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ON RIBOSOME CONSERVATION AND EVOLUTION

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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- \rightarrow P-site passage of the tRNA terminus in the peptidyl transferase center is performed by a rotatory motion, synchronized with the overall tRNA/mRNA sideways movement. Guided by the PTC, the rotatory motion leads to stereochemistry suitable for peptide bond formation, as well as for substrate-mediated catalysis, consistent with quantum mechanical calculations elucidating the transition state mechanism for peptide bond formation and indicating that the peptide bond is being formed during the rotatory motion.

Analysis of substrate binding modes to inactive and active ribosomes illuminated the significant PTC mobility and supported the hypothesis that the ancient ribosome produced single peptide bonds and non-coded chains, utilizing free amino acids. Genetic control of the reaction evolved after polypeptides capable of enzymatic function were created, and an ancient stable RNA fold was converted into tRNA molecules. As the symmetry relates only the backbone fold and nucleotide orientations, but not nucleotide sequence, it emphasizes the superiority of functional requirement over sequence conservation, and indicates that the PTC has evolved by gene fusion, presumably by taking advantage of similar RNA fold structures.

Keywords: ribosome evolution, gene fusion, ribosomal symmetrical region, peptide bond formation, transition state

INTRODUCTION

Ribosomes are universal giant ribonucleoprotein cellular assemblies that act as polymerases translating the genetic code into proteins. They are built of two subunits of unequal size that associate upon the initiation of protein biosynthesis to form a functional particle and dissociate once this process is terminated. The bacterial ribosomal subunits are of molecular weights of 0.85 and 1.45 Mega Dalton. The small subunit (called 30S in

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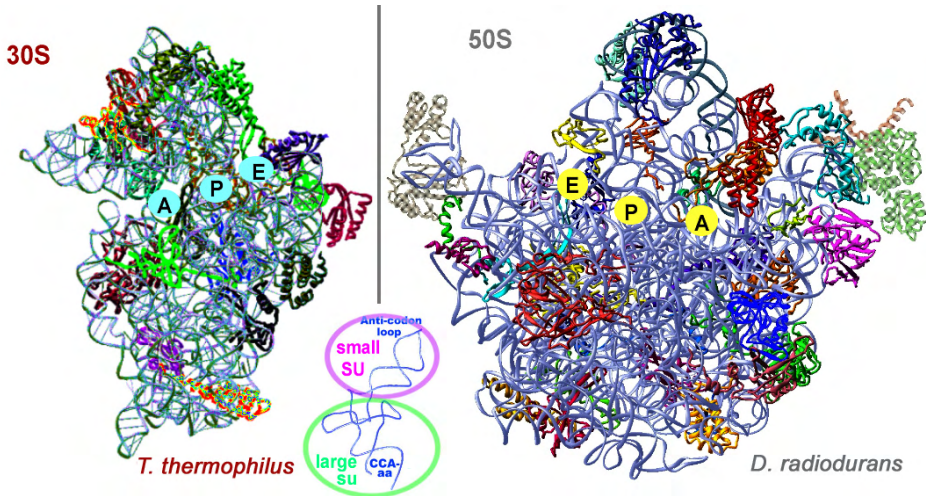


Fig. 1. The two ribosomal subunits. View at the intersubunit interfaces of the small (30S) and the large (50S) ribosomal subunit, from *Thermus thermophilus* (Schluenzen et al., 2000) and *Deinococcus radiodurans* (Harms et al., 2001), respectively. In both, the ribosomal RNA is shown as silver ribbons, and the ribosomal proteins main chains in different colors. A, P, and E designate the approximate locations of the A-, P-, and E-tRNA binding sites on the small and the large subunits. The regions of tRNA interactions with each subunit are shown on the tRNA molecule, inserted in the middle.

prokaryotes) contains an RNA chain (called 16S) of about 1500 nucleotides and 20–21 proteins, the larger one (called 50S in prokaryotes) having two RNA chains (23S and 5S RNA) of about 3000 nucleotides in total, and 31–35 proteins. Protein biosynthesis is performed cooperatively by the two ribosomal subunits (Fig.1). While elongation proceeds, the small subunit provides the decoding-center and controls translation fidelity, and the large one contains the catalytic site, called the peptidyl-transferase-center (PTC), as well as the protein exit tunnel.

mRNA carries the genetic code to the ribosome, and tRNA molecules carry the amino acids to the ribosome. tRNA molecules are L-shaped and are built mainly of double helices, but their two functional sites, namely the anticodon loop and the CCA 3'end, to which the amino acids are bound, are single strands. The ribosome possesses three tRNA binding sites, the A-(aminoacyl), the P-(peptidyl), and the E-(exit) sites. The tRNA anticodon loop interacts with the mRNA on the small subunit, whereas the tRNA acceptor stem, together with the aminoacylated or peptidylated tRNA 3'end, interacts with the large subunit (Fig. 1). Hence, the tRNA molecules are the entities combining the two subunits, in addition to the intersubunit bridges. The elongation cycle involves decoding, the creation of a peptide bond, the detachment of the P-site tRNA from the growing polypeptide chain, the release of a deacylated tRNA molecule, and the advancement of the mRNA, together with the tRNA molecules from the A- to the P- and then to the E-site, driven by GTPase activity. Being a ribozyme, decoding and peptide

bond formation, which are the main ribosomal functions, are performed in protein-free environments. Nevertheless, ribosomal proteins (r-proteins) situated in proximity to the active sites may support these functions by controlling their fidelity and/or facilitating specific activities, such as the ribosomal polymerase function. Examples are proteins S5, S12, L16, L27, and L2 (throughout, the numbering system is of *Escherichia coli* ribosome; r-proteins are named S or L according to their positions in the small or in the large subunit, respectively). S12 and S5 effect mRNA binding accuracy (Lodmell and Dahlberg, 1997); proteins L16 and L27 (Maguire et al., 2005) seem to assist the accurate positioning of the A-site tRNA in the PTC, and protein L2 allows for the polymerization of the nascent chains (Diedrich et al., 2000; Agmon et al., 2004, 2005).

Comparative sequence analysis identified regions of the ribosome that have been evolutionarily conserved to various extents. The highest level of conservation was detected mainly in functional domains. Conservation was detected in several levels: in the sequences of the rRNA, in the two-dimensional representations of the ribosomal RNA, and in three dimensions of the so far available crystal structures: that of the whole ribosome from *Thermus thermophilus* (T70S) in complex with tRNA molecules (Yusupov et al., 2001; Korostelev et al., 2006; Selmer et al., 2006); the empty ribosome from *E. coli*, E70S (Schuwirth et al., 2005); the small ribosomal subunit from *T. thermophilus* (Schluenzen et al., 2000; Wimberley et al., 2000), and the large subunits of an archaeon, *Haloarcula marismortui*, H50S (Ban et al., 2000), and from a eubacterium, *Deinococcus radiodurans*, D50S (Harms et al., 2001). Based on the ribosomal RNA sequences, extrapolation of the available structures from eubacteria and archaea to eukaryotic ribosomes showed that within the ribosome, the largest differences between widely diverged species are at the periphery, away from the central core (Mears et al., 2002).

This review article discusses features of the ribosome structure that may implicate its evolution. It focuses on ribosomal architectural elements that govern its function as a polymerase, portrays the quantum mechanical transition state of peptide bond formation in the ribosome, indicates where and when the peptide bond is formed, and suggests several stages along the sequence of events leading from primitive RNA chains to a highly complex molecular machine.

INTERNAL SYMMETRY — A UNIVERSAL FEATURE FACILITATING PEPTIDE BOND FORMATION

In all known structures of ribosomal complexes with substrate analogs (Nissen et al., 2000; Hansen et al., 2002; Schmeing et al., 2002, 2005a,b; Bashan et al., 2003a), the PTC is located at the bottom of a V-shaped cavity at the subunit interface (Fig. 2A) and is made exclusively of RNA, with dimensions suitable for accommodating the 3' ends of the A- and the P-site tRNAs. The PTC is situated in the middle of a universal sizable symmetrical region (Fig. 2B), which contains about 180 nucleotides and is divided into two subregions, called A- and P-regions according to their main components, namely the A- and P-loops (Agmon et al., 2003, 2005; Bashan et al., 2003). This symmetrical region extends far beyond the vicinity of the PTC and interacts, directly or through its exten-

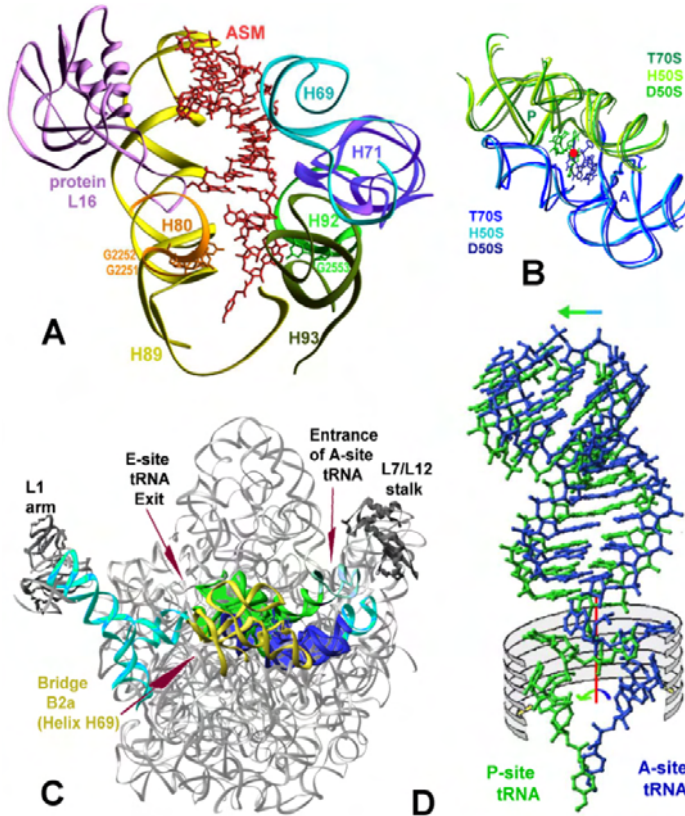


Fig. 2. The PTC and the symmetrical region within the large ribosomal subunit. (A) The cavity containing the PTC pocket (at its bottom), including ASM, a 35-nucleotide A-site substrate analog including the aa-CCA end and the tRNA acceptor stem (in red). Note the extensive network of remote interactions of the substrate, including the universal 3' end base pairs, a single base pair at the A-site, and two in the P-site, are shown. (B–D) Throughout, the subregion of the symmetrical region containing the A-loop, as well as A-site tRNA, are shown in blue, and that containing the P-loop, as well P-site tRNA, are green. The symmetry axis is shown in red. (B) Superposition of the backbone of the symmetrical regions in the entire ribosome, T70S (PDB 1GIY), and the large ribosomal subunits D50S (PDB 1NKW) and H50S (PDB 1JJ2). Note the A-site mimic ASM (PDB 1NJP) and the derived P-site, which are incorporated into this view. (C) The symmetry-related region within the large subunit (PDB 1NJP). Ribosomal RNA is shown as gray ribbons. The direct extensions of the symmetrical region are shown in cyan. The approximate positions of the entrance and exit of the tRNA molecules are marked. The gold feature is the intersubunit bridge (B2a) that combines the two ribosomal active sites. (D) A schematic presentation of the combined linear and rotational motions involved in the passage of the A-site tRNA from the A- to the P-site. The approximate contour of PTC walls is shown as gray-white ribs. The 2-fold symmetry axis is shown in red. The apparent overlap of the 2 tRNA stems is a result of the specific view (diagonal towards the back of the paper plane), chosen to show best the concerted motions.

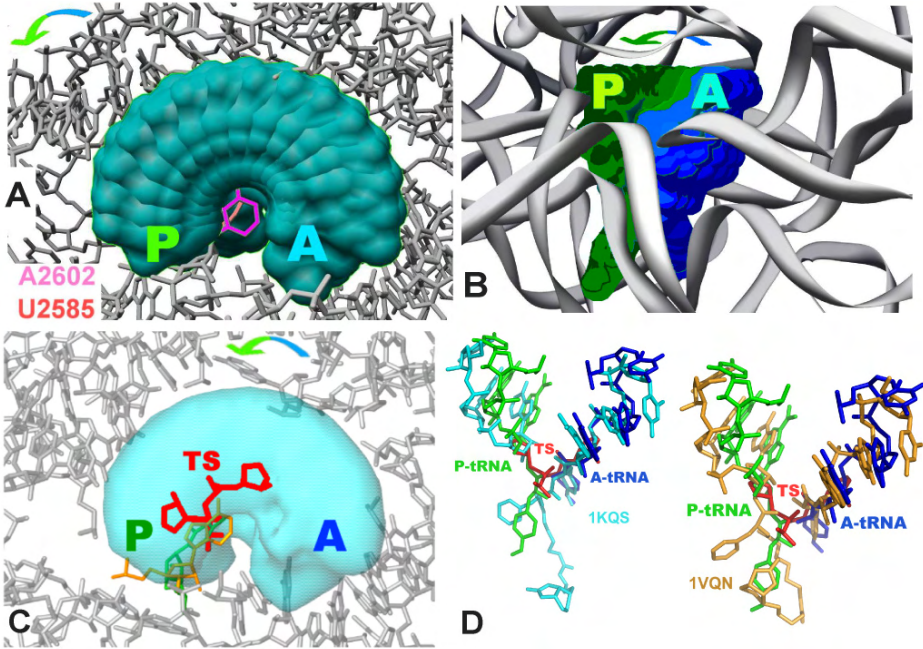


Fig. 3. The peptide bond is formed during the rotatory motion. Throughout, the ribosomal nucleotides are shown in gray, except for specific ones (whose colors are mentioned in the legend). A-site tRNA and the subregion containing the A-loop are shown in blue. P-site tRNA and the subregion containing the P-loop, are green. The symmetry axis is shown in red. The blue-green arrows indicate the approximate direction of the rotatory motion. (PDB 1NJP). (A–B). Two orthogonal views of the volume occupied by the rotatory motion within the ribosome environment, looking approximately down (A), and perpendicular to the 2-fold symmetry axis (B), empathizing the confinement of the rotatory path by the ribosomal components. In (A), all atoms are shown, whereas (B) shows only the RNA backbone. In (A), the two flexible nucleotides that anchor the rotatory motion, A2602 and U2585, are magenta and pink, respectively. Both views were produced by simulating the rotatory motion, every 15°, as described in Bashan et al. (2003a). (C) The TS position within the volume occupied by the rotatory motion in the peptidyl transferase center of the ribosome (same view as in A). The transparent cyan “cloud” shows the rotatory space (as in A), and the ribosomal nucleotides are shown in gray. The TS is shown in red and nucleotides A2602 and U2585 are yellow and light green, respectively. (D) The transition state (TS, red) and the CCA ends of the A- and P-tRNAs in D50S (PDB 1NJP), shown in blue and green, respectively, viewed from a direction similar to that shown in (B). Superposed are the structures observed in H50S bound to the substrate analogs representing (left) the “pre-translocation” state (PDB 1KQS, in cyan, Schmeing et al. (2002)), and (right) the “induced fit” product (PDB 1VQN, in gold, Schmeing et al. (2005)). Note that in the right the “meeting point” of both substrates resembles the TS orientation (Gindulyte et al., 2006) and the nascent chain seems to originate from the P-site, whereas on the left, in the “pre-translocation” state, the nascent chain seems to originate at the A-site.

sions, with all ribosomal functional features that are relevant to the elongation process: the stalks facilitating tRNA entrance and exit, the PTC, and the bridge (B2a) connecting the PTC cavity with the vicinity of the decoding center in the small subunit (Fig. 2C). The spatial organization of this region and its central location may enable signal transmission between the remote locations on the ribosome (Agmon et al., 2003, 2005).

Analysis of the crystal structures of complexes of substrate analogs with D50S (Bashan et al., 2003a) showed a clear linkage between substrate binding and ribosomal symmetry, namely, an approximate overlap of the bond connecting the tRNA 3' end with the rest of the molecule and the symmetry axis. This unique structural element indicates that tRNA A- to P-site passage is performed by a combination of two independent, albeit synchronized, motions: a sideways shift of most of the tRNA molecules, performed as a part of the overall mRNA/tRNA translocation, and a rotatory motion of the A-tRNA 3' end (Fig. 2D) along a path confined by the PTC walls and by two ribosomal nucleotides that bulge from the front wall into the PTC center (Fig. 3A,B). Simulation of the rotatory motion showed that the striking architecture of the PTC navigates and guides this motion and provides all structural elements enabling ribosome function as an amino acid polymerase, including ensuring proper elongation of nascent protein chains (Bashan et al., 2003a,b; Agmon et al., 2004, 2005) and eliminating D-amino acid incorporation in the growing protein chains (Zarivach et al., 2004). Importantly, the same nucleotides identified to be involved in the rotatory motion and peptide bond formation by the structural analysis have been classified as essential by a recent comprehensive genetic selection analysis (Sato et al., 2006).

The entire symmetrical region is highly conserved, consistent with its vital function. Sampling of 930 different species from three phylogenetic domains (Cannone et al., 2002) shows that 36% of all of the *E. coli* 23S RNA nucleotides, excluding the symmetrical region, are "frequent" (namely, found in >95% of the sequences), whereas within the symmetrical region, 98% of the nucleotides are categorized as such. Furthermore, 75% of the 27 nucleotides lying within 10 Å distance from the symmetry axis are highly conserved, among them, seven are absolutely conserved. Importantly, the ribosomal internal symmetry relates nucleotide orientations and RNA backbone fold, but does not apply to the sequences of the related nucleotides in the A- and the P-regions. 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 different RNA chains (Baram and Yonath, 2005).

FROM SINGLE PEPTIDE BOND FORMATION TO AMINO ACID POLYMERIZATION

Within the ribosome, peptide bonds are products of nucleophilic attack of the free amine of the amino acid attached to the A-site tRNA on the carbonyl carbon of the peptidyl tRNA. A cardinal question in this matter is: when and where is the peptide bond formed? In reactions terminating by the formation of a single peptide bond, the A- and P-site

tRNAs can remain in their positions. However, for maintaining the processivity of the reaction, namely for performing amino acid polymerization, this reaction is accompanied with the translocation of the tRNA from the A- to the P-site by the rotatory-shift motion. In principle, peptide bond can be formed either while A-site tRNA is still occupying the PTC A-site, during the rotatory motion, or after the A-site tRNA reaches the P-site. Based on biochemical and crystallographic experiments with minimal substrates (e.g., derivatives of puromycin, a natural product resembling the tip of the aminoacylated tRNA, which has been used extensively as substrate analogs and/or as ribosome inhibitors), it was suggested that the peptide bond is being formed at the A-site in a functional state called “the pre-translocation” (Schmeing et al., 2002). This implies that in each elongation cycle the A-site tRNA translocates to the P-site after peptide bond formation, together with the peptidyl bound to it, and then, following the release of the free tRNA from the P-site, the peptidyl elongating chain that temporarily resides at the P-site, is transferred to the A-site for the next peptide bond formation. Furthermore, according to this suggestion, such a mode of operation requires a rotation of the entire nascent chain by 180° in each elongation step, since A- to P-site translocation of the tRNA 3' end involves a rotatory motion. Such suggested mode of operation would be costly in energy and expensive in space, both rather limited considering the availability of energy and the size of the tunnel entrance.

To address this cardinal issue, we investigated the formation of the transition state (TS) of peptide bond formation using Quantum Crystallography. These studies showed that the TS is formed during the rotatory motion (Fig. 3C), and that the TS is stabilized by its interactions with the PTC walls, which make and break along the rotatory path (Gindulyte et al., 2006). These findings provide the following scenario: while rotating, the A-site tRNA becomes increasingly free; hence the new aminoacylated A-site tRNA can gradually slide into the evacuated space, in conjunction with the entire translocation motion. The TS is being formed and the bond connecting the P-site tRNA to the peptidyl breaks, enabling the release of P-site tRNA and the accommodation of the rotating A-site. This scenario can also explain why dipeptides produced by minimal substrates reside at the A-site when the initial orientation of the substrate allows peptide bond formation, but is not suitable for the rotatory motion (Yonath, 2003), and hence would not have undergone translocation.

Positioning reactants in orientation suitable for chemical reactions is performed by almost all bio-catalysts (Jencks, 1969, reissued 1987). Different from enzymes catalyzing a single chemical reaction and similar to polymerases, the ribosome provides the means not only for the chemical reaction, but also for the substrate motion required for the processivity of the reaction, namely for amino acid polymerization. As shown previously, a prerequisite for achieving the ribosome contribution is accurate substrate placement (Yonath, 2003), and obtained by remote interactions, as well as by the formation of two symmetrical universal base pairs between the tRNAs and the PTC (Kim and Green, 1999; Dorner et al., 2002; Bashan et al., 2003a; Weinger et al., 2004; Agmon et al., 2005). Since remote interactions cannot be formed by substrate analogs that are too short to reach the PTC cavity upper region (Nissen et al., 2000; Hansen et al., 2002;

Schmeing et al., 2002; Bashan et al., 2003a), their orientation in the PTC may not allow the rotatory motion. Nevertheless, owing to the PTC mobility (Harms et al., 2001; Yonath, 2003), semi-correct orientations may lead to a single peptide bond formation. As expected, owing to the elimination of the rotatory motion, the products of such reaction are likely to reside at the A-site (Fig. 3D, *left*) and be considered as “pre-translocational intermediates” (Schmeing et al., 2002).

Recent studies, performed on H50S complexes, suggested that the PTC undergoes induced fit when accommodating its substrates. Comparison between this induced fit conformation and those of native and complexed D50S (Harms et al., 2001; Bashan et al., 2003a) showed that part of the rearrangements of the PTC in H50S led to a conformation similar to that observed in native and/or substrate-bound D50S (Fig. 4), despite the differences in the specific substrate–PTC interactions in the two ribosomes. These findings confirm previous indications that the PTC in the crystal structure of H50S assumes an inactive conformation (Zamir et al., 1971; Bayfield et al., 2001), presumably because the H50S crystals were obtained under salt conditions remote from those suitable for efficient protein biosynthesis (Harms et al., 2001). Importantly, in a complex of H50S with A- and P-site minimal substrates that may mimic the actual reaction of peptide bond formation, the growing polypeptide originates from the P-site (Schmeing et al., 2005b) (Fig. 3D), consistent with the rotatory motion mechanism (Bashan et al., 2003a; Agmon, 2005), as well as with the TS calculations (Gindulyte et al., 2006).

In short, analysis of the differences between single peptide bond formation by minimal substrates and amino acid polymerization highlighted the remarkable ability of the PTC to rearrange itself from inactive conformation into an active form upon substrate binding. This, together with the significant tolerance of the PTC, is consistent with the suggestion that the ancient ribosome produced single peptide bonds (see below), exploiting a mechanism resembling, to some extent, that used for single peptide bond formation by minimal substrates.

THE EVOLVING COMPLEXITY OF THE RIBOSOME

Below, we portray a possible 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. In support of this hypothesis is the functional significance, as indicated by the similarity between the symmetrical regions of eubacteria and archaea (Fig. 2b). Analysis of the composition of mitochondrial ribosomes, in which a large part of the ribosomal RNA is replaced by ribosomal proteins, supported this hypothesis. Thus, the assumption that the ancient ribosome was made exclusively from RNA that could form peptide bonds implies that no essential rRNA would be converted into proteins in the mitochondrial ribosomal RNA. Indeed, the 3' half of the mitochondrial ribosomal RNA retains only the highly functional regions, such as the symmetrical region, the helical part that stabilizes the P-site, the region responsible for factor accommodation (called the sarcin–ricin loop), the region stabilizing the tRNA binding, and the bridge connecting the PTC and the small subunit. In contrast, the RNA extensions of the symmetrical

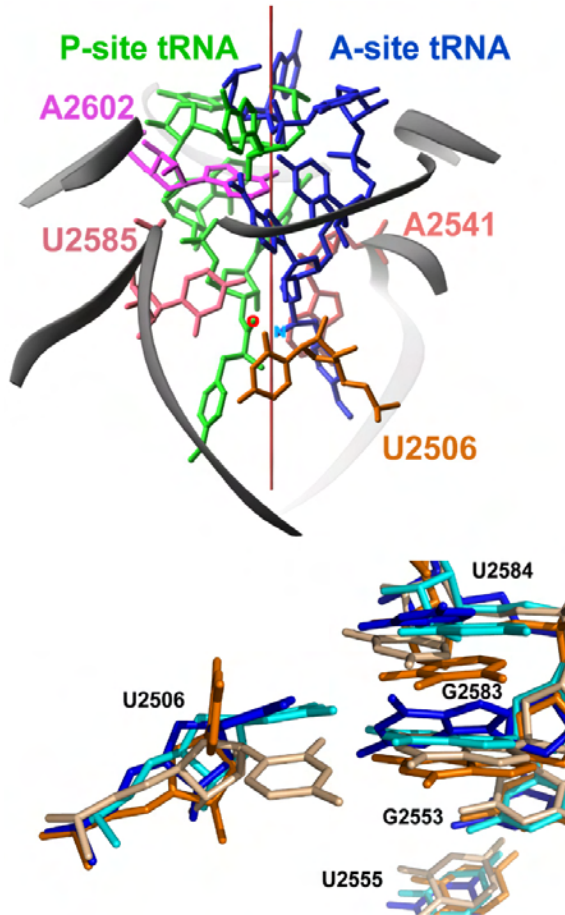


Fig. 4. The conformations of “induced fit” H50S and native D50S. *Top*: A view perpendicular to the symmetry axis of the D50S PTC backbone (in gray) together with the tip of the ASM substrate analog and its derived P-site (PDB 1NJP). Four of the nucleotides situated at the vicinity of the PTC are marked. *Bottom*: the H50S “induced fit” structure (PDB 1VQN, in brown), superposed on H50S native structure (PDB 1JJ2, in beige), D50S native (PDB 1NKW, in blue), and ASM bound (PDB 1NJP, in cyan) structures. Note the motion that U2506 underwent from its original position in H50S to the induced fit one, via the positions of this nucleotide in D50S.

region are mostly missing in the mitochondrial large subunit, suggesting that peptide bond formation could be formed without them, and that their additional functions can be performed by the proteins replacing them.

It is conceivable that among other substituents, the primordial soup contained single nucleotides, short RNA segments, and RNA chains of significant size, around 50–60 nucleotides (Huang et al., 2006), some of which survived since they acquired a stable

conformation, presumably similar to that of half the symmetrical ribosomal region. Similar to the tendency of small RNA molecules to form dimers (Kholod, 1999; Roy et al., 2005), these surviving ancient RNA chains underwent dimerization.

The products of the dimerization yielded structures with three-dimensional symmetry. Some of these dimers contained a symmetrical pocket that could accommodate two amino acids facing each other. Once amino acids became available (Miller, 1953), they could be trapped in this pocket. The spontaneous reaction of formation of the peptide bond followed, and the symmetrical structures that included the appropriate pockets for catalyzing this reaction thus became the ancient ribosomes. As RNA chains can act as gene-like molecules coding for their own reproduction, it is conceivable that the ancient pocket was made by homodimerization of two symmetrical accommodating sites so that each of them could accommodate the first amino acid. In later stages, these genes underwent fusion to produce a more defined, relatively stable pocket. Then, when a clear distinction was made between the amino acid and the growing peptidyl sites, each of the two halves was optimized for its task so that their sequences evolved differently.

Similarly, the substrates of the ancient ribosomes evolved to materials allowing higher specificity. Initially, the two termini of the dipeptides (the products of peptide bond formation) were free for subsequent reactions. Hence, longer products, polypeptides of various sizes, could be produced. To gain more control on the reaction and its products, the original substrates, the amino acids, were no longer suitable. Consequently, these were converted into compounds that could ensure their usage. Such compounds could be formed by the fusion between a nucleic acid and an amino acid, resulting in a structure with a contour that can complement the inner surface of the reaction pocket. An increase of the binding affinity could occur by enlarging the nucleic acid component, exploiting double or triple nucleotides, similar to the universal CCA end of the modern tRNA molecules. Later, for increasing binding accuracy, these short RNA segments were extended to larger structures by their fusion with RNA stable features to form the ancient tRNA.

Simultaneously, RNA chains capable of storing, selecting, and transferring instructions for producing useful proteins became available. Subsequently, the decoding process was combined with peptide bond formation. A single molecule evolved, 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 ancient tRNA structure. It is plausible that this ancient decoding–transporting molecule is the basis for the modern tRNA, consistent with the notion that RNA fragments showing tRNA-like structures existed in the primordial soup (Pan et al., 1991).

The availability of amino acids alongside ancient ribosomes and tRNA molecules, and the occasional production of functional proteins, can be associated with the creation of the ancient living cells. When cell complexity increased, a linkage between decoding and peptide bond formation allowing smooth operation of the ribosome became crucial. A second ribosomal particle, able to offer a path for binding the molecule containing the genetic information (mRNA), evolved. Efficient, concerted functions of the two ribosomal particles demanded full synchronization between them. Hence, larger particles with complimentary surfaces evolved, and hence, the fold of the ribosomal RNA chains

became more complex, thus requiring support. Such support could be provided by proteins. Consequently, the ribosome became a riboprotein assembly.

In addition, ribosomal function within the cell is dictated by cellular requirements, which are transmitted to the ribosomes by interactions with cellular components. Proteins were found more suitable than RNA for the selection between such interactions, since they provide molecular regions with different affinities. Consequently, increasing numbers of proteins became associated with the ribosome; most of them are multifunctional: stabilizing the RNA fold, performed mainly by their long tails and extended loops; interactions with cellular components, performed mainly by their globular domains residing on the surface; and involvement in ribosomal control mechanisms, which was found to be performed by both the globular and the extended domains. Examples are protein S18, which interacts with initiation factor 3 (Pioletti et al., 2001); protein L22, that is involved in tunnel gating (Berisio et al., 2003); and protein L23, which may control nascent protein trafficking (Baram et al., 2005).

Regarding amino acid polymerization, the focus of this article, proteins L16, L2, and L27 seem to be directly involved in it, although the three of them are not essential for peptide bond formation by minimal substrates. Proteins L27 (Maguire et al., 2005) and L16 are likely to assist A-site tRNA positioning, and therefore may be critical for enabling the rotatory motion, hence for the formation of the TS, as well as for nascent protein elongation. The contributions of L16 to tRNA positioning (Fig. 2A) can be described rather accurately by its interactions with substrate analogs containing the tRNA acceptor stem (Bashan et al., 2003a), but the way protein L27 influences substrate positioning is still unclear since its N-terminus, which was found to play the major role in protein biosynthesis, is not resolved in any of the D50S crystal structures (e.g., Harms et al., 2001). Nevertheless, based on preliminary modeling, it is possible that under specific conditions this region may reach the vicinity of the junction between A-site tRNA CCA end and its acceptor stem (Zarivach and Yonath, unpublished data).

Similar to the symmetrical region, function, rather than sequence conservation, is exhibited by ribosomal protein L16, as it displays conserved tertiary structure (Fig. 5) alongside diverged primary sequence. Consistently, results of recent experiments addressing the functional conservations of the ribosome, show that the translational factors functioning at the subunit-subunit interactions are conserved in *E. coli* and *T. thermophilus*, despite the highly divergent environments to which these species have adapted (Thompson and Dahlberg, 2004). Likewise, mutations in *T. thermophilus* 16S and 23S rRNAs, within the decoding site and the PTC, produced phenotypes that are largely identical to their mates in mesophilic organisms (Gregory et al., 2005).

The contribution of protein L2 to ribosomal polymerase activity may shed some light on ribosome evolution. Protein L2 is the only protein interacting with both the A- and the P-regions (Agmon et al., 2005) (Fig. 5). Among its residues involved in these interactions, one was shown to be essential for the elongation of the nascent chain (Cooperman et al., 1995). It appears, therefore, that the main function of L2 is to assist the elongation step, most likely by the stabilization of the symmetrical region conformation. Maintaining the ribosomal frame is mandatory for obtaining accurate substrate positioning. It is

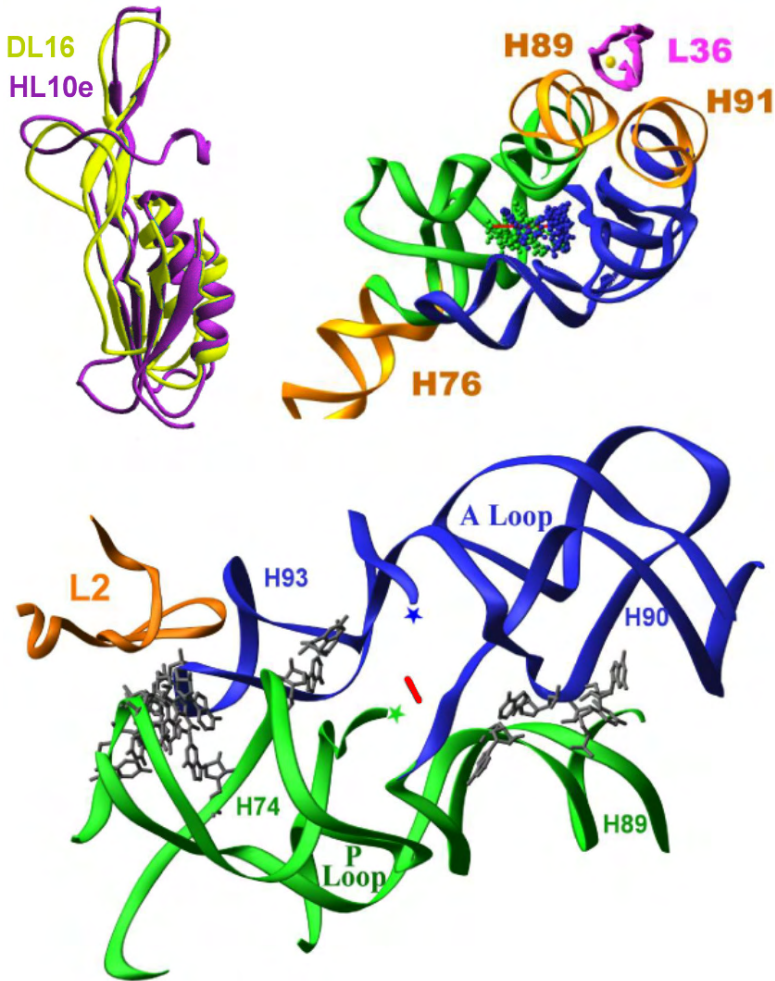


Fig. 5. Ribosomal proteins assisting amino acid polymerization. In all, A-site tRNA and the sub-region containing the A-loop are shown in blue. P-site tRNA and the subregion containing the P-loop, are green. The symmetry axis is shown in red (PDB 1NJP). *Top left*: the eubacterial L16 (as in D50S, PDB 1NJP, in dark yellow) and its mate (called HL10e) in the archaeon H50S (PDB 1JJ2, in purple). *Top right*: Protein L36 at its location between the extensions of the symmetrical region (in gold). *Bottom*: Protein L2 at its location at the contact area between the A- and the P-subregions. The gray nucleotides, as well as the stars, indicate direct contact between the 2 regions.

required for enabling the rotatory motion, but irrelevant to single peptide bond formation (Yonath, 2003).

Remarkably, computational methods showed that the most ancient ribosomal proteins are S12 and L2 (Sobolevsky and Trifonov, 2005). S12 is known to control the

correctness of mRNA binding, and L2 is essential for nascent chain polymerization, the two fundamental and essential ribosomal functions, allowing it to act as a polypeptide polymerase. The addition of these two proteins to the ancient RNA-only ribosomes is consistent with our hypothesis that the first proteins were incorporated in order to increase the fidelity and efficiency of the RNA-only ribosome.

Involvement in maintaining the symmetry region architecture can also be attributed to protein L36. This small Zn-containing protein is situated in the middle of four parallel helices that radiate from the symmetrical region and seems to stabilize their overall conformation (Fig. 5). The availability of an alternative route for signaling and/or alternative means for conformation preservation may account for the absence of L36 in some species, such as *H. marismortui*.

To summarize: we suggest that the ancient ribosome was a simple ribozyme that produces peptide bonds, utilizing free amino acids. The formation of single peptide bonds seems to be accidental or sporadic, thus not controlled by genetic instructions. Since the products of this reaction may be substrates for it, elongation of the dipeptides could occur. Only when these polypeptides acquired capacity to perform enzymatic tasks was the information about their desired structure stored in genes. Once a molecule capable of decoding this information simultaneously with transporting the cognate substrates evolved, the primitive ribosome acquired properties enabling the smooth translation of genetic information into proteins.

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