evolution, a mechanism, enabling some type of decoding genetic control triggered the emergence of the small ribosomal subunit or part of it. This seems to be the result of the appearance of reaction products that could have evolved after polypeptides capable of enzymatic function were generated sporadically, while an ancient stable RNA fold was converted into an old version of a tRNA molecule. Since in the contemporary ribosome, the symmetry relates only to the backbone fold and nucleotide orientations but not nucleotide sequences, it emphasizes the superiority of functional requirement over sequence conservation, and indicates that the PTC may have evolved by gene fusion or gene duplication.

Keywords: evolving ribosomes · peptide bond formation · polymerase · RNA · ribozyme

1. Introduction

Ribosomes are the nano-machines common to known extant cellular life. They are the key players in the translation of the genetic code into proteins, providing the molecular framework for the proper positioning of all other translation participants. Ribosomes are giant macromolecular assemblies, composed of long ribosomal RNA (rRNA) chains and many different proteins (r-proteins). All ribosomes are constituted by two unequal subunits. In prokaryotes, the small subunit, denoted as 30S, contains an RNA chain (termed 16S in prokaryotes) of about 1500 nucleotides and 20–21 different proteins, whereas the large subunit (termed 50S in prokaryotes) has two RNA chains (23S and 5S RNA) of about 3000 nucleotides in total, and 31–35 different proteins. In all organisms the two subunits exist independently and associate to form functionally active ribosomes. The small subunit provides the path along which the mRNA progresses and the decoding center which is the mechanism controlling translation fidelity. The large subunit provides the site for the main ribosomal catalytic function, polymerization of the amino acids, and the protein exit tunnel.

The three-dimensional structure of all tRNA molecules from all living cells is alike, although each of them is specific to its amino acid. They are all built mainly of double

helical L-shape molecules, made by a stem-elbow-stem organization, but each contains an anticodon loop matching its complementary three-nucleotide codes on the mRNA, on one of its edges. About 70 Å away, at their 3' end, the tRNA molecules contain a single strand portion with the universal sequence CCA, to which the cognate amino acid is bound via an ester linkage. These tRNA molecules are the non-ribosomal entities bridging the two subunits, as two of their three binding sites (A- (aminoacyl), P- (peptidyl), and E- (exit) sites) reside on both subunits. At the A- and P-sites, the tRNA anticodon loops interact with the mRNA on the small subunit and the acceptor stem with the aminoacylated or peptidylated 3' end are located on the large subunit. Peptide bonds are formed during A- to P-site tRNA translocation, a motion that is comprised of sideways shift and a ribosomal navigated rotary motion. Nascent proteins progress along a

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Anat Bashan earned her B.Sc. degree in chemistry at The Hebrew University of Jerusalem in 1987, and received her M.Sc. (1989) and Ph.D. (1995) degrees at the Department of Structural Biology of the Weizmann Institute of Science. Between 1995 and 1998 she was a Post-Doctoral Fellow at the Weizmann Institute, and since then she has been a staff scientist in the group of Prof. Ada Yonath at the Department of Structural Biology, Weizmann Institute. Her research activities are focused on structure and function of ribosomal components, and the mode of action of ribosomal antibiotics.



Ada Yonath is a structural biologist who is using X-ray crystallography. She earned her B.Sc. (1962) and M.Sc. (1964) degrees in chemistry at The Hebrew University of Jerusalem and her Ph.D. (1968) at the Weizmann Institute of Science. She conducted her postdoctoral studies at Carnegie Mellon University and at the Massachusetts Institute of Technology, and in 1970 she established the first protein crystallography laboratory in Israel. She pioneered ribosomal crystallography in 1980 and together with her coworkers, some of whom are coauthors of this manuscript, determined the high resolution structure of the small ribosomal subunit from *Thermus thermophilus* (2000) and of the large ribosomal subunit from *Deinococcus radiodurans* (2001). She is the Martin S. and Helen Kimmel Professor of Structural Biology at the Weizmann Institute of Science, the Director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly, a member of the Israeli, European, and US National Academies of Sciences, and the 2009 Nobel Prize Laureate in Chemistry.



dynamic tunnel and in eubacteria they emerge into a shelter formed by ribosome-bound trigger-factor which acts as a “chaperone” to prevent aggregation and misfolding.

The peptidyl transferase center (PTC) is the primary catalytic core of the ribosome. It is responsible for promoting the chemical catalysis required for peptide bond formation. We aim to define the minimal ribosomal component required for maintaining PTC structure and function, in order to shed light on the evolution of the ribosome. Since the discovery made in the 1960s that the ribosome can catalyze peptide bond formation between minimal substrates, e.g. puromycin and fMet-tRNA^[1] or CAACAA-formyl-methionine,^[2] in a process called the “fragment reaction”, efforts were made to identify the smallest set of ribosomal components that are capable of carrying out such a reaction. In reconstitution experiments performed in 1980, it was found that the large ribosomal subunit can be assembled in vitro from its isolated components to achieve active particles.^[3,4] Furthermore, it was found that the large ribosomal subunit per-se possesses peptidyl transferase activity even in the presence of only the 23S rRNA and 15 r-proteins, out of its “34 distinct components”.^[5]

A decade later it was shown that the large ribosomal subunit has catalytic activity even after vigorous treatment with proteinase K and SDS.^[6] Partial activity was also observed in reconstituted large subunits from T7 RNA polymerase transcribed 23S and 5S rRNA and r-proteins that were extracted from the 50S subunit.^[7] The smallest active sub-ribosomal particle obtained in those studies included both 5S and 23S rRNA and 8 ribosomal proteins.^[8] Yet, while the combination of several intact ribosomal components was shown to maintain some level of ribosomal activity, trials to obtain peptidyl transferase activity by designing minimal construct including only a small portion of 23S rRNA from the vicinity of the PTC were unsuccessful.^[9-11]

2. A Symmetrical Region at the Active Site

The ribosome provides the means for peptide bond formation, as well as the processivity of this reaction for amino acid polymerization. Both tasks are governed by the striking internal architecture of the ribosome; containing a region of 180 nucleotides, relating the rRNA fold and the orientations of its nucleotides by pseudo two-fold symmetry, but not their sequences. This sizable intra-ribosomal region is located (Figure 1) within the otherwise asymmetric ribosomal particle. It has been identified in all known structures of the whole ribosome and of the large ribosomal subunits, regardless of their source, their functional state, or their kingdom of life. Particularly, the same sub-structure was identified in the cores of ribosomes from mesophilic, thermophilic, radia-

tion resistant eubacteria, and halophilic archaea; in assembled empty ribosomal particles or in complexes of them with substrates; in unbound and complexed large subunit, including complexes with ribosomal antibiotics and non-ribosomal factors involved in protein biosynthesis.^[12,13] Furthermore, despite the significant size differences between ribosomes from various kingdoms of life, their functional regions are rather well conserved, sharing the highest sequence conservation level at their central core, whereas the largest structural differences are found at the periphery. Remarkably, this core contains the symmetrical region in which 98 % of the nucleotides are found in >95 % of sequences (from 930 different species from the three domains of life), whereas only 36 % of all *E. coli* nucleotides, excluding the symmetrical region, can be categorized as such. Importantly, 75 % of the 27 nucleotides lying within 10 Å from the symmetry axis are highly conserved. Among them, seven are completely conserved.^[13] This exceptional high conservation of the ribosome core, namely the symmetrical region, throughout evolution indicates that during its evolution it was less sensitive to environmental conditions, contrary to the large differences detected at the periphery of the ribosome, far away from the central core.^[14]

This symmetrical region includes the PTC and its environs, and connects all ribosomal functional regions involved in amino-acid polymerization, namely the tRNA entrance/exit dynamic stalks, the PTC, and the bridge connecting the PTC cavity with the vicinity of the decoding center in the small subunit and the nascent protein exit tunnel. Hence it appears that the internal symmetrical region is a universal feature facilitating peptide bond formation. Furthermore, as it is located at the heart of the ribosome and connects all of the functional regions involved in protein biosynthesis, it can serve as the central feature for signaling between them. Even those that are remotely located from each other (up to 200 Å away) must still communicate during the elongation process.^[12,13] A suitable example is the direct connection between peptide bond formation in the large subunit and the formation of the Shine-Dalgarno interactions in the small one.^[15]

3. Translocation of tRNA 3' End and Peptide Bond Formation

The PTC is located in the midst of this symmetrical region, at the bottom of a V-shaped cavity, and is built as an arched void. The complex of *D. radiodurans* 50S subunit with an acceptor stem mimic (D50S-ASM) demonstrates the significance of the interactions between the tRNA acceptor stem and the cavity's walls. These key interactions seem to govern accurate substrate positioning at the PTC, which has dimensions suitable for accommodating the 3' ends of the A- and P-site tRNA molecules^[16]

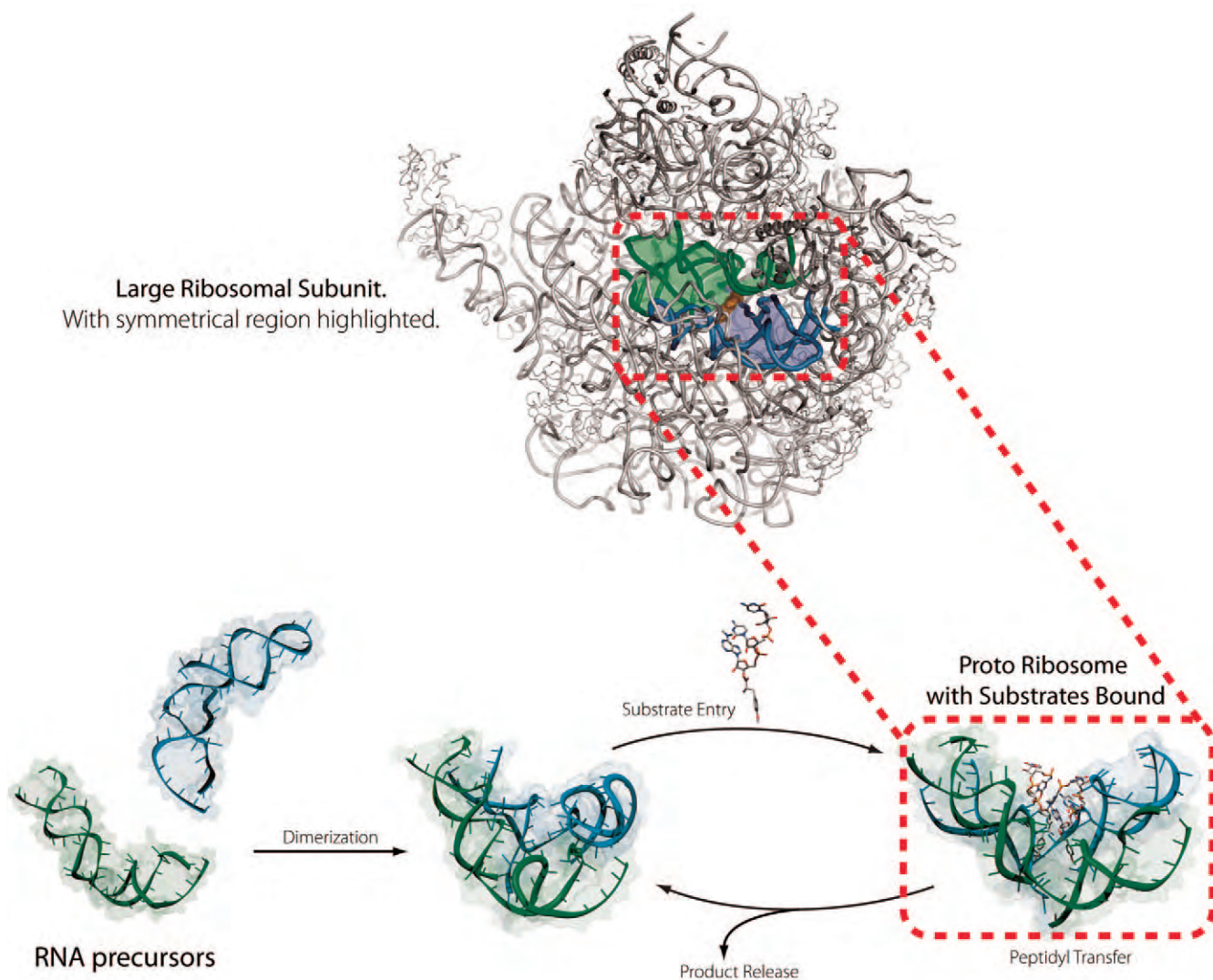


Figure 1. Top: the symmetrical region within the large ribosomal subunit. Bottom: Schematic representation of “pocket-like” proto-ribosome formation from a RNA precursor, showing simple catalytic peptidyl transferase activity.

with significant tolerance^[17] in configuration, allowing for peptide bond formation. The linkage between the elaborate architecture of the symmetrical region and the position of the A-site tRNA, as observed crystallographically,^[16] indicates that the translocation of the tRNA 3' end is performed by a combination of two independent, albeit synchronized, motions: a sideways shift, performed as a part of the overall mRNA/tRNA translocation, and a rotary motion of the A-tRNA 3' end along a path confined by the PTC walls (Figure 2).

This rotary motion is navigated and guided by the ribosomal architecture, mainly the PTC rear wall that confines the rotary path and the flexible nucleotides 2602 and 2585 (*E. coli* nomenclature) that anchor and seem to propel it (Figure 2). Hence, it appears that the ribosomal architecture and its mobility provides all structural elements enabling the ribosome to function as an amino acid

polymerase, including the formation of two symmetrical universal base pairs between the tRNAs and the PTC,^[12,16] a prerequisite for substrate mediated acceleration^[18] and for the direction of the nascent protein into the exit tunnel. Importantly, all nucleotides involved in this rotary motion have been classified as essential by a comprehensive genetic selection analysis.^[19] Furthermore, the rotary motion positions the proximal 2'-hydroxyl of P-site tRNA A76 in similar position and orientation to that found in crystals of the entire ribosome with mRNA and tRNAs, as determined independently in two laboratories,^[20,21] and allows for chemical catalysis of peptide bond formation by A76 of the P-site tRNA.^[18]

Remarkably, a high level of conservation of components of the symmetrical region was detected even in mitochondrial ribosomes, in which half the ribosomal RNA is replaced by proteins. This, together with the ability of

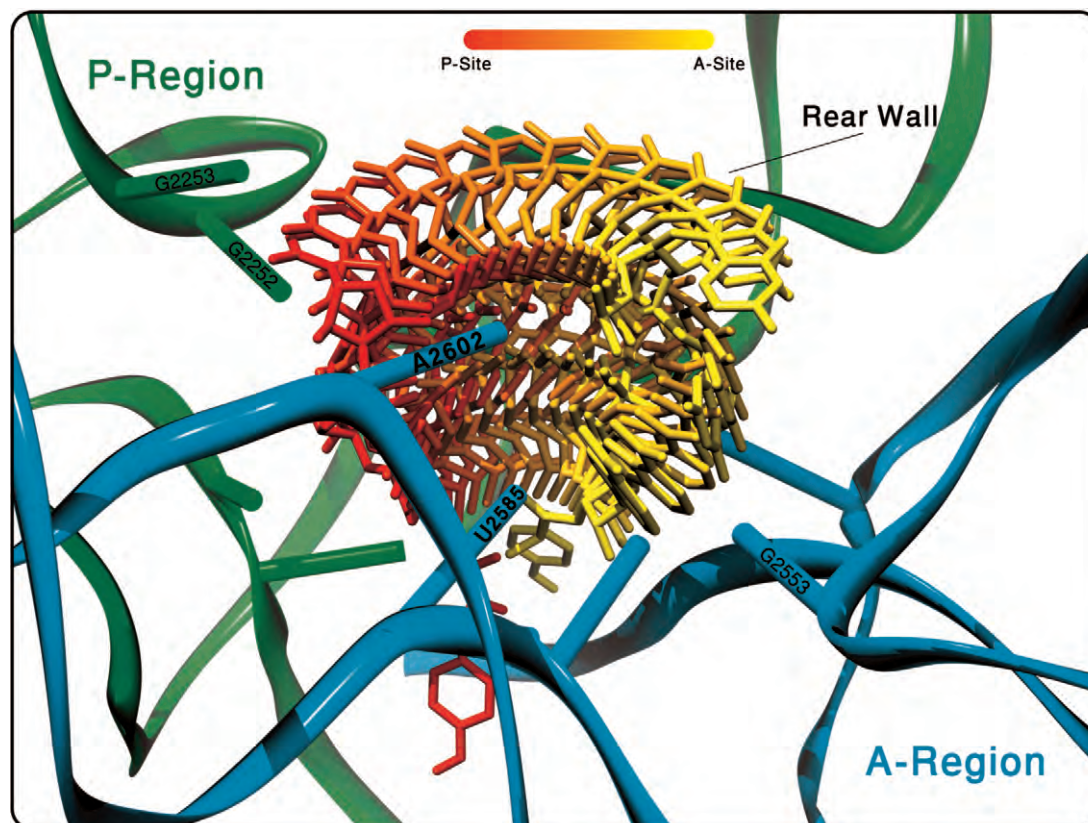


Figure 2. Snapshots of the rotary motion, obtained by successive 18° rotations from A- to P-site (yellow to red color) of the 3' end of ASM, around the bond connecting the 3' end with the rest of the tRNA molecule. The PTC boundaries scaffold the path of the rotary motion. The positions of A2602 and U2585, the nucleotides that anchor the rotary motion, are marked, as are the nucleotides that are in hydrogen bond contact with the tRNA 3' end at the A- and P-sites. The amino acids connected to the CCA end were removed from all snapshots, except for the initial and terminal ones, in order to minimize overlapping.

the symmetrical region to provide all structural elements required for performing polypeptide elongation, suggests that the modern ribosome evolved from a simpler entity that can be described as a pro-ribosome, by gene fusion or gene duplication. In particular, the preservation of the three-dimensional structure of the two halves of the ribosomal frame — regardless of the sequence — demonstrates the rigorous requirements of accurate substrate positioning in stereochemistry supporting peptide bond formation. This, as well as the universality of the symmetrical region, led to the assumption that the ancient ribosome contained a pocket confined by two RNA chains, which formed a dimer.

4. Presumed Sequence of Evolutionary Events

A feasible sequence of events, based on the hypothesis that the two halves of a structure resembling the symmetrical region were the core of the ancient ribosome, may shed light on the evolving complexity of the ribosome. The main assumption for this hypothesis is that the ancient machinery that could form peptide bonds was made

exclusively from RNA chains that dimerize. It is conceivable that among other substituents, the primordial soup contained single nucleotides, short RNA segments, and RNA chains of significant size (around 50–90 nucleotides) which survived because they acquired a stable conformation. These surviving ancient RNA chains could have been the ancestors of RNA chains which underwent dimerization, thus constructing a moiety resembling the symmetrical region.^[13]

The products of the dimerization yielded three-dimensional structures with a symmetrical pocket which could have performed chemical reactions. In particular, it could have accommodated two substrates facing each other. Substrates could presumably be amino acids, chemically activated by conjugation to oligonucleotide. This would have formed an enrichment trap for the amino acids once they became available. The spontaneous reaction of peptide bond formation followed, and the structures that include the appropriate pockets for accommodating this reaction continued to exist (Figure 1). Since RNA chains can act as gene-like molecules coding for their own reproduction, it is conceivable that the surviving ancient pockets became the templates for the ancient ribosomes. In a

later stage these primitive RNA genes underwent initial optimization to produce a more defined, relatively stable pocket, and when a clear distinction was made between the sites of the precursors — amino acids — and of the products — growing peptidyl chains — each of the two halves was further optimized for its task so that their sequences evolved differently. We assume that the modern ribosome evolved around the symmetrical region, in accord with results of analysis of the A-minor motif distribution within the ribosome performed elsewhere.^[22]

In parallel, the presumed substrates of the ancient ribosome evolved to allow more accurate binding to the RNA pocket. Compounds mimicking the termini of the modern tRNA molecules (part or the entire amino acylated CCA 3' end)^[23] provide more control of the reaction, its reactant, and its products. Consequently, these were converted into compounds with a contour that can complement the inner surface of the reaction pocket. The binding affinity could be increased by enlarging the nucleic acid component, exploiting double or triple nucleotides, similar to the universal CCA end of the modern tRNA molecules. Consequently, for increasing specificity, these short RNA segments were extended to larger structures by their fusion with stable RNA features similar to the tRNA acceptor stem, to form an ancient tRNA (called here proto-tRNA). Later, RNA chains capable of storing, selecting, and transferring instructions for producing useful proteins became available, and upon emergence of the small ribosomal subunit and decoding center those could code for the peptides that were more favorable, namely, useful. Subsequently, the decoding process was combined with peptide bond formation. The tRNA co-evolved as molecules capable of not only carrying the amino acids while bound to them, but also translating the genomic instructions, by adding a feature similar to the modern anticodon arm to the proto-tRNA.

5. Summary

The ancient ribosome appears to be a simple ribozyme that produces peptide bonds, utilizing activated amino acids. The formation of single peptide bonds could have been accidental or sporadic, thus not controlled by genetic instructions. Since the products of the simple peptide bond formation reaction may also be the substrates for it, elongation of the dipeptides could occur. In a later scenario, simple peptide-coding genes were co-evolved with molecules capable of decoding genetic information. These molecules gained capability of transporting the cognate pro-ribosome substrates evolved and the pro-ribosome acquired properties enabling the smooth translation of genetic information into proteins. This mechanism permitted faster and more robust evolution of useful polypeptides and proteins.

Our efforts are toward developing RNA constructs mimicking the function of the PTC, although possibly at lower efficiency. The uniqueness of our approach, compared to other attempts to define the minimal ribosome, is that we are constructing the proto-ribosome as an autonomic apparatus,^[24,25] from its core outward, whereas all others attempted to create a minimal machine by stripping off the ribosome r-proteins and/or segments of rRNA. Hence, we expect not only to construct the “minimal architecture” but also to aid understanding of ribosomal evolution and assembly.

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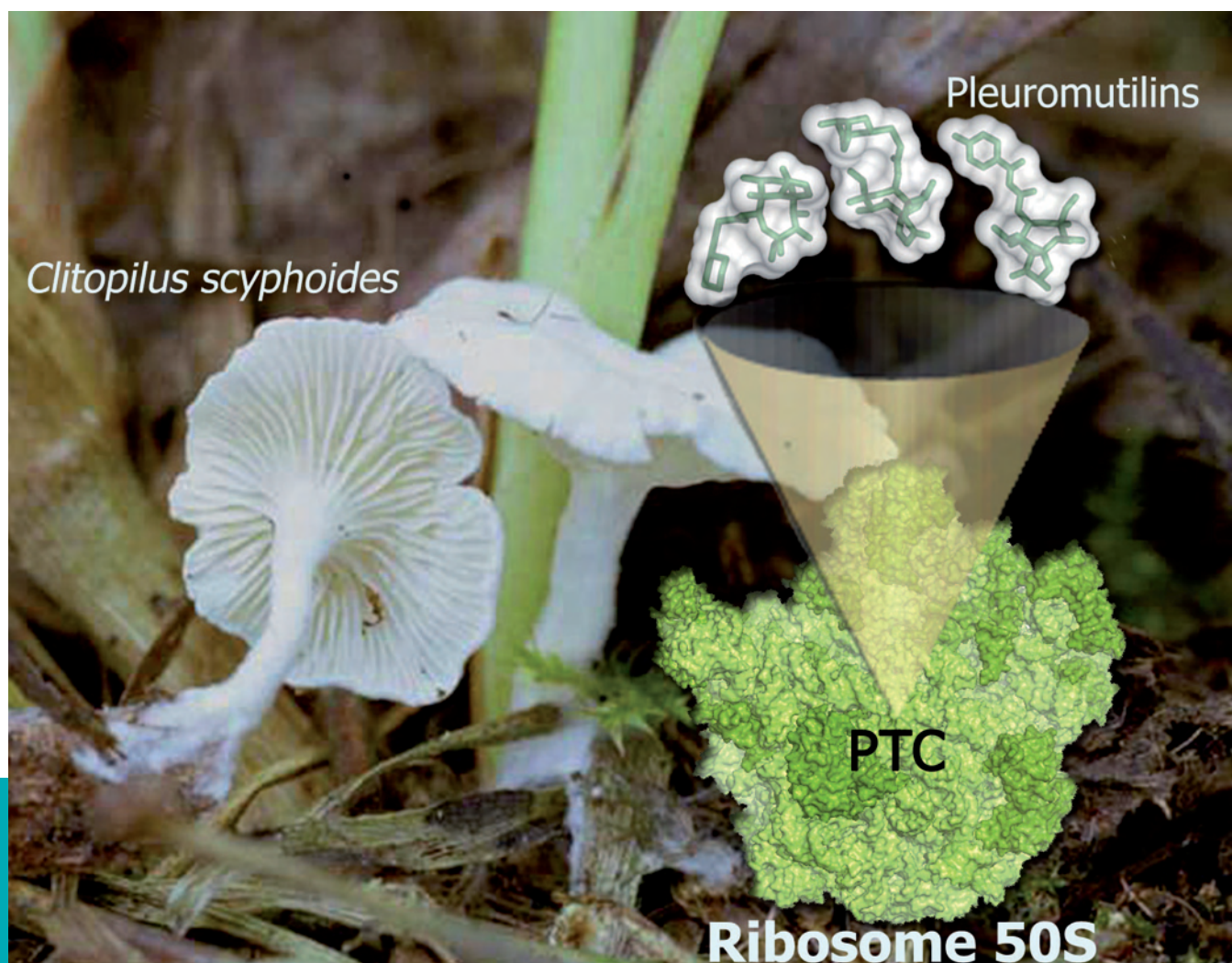
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Cover Picture

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The cover picture shows the ribosome 50S subunit on a fungal background. Represented fungi, *Clitopilus scyphoides*, produce antibiotics of the pleuromutilin family. Pleuromutilins “moving” into the ribosome Peptidyl Binding Centre (PTC) are also shown. The figure was contributed by Anat Bashan and Ada Yonath (see The Proto-Ribosome: An Ancient Nano-machine for Peptide Bond Formation, Davidovich et al., p. 29 in this issue).

