RNA, the first macromolecular catalyst: the ribosome is a ribozyme

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Recently, the atomic structures of the large ribosomal subunit from *Haloarcula marismortui* and its complexes with substrates have been determined. These have provided exciting new insights into the principles of RNA structure, the mechanism of the peptidyl-transferase reaction and early events in the evolution of this RNA–protein complex assembly that is essential in all cells. The structures of the large subunit bound to a variety of antibiotics explain the effects of antibiotic resistance mutations and provide promise for the development of new antibiotics.

The RNA world hypothesis gained great support and prominence from the discovery that the RNA molecules of tetrahymena Group I intron and RNase P catalyze enzymatic reactions [1,2]. These results established that, in addition to its capacity to function as a carrier of genetic information, RNA can serve as an enzyme. The existence of these ribozymes made the RNA world hypothesis plausible, namely that an early biological world once existed in which all enzymes were made of RNA rather than protein. However, given the functions of these ribozymes and those discovered subsequently, it could still be argued that they are not relics of an early stage of evolution but, rather, entities that evolved later. Although the existence of these ribozymes proves that RNA can be a catalyst and, thus, offers evidence for the possibility of a pre-protein RNA world, it does not prove it. Now that the ribosome has been shown to be a ribozyme there can be no doubt that there was a pre-protein RNA world.

The atomic structures of the large ribosomal subunit and its complexes with substrates and products prove that RNA is the catalytic component of the macromolecular assembly that synthesizes proteins [3–6]. Because the catalytic element that synthesizes proteins must have preceded proteins, the ribosome and, thus, ribozymes must have preceded protein enzymes. The ribosomal structures enable us to explore how these large RNA–protein machines are put together, to ponder how they might have evolved and suggest how they catalyze peptide-bond formation.

The ribosome is a macromolecular machine that carries out the mRNA-directed synthesis of proteins. It has two subunits, the larger of which sediments at 50S and has a mass of 1.5 megadaltons (MDa) in prokaryotes, and the smaller of which sediments at 30S and has a mass of 0.8 MDa; together they form an assembly that sediments at 70S. In *Escherichia coli* the ribosome is approximately two-thirds RNA, and the large subunit contains 34 proteins, and the small subunit 21 proteins. The small subunit contains the messenger decoding site, where interactions between codons in the mRNA and the anticodons of tRNAs determine the order in which amino acids will be assembled into protein. The larger ribosomal subunit contains the site of peptide-bond formation – the peptidyl transferase centre. The ribosome has three tRNA-binding sites: the A-site binds aminoacyl-tRNA, the P-site binds peptidyl-tRNA and the E-site interacts with deacylated tRNAs that are being discharged from the ribosome. In addition to requiring a small subunit, which specifies the identity of the tRNA to be bound, and a large subunit, which stitches the polypeptide together, this assembly line machine makes use of two protein-factor components: (1) protein elongation factor Tu (EF-Tu), which delivers the aminoacylated-tRNAs to the ribosome and (2) protein elongation factor G (EF-G), which moves the assembly line device along its mRNA subsequent to peptide-bond formation. EF-Tu delivers aminoacyl-tRNA molecules to the ribosome and leaves upon hydrolysis of GTP only when the correct cognate tRNA has been delivered. EF-G, which is also GTP driven, facilitates the translocation of tRNA and mRNA after peptide-bond synthesis. Recent atomic structures of the large and small ribosomal subunits together with decades of biochemical and genetic studies of the ribosome have begun to elucidate, in atomic detail, how this 2.4 million molecular weight RNA–protein machine carries out a function that is central to all biology.

Structural studies of the ribosome

Electron microscopy played a key role in the early structural studies of the ribosome, beginning with the pioneering work of Palade [7] that contributed to the discovery of the ribosome, the first determination of the shapes of the large and the small subunits in the early 1970s [8], and continuing with the cryo-electron microscopic investigations that have advanced to increasingly higher resolution [9,10]. At present, however, the only way to obtain an atomic structure of an assembly as large as the ribosome is by X-ray crystallography, and because this has

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long been evident, one might understandably ask why it took so long and what the events were that ultimately led to atomic structure determination. The essential first step – the growth of the first crystals of ribosomal subunits – was accomplished by Yonath and Wittmann in the early 1980s [11]. The first crystals, although exciting in their prospect, diffracted poorly. Greater success was achieved with subunits obtained from other extremophiles, either thermophiles or halophiles, possibly because they are more robust to purification and perhaps because they are less flexible. Crystals of the 50S ribosomal subunit from *Haloarcula marismortui* (an archaeal halophile from the Dead Sea), first grown by Yonath and her collaborators in the mid-1980s, and their diffracting qualities were improved and by 1991 they diffracted to a resolution of 3 Å [12]. Although these crystals possessed the marvellous property of diffracting to a resolution that makes an atomic-level structure determination possible in principle, they retained several pathologies. They were extremely thin, multiple, prone to non-isomorphism, radiation-sensitive and, often, as it turned out, twinned.

Although crystals are essential for structure determination, the challenge in solving the structure of such a large assembly is obtaining the phases associated with the hundreds of thousands of diffraction amplitudes their crystals yield. Just as the determination of the structure of myoglobin was a challenge because it was approximately one order of magnitude larger than the largest molecular structure that had been determined previously (in the 1950s), the determination of the ribosome structure posed a similar challenge in the mid-1990s because it too was approximately one order of magnitude larger than the next largest asymmetric macromolecular structure that had been solved up to then. The only approach that appeared likely to succeed was the method of heavy-atom isomorphous replacement pioneered by Max Perutz, in which heavy atoms are bound to specific sites in the crystal to make a derivative, and to follow the strategy of Kendrew, who began his myoglobin studies at low (5.5 Å) resolution before proceeding to higher resolution. The Yale group (Nenad Ban, Poul Nissen, Jeffrey Hansen, Peter Moore and Thomas Steitz) began its crystallographic studies of the *H. marismortui* 50S subunit at very low resolution (16 Å), which vastly decreased the effort required to assure that heavy-atom-derivative data were correctly analyzed and increased the diffraction signal obtained from heavy-atom-cluster compounds [13].

Because the macromolecular assembly to be solved was supersized, a supersized heavy-atom compound was required. An 18-tungsten cluster compound – which, at the low resolution of 16 Å, has a diffraction signal that approaches 300 times that of a single tungsten atom – was used for the first derivative. To check that the positions of the tungsten-cluster compound bound to the 50S subunit had been correctly determined crystallographically, a cryo-electron microscopic reconstruction of the subunit determined at 20 Å resolution was used to phase the low-resolution diffraction from the 50S crystals by molecular replacement. A difference electron-density map of the tungsten derivative calculated using these phases established that the heavy atoms were indeed correctly positioned [13].

The first electron-density map of a ribosomal subunit that showed features expected of RNA was the 9 Å resolution map of the *H. marismortui* 50S ribosomal subunit that was published in 1998 by the Yale group [13]. This map proved that it was possible to solve the phasing problem for diffraction from crystals of objects as large as the ribosome. The strategies developed for the large ribosomal subunit were quickly followed by Venki Ramakrishnan [14], who was studying the 30S subunit, and James Cate and Harry Noller [15], who were investigating the 70S ribosome. The structure of the large subunit was eventually refined at 2.4 Å resolution (Fig. 1) and ~100 000 atoms of RNA, protein, water and metal ions were placed with an accuracy approaching that of the best determined protein structures [3]. The coordinates deposited for the 3000 nucleotides of RNA and 27 proteins increased the known structural database for RNA by an approximate factor of four or five and revealed a surface area of protein–RNA interaction that is 30 times the surface area of $tRNA^{Gln}$ that contacts Gln-tRNA synthetase. The structure determination has also made it possible to examine the structures of substrate and product-like ligands bound to the peptidyl transferase active site as well as a variety of antibiotics that target the peptidyl transferase centre and inhibit protein synthesis. These structures have provided insights into the principles of RNA architecture, the mechanism of the peptidyl transferase reaction and the evolution of this ancient macromolecular machine.

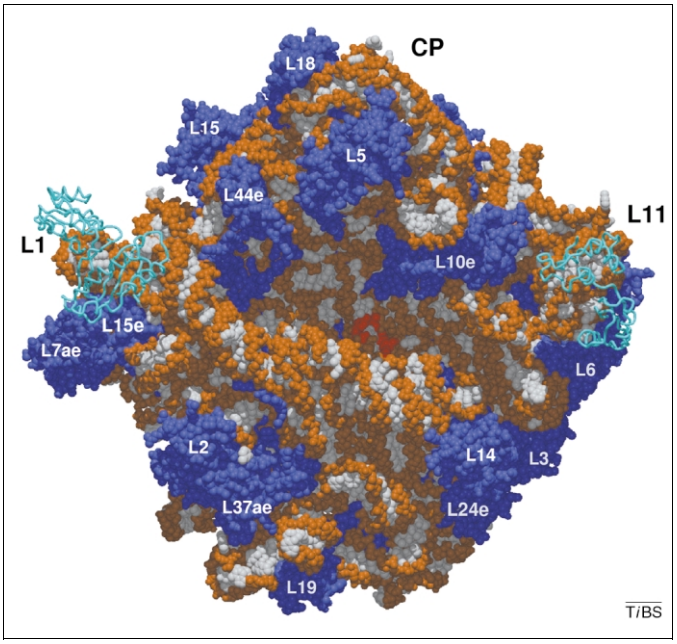

Fig. 1. A space-filling model of the large ribosomal subunit from *Haloarcula marismortui* with a transition-state analogue bound viewed down the active site cleft. Bases are white, the sugar-phosphate backbone is orange and the substrate analogue is red. Proteins whose structures are established by the 2.4 Å resolution map are blue. Cyan ribbons represent proteins whose structures are independently known and have been approximately positioned using lower-resolution electron-density maps. Identification numbers are provided for all proteins, and 'CP' designates the central protuberance. Reprinted, with permission, from Ref. [4]. © (2003) American Association for the Advancement of Science (http://www.sciencemag.org).

Organization and stablization of the 50S subunit structure

Early investigators of 23S rRNA could only wonder in amazement that this large polyanion, of nearly 3000 negative charges, could fold to form a compact and stable structure [16]. We can now see how this is achieved. Three kinds of interactions stabilize the tertiary structure of 23S and 5S rRNA: (1) Mg^{2+} bridges, (2) RNA–RNA interactions that are largely of two types – long-range base pairs and a newly identified interaction called the A-minor motif, and (3) RNA–protein crosslinks. The 23S rRNA can be divided into six domains on the basis of a secondary structure [17], and the 5S rRNA can be thought of as the seventh domain. of the subunit. The shapes of all of these domains are highly irregular, although they fit together like the pieces of a jigsaw puzzle to form a compact object whose overall shape is essentially that of the entire subunit [3]. The interactions between rRNA domains and the large subunit are so extensive and intimate that it is not possible to tell by visual inspection where one domain ends and the next begins; the RNA structure of the subunit is monolithic, which is different from the domain organization of the small ribosomal subunit (Fig. 1).

Perhaps not surprisingly, magnesium ions play an important role in stabilizing the compact structure of 23S rRNA by interacting with two or more phosphate groups from secondary-structure elements that are remote in the sequence, thereby allowing their close proximity [3]. These shared Mg^{2+} ions form a neutralizing link between phosphate groups whose non-bridging oxygens may be either inner- or outer-sphere ligands. Approximately 65 of the 108 Mg^{2+} ions identified thus far in the *H. marismortui* large ribosomal subunit stabilize the tertiary structure of 23S rRNA this way. In addition to single Mg^{2+}-bridging sugar–phosphate backbones that are remote in the sequence, there are clusters of two or three Mg^{2+} ions that function the same way. There are also numerous monovalent cations bound to specific locations where they neutralize the negative charge of the phosphate and, thus, assist in specific rRNA positioning.

A major portion of both the stability and specificity of the tertiary structure arises from specific RNA–RNA interactions, which largely involve the bases. There are base-pairs between nucleotides associated with different secondary-structure elements, many of which had previously been predicted by phylogenetic sequence comparisons [18]. There are ~100 such long-range base-pairs in *H. marismortui* 23S rRNA. An even more significant contribution to the RNA structure is made by a newly recognized interaction between adenine and the minor groove of RNA helices – an interaction that we term the 'A-minor motif' [19] (Fig. 2).

Adenines are disproportionately abundant in the non-helical sequences of 23S rRNA, and many of these are completely conserved among the three kingdoms of life [20,21]. The adenine in an A-minor motif inserts its minor-groove face into the minor groove of a base pair in a helix, most often a GC pair, where it forms hydrogen bonds with one or both of the backbone 2′ OH groups of the duplex (Fig. 2). Often, two or three consecutive adenines in a single-stranded region interact with successive base pairs of a helix in this way. There are 186 A-minor interactions in the *H. marismortui* large ribosomal subunit, and 68 of them involve adenines that are conserved across all three kingdoms [19]. A-minor motifs have both functional and structural significance. For example, the 3′-terminal adenines of tRNAs bound in either the A-site or the P-site make A-minor interactions with 23S rRNA base pairs in the peptidyl transferase region of the large ribosomal subunit [19]. A-minor interactions also play an important role in assuring the fidelity of messenger decoding by the small ribosomal subunit, where A1492 and A1493 in 16S rRNA interact via the minor groove with

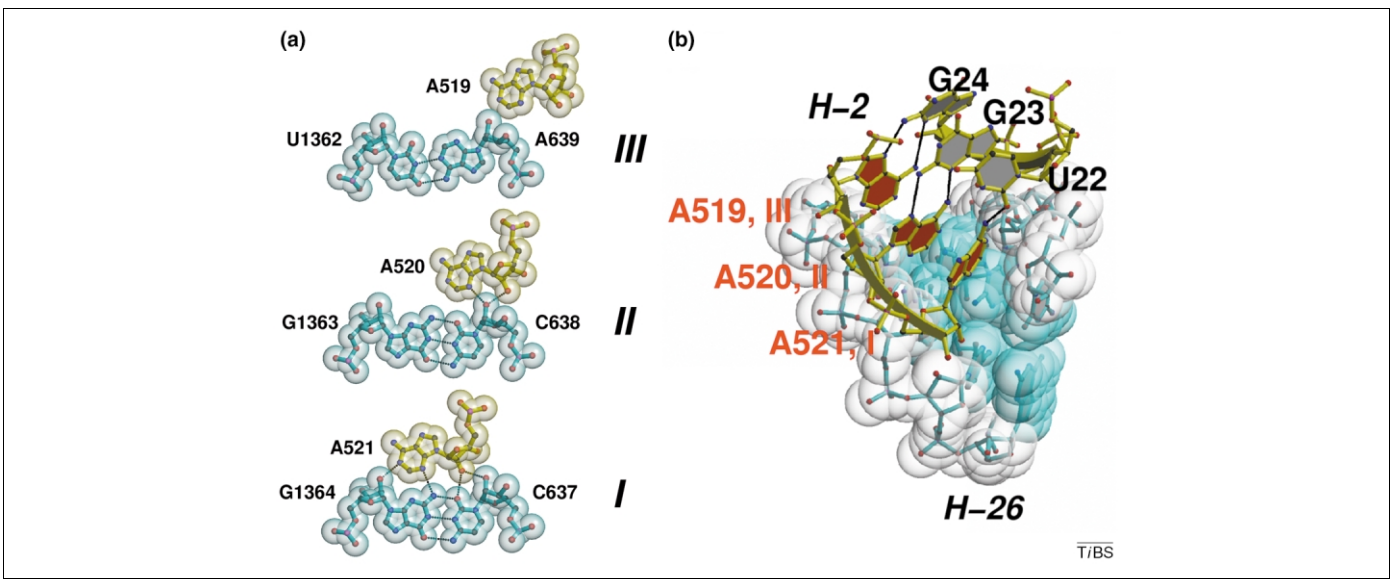

Fig. 2. The A-minor motif, an RNA–RNA interaction involving adenosines interacting in the minor groove of helices. (a) Examples of the three most prevalent kinds of A-minor motifs from the 23S rRNA of *Haloarcula marismortui*. Types I and II can only be formed by an adenine that makes specific interactions. Type III interactions can be made by other bases, but adenine is preferred. (b) The interaction between three consecutive adenines (an 'A patch') in helix (H-2 in yellow ball and stick with adenine bases shaded red) and the minor groove of helix 26 (H-26) shown in space-filling representation with underlying stick. A519, A520 and A521 of helix 2 make type III and type II and type I interactions, respectively. Reprinted, with permission, from Ref. [19]. © (2003) National Academy of Sciences, U.S.A.

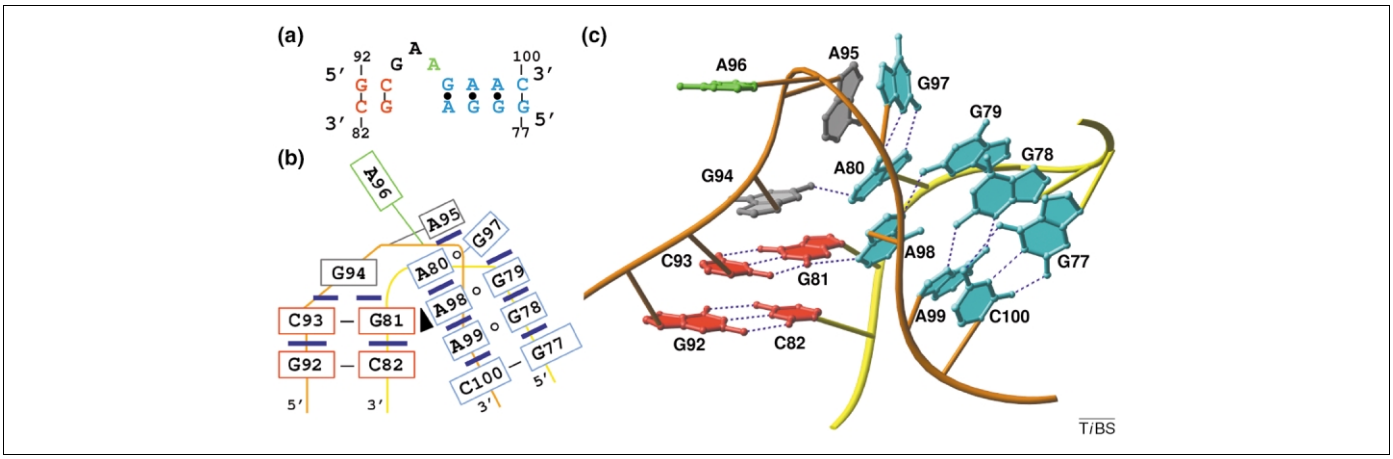

Fig. 3. The structure of kink-turn 7 (KT-7) in the 23s rRNA of the *Haloarcula marismortui* ribosome. (a) The secondary structure of KT-7. The GC base-paired stem is red, the non-canonical base-paired stem is blue, and the bulged nucleotide is green. (b) Base pairing and stacking interactions in kink. The black triangle identifies an A-minor interaction. (c) KT-7 in three dimensions. The backbone of the kinked strand is orange, and that of the unkinked strand is yellow. Broken lines indicate hydrogen bonds. Reprinted, with permission, from Ref. [23].

a correctly paired codon–anticodon but not incorrectly paired codons and anticodons [22].

RNA–protein interactions

Interactions between 27 of the 31 proteins of the large subunit and rRNA are clearly crucial for the specific folding and stability of the large ribosomal subunit. Unlike proteins that bind to specific DNA sequences, ribosomal proteins bind to their specific RNA sites by recognizing unique RNA shapes through interactions that are largely with the sugar–phosphate backbone rather than through interactions with bases that would be sequence specific [3]. Twenty-three of the 30 proteins that interact with RNA contact two rRNA domains or more. The 'champion' in this regard is L22, which is the only protein that interacts with sequences belonging to all six of the 23S rRNA domains. Among the unique secondary structures recognized by ribosomal proteins is a new RNA-secondary-structure motif that we call the 'kink-turn' or 'K-turn' [23] (Fig. 3). There are six K-turns in the 23S rRNA of *H. marismortui*, and they associate with about a third of the proteins in the large subunit. The RNA sequences that form K-turns have an asymmetric internal loop that is flanked by GC base pairs on one side and sheared GA base pairs on the other; an A-minor interaction occurs between these two helical stems. This structural motif has a kink in the phosphodiester backbone that causes a sharp turn in the RNA helix. The K-turns interact with proteins of unrelated structures in different ways, but interact with L7Ae and two homologous non-ribosomal proteins in the same way.

The most unexpected features of ribosomal proteins are the tails that many of them possess, which penetrate into a forest of RNA helices within the interior of the ribosome [3] (Fig. 4). Although the globular regions of all large subunit proteins are partially exposed to solvent on one side and interact extensively with RNA on the other, 12 proteins include at least one sequence of significant length that has an extended, non-globular structure. Viewed in isolation these protein tails, which comprise ~26% arginine plus lysine and with abundant glycine and proline residues, look like random coils, and probably assume the conformations they display in the ribosome only upon interacting with rRNA. However, they are not random because the sequences of these protein tails are even more conserved than the sequences of the globular domains to which they are attached. They extend into the interior of the ribosome filling the gaps between RNA helices where they interact intimately and specifically with RNA groups all over their entire lengths.

Only 19 of the 31 proteins in this archaeal large ribosomal subunit show clear sequence homology to proteins in the eubacterial large subunit, but all 31 proteins are homologous to eukaryotic ribosomal proteins [3]. Almost all of the 19 proteins that are conserved across kingdoms have known important functions. In the recent structure of a eubacterial large ribosome subunit from *Deinococcus radiodurans* [24], six proteins that are not homologous to any *H. marismortui* proteins are bound in similar locations to the six *H. marismortui* proteins that are homologues of eukaryotic proteins. A few of the eubacterial and archaeal proteins are located in partial or non-overlapping positions. Thus, it appears that, at the time that the eubacterial kingdom diverged from the archaea and eukaryotes, the large ribosomal subunit had only 20 proteins.

The ribosome is a ribozyme

Of all the observations that have arisen from these structural studies of the large ribosomal subunit, the one that has the greatest functional and evolutionary significance is the finding that the site of peptide bond synthesis – the peptidyl transferase centre – is composed entirely of RNA [4]. Because the ribosome will catalyze peptide-bond formation using substrates that are small fragments of the aminoacyl- and peptidyl-tRNA substrates used by the full ribosome (Fig. 4), it has been possible to diffuse these substrates, as well as analogues of the reaction intermediate, into crystals and to establish their structures bound to the peptidyl transferase centre. Indeed, when an aminoacyl-CCA, which binds to the A-site, and a peptidyl-CCA, which binds to the P-site, are diffused into the crystals, the product CCA is observed in the P-site and an elongated peptidyl-CCA is observed in the A-site (Fig. 5), showing

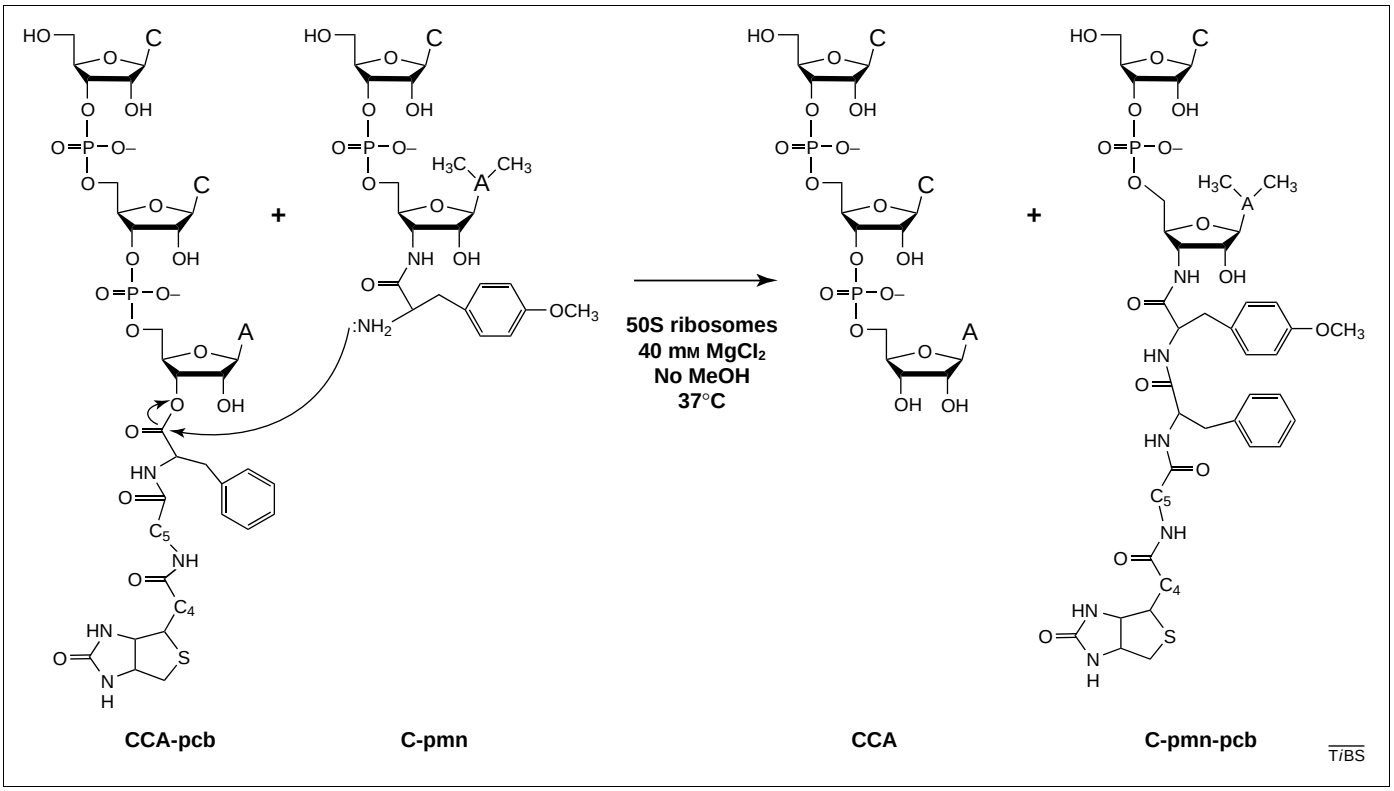

Fig. 4. A ribosome-catalyzed peptide-bond-forming reaction involving low molecular weight substrates. The reaction of CCA-phenylalanine-caproic acid-biotin (CCA-pcb), and C-puromycin (C-pmn) that yields CCA and C-puromycin-phenylalanine-caproic acid-biotin (C-pmn-pcb) is catalyzed by large ribosomal subunits. Reactions of this type are analogous to the peptidyl transferase reaction that occurs on intact ribosomes *in vivo*, and is referred to as the 'fragment reaction', because its substrates resemble the 3′ termini of aminoacyl- and peptidyl-tRNAs. Reprinted, with permission, from Ref. [5] (http://www.nature.com/nsb/).

that the ribosome subunit is catalytically active in the crystals [5].

The crystal structures of the large ribosomal subunit complexed with substrate and product analogues show that only rRNA is involved in the positioning of the A- site and P-site substrates, and only RNA is in a position to chemically facilitate peptide-bond formation [4–6]. A peptidyl-CCA bound in the P-site has its C74 and C75 base-paired with two guanine residues of the P-loop and, correspondingly, the aminoacylated-CCA bound at the A-site has its C75 base-paired with a guanine residue of the A-loop. Furthermore, the A76 bases of the tRNA molecules bound in both the A- and P-sites make A-minor interactions. The orientations of the two single-stranded CCAs bound in these two sites are related by a twofold rotation axis in spite of the fact that the tRNA molecules to which they are attached are related to each other by a translation. The proposal [4] that this difference in the orientations of the 3′ termini of the two tRNA molecules might help facilitate their translocation after peptide-bond formation is, as yet, untested.

Although the structure of most of the subunit, including the peptidyl transferase centre, remains unchanged upon substrate binding, several nucleotides exhibit significant alterations in their positions. The most dramatic change is A2637 (A2602 in *E. coli*) whose base becomes positioned between the A-site and P-site CCAs and interacts with both. Likewise, the base of U2620 (U2585 in *E. coli*) moves and lies adjacent to the nascent peptide bond, and a possible role in peptide-bond formation is not ruled out.

Although the structure of the large subunit containing both substrates bound simultaneously has not yet been established, an approximation of this complex can be achieved by superimposing the structures of the separately determined A-site and P-site substrate complexes (Fig. 5c). In this hypothetical two-substrate complex, the α-amino group of the A-site amino acid is adjacent to the ester-linked carbonyl carbon of the peptidyl-tRNA it is to attack [6]. This positioning of the reactants by the ribosome alone might explain most of the catalytic-rate enhancement provided by the peptidyl transferase centre.

We still do not know the extent to which or how the peptidyl-transferase centre might also chemically enhance the rate of peptide-bond formation. Importantly, no protein moiety is observed closer than 18 Å to the site of peptide-bond formation. Thus, protein cannot contribute to catalysis and, at present, there is no evidence for metal ion involvement. However, in the current model for A-site and P-site substrates bound to the centre (Fig. 5c) there are three RNA groups close enough to the reaction site to form a hydrogen bond with the attacking α-amino group: (1) the 2′ OH of A76 of the tRNA in the P-site, (2) the N3 of A2486 (A2451 in *E. coli*) of 23S rRNA, and (3) the 2′ OH of A2486. In part, these hydrogen-bond interactions help align the α-amino group for its nucleophilic attack, and there is reason to believe that hydrogen-bond formation by itself could enhance the reactivity of the α-amino group by two orders of magnitude [25]. A P-site substrate containing a 2′ deoxy A76 is inactive in peptide-bond formation [26], consistent with a possible role for that 2′ OH in catalysis. Furthermore, mutation of A2486 (A2451 in *E. coli*) to a

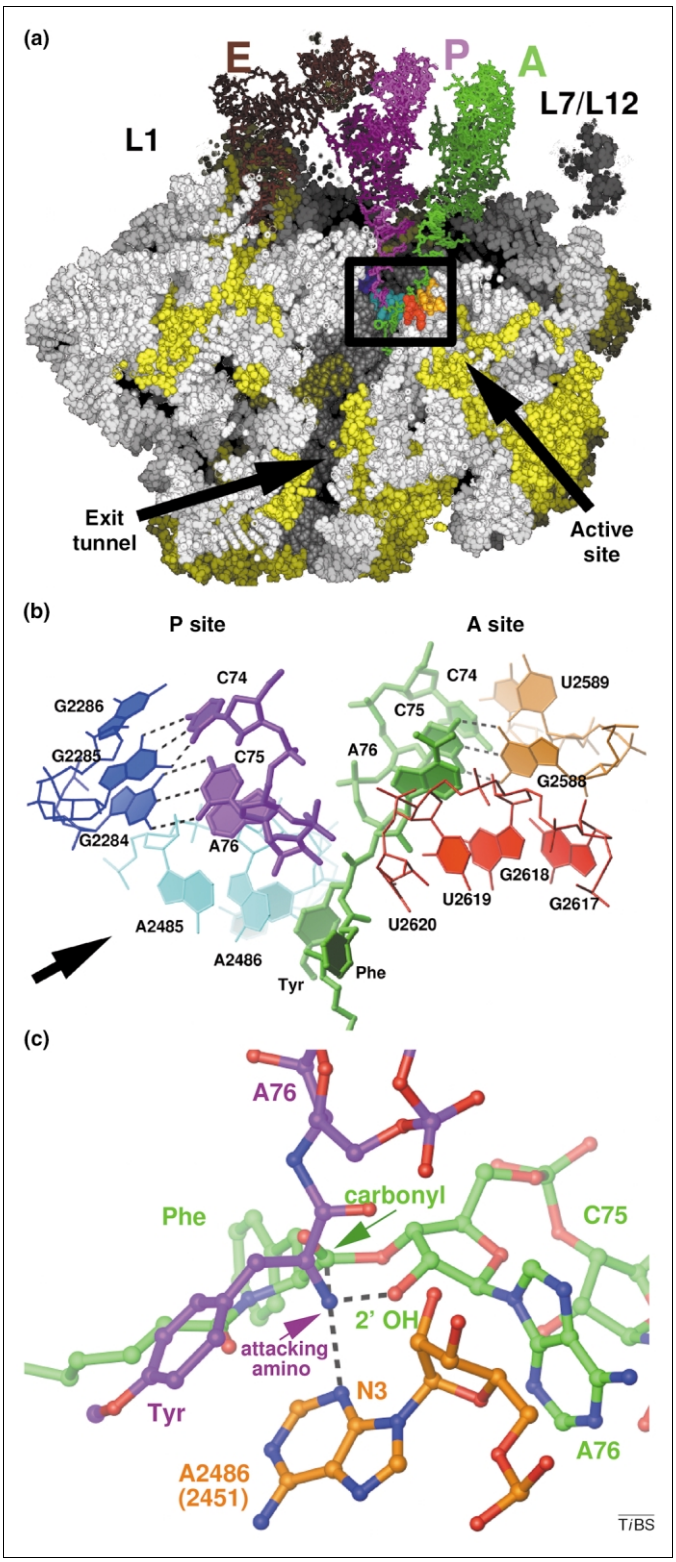

Fig. 5. Structural insights into peptide-bond formation. (a) A space-filling representation of the complex between the *Haloarcula marismortui* large subunit and three intact tRNAs added in the positions that the tRNAs assume when bound to the A, P and E sites of the 70S ribosome [5]. rRNA is white, and ribosomal proteins are yellow. The subunit, which is oriented in the crown view, has been cut in half along a plane that passes through the peptide exit tunnel, and the front of the structure has been removed to expose the polypeptide exit tunnel, which is ~100 Å long and 12–20 Å wide. The active-site area is boxed. (b) A close-up of the active site showing the peptidyl product CC-puromycin-phenylalanine-caproic acid-biotin (CC-pmn-pcb; green) bound to the A-loop (tan), and the deacylated product (CCA; violet) bound to the P-loop (blue). The N3 of A2486 (A2451 in *Escherichia coli*; light blue) is close to the 3′ OH of the CCA, and the base of U2620 (U2585 in *E. coli*; red) has moved close to the new peptide bond and the 3′ OH of A76 [5]. (c) A model of the peptidyl transferase centre of the large ribosomal subunit from *H. marismortui* with substrates bound to both the A-site and P-site. This model was obtained by superimposing the structure of an A-site substrate complex (PDB code: 1FGO) on the structure of a P-site substrate complex (PDB code: 1M90) [6]. The α-amino roup of the A-site substrate (purple) is positioned for a pro-S attack on the carbonyl carbon of the ester linking the peptide moiety of the P-site substrate (green). Possible hydrogen-bonding interactions involving the α-amino group and the N3 of A2486 (A2451 in *E. coli*) and the 2′ OH of A76 are indicated. The 2′ OH of A2486 (A2451 in *E. coli*) is also close enough so that is might interact. Panels (a) and (b) reprinted, with permission, from Ref. [5] (http://www.nature.com/nsb/). Panel (c) reprinted, with permission, from Ref [6]. © (2003) National Academy of Sciences, U.S.A.

uracil reduces the rate of the chemical step of peptide-bond formation by two orders of magnitude and removes the pH dependence with a pK of 7.5 [27], again consistent with the possibility that A2486 plays a small but significant role in catalysis.

Further insights into the structural basis for the catalysis of peptide-bond formation will require two experiments (at least). First, a substrate complex containing both A- and P-site substrates bound simultaneously will be essential to obtain a more precise view of their relative orientation and to see if there are any conformational changes in the centre produced by the presence of both. Second, it is imperative to have a structure for the 70S ribosome complexed with A- and P-site tRNA substrates at a resolution high enough (3 Å) to accurately and independently position all of the atoms at the site of peptide-bond formation. Presumably, such a structure will explain why the 70S ribosome exhibits a 10^3 to 10^4-fold higher rate of peptide-bond formation than does the 50S subunit [28].

Inhibition of the peptidyl transferase reaction by antibiotics

Microorganisms conduct a form of bacterial warfare by making small molecule compounds that inhibit or kill other bacteria. Many of these bactericidal compounds work by blocking protein synthesis, targeting either the large or the small ribosomal subunit. Although many of these antibiotics will inhibit protein synthesis in all three kingdoms, a few are specific for eubacterial protein synthesis and are, therefore, useful in treating bacterial diseases in humans and animals. Medicinal chemists have improved several natural antibiotics by further chemical modifications.

The crystal structures of 12 complexes between the large ribosomal subunit and antibiotics have been determined (Fig. 6), and they demonstrate at least two modes by which they inhibit protein synthesis [29–31]. One class of antibiotics called macrolides (e.g. erythromycin, tylosin and azythromycin) bind to a site in the proximal part of the polypeptide exit tunnel adjacent to the peptidyl-transferase centre, and all appear to inhibit protein synthesis largely by blocking the passage of nascent polypeptide down the exit tunnel. This location is consistent with the observation that some macrolide antibiotics, such as erythromycin, enable the synthesis of di- or tri- peptides [32]. The macrolides tylosin, carbomycin and azythromycin bind to the *H. marismortui* 50S subunit [30] in the same location that erythromycin and other macrolides bind to the eubacterial *D. radiodurans* large subunit [29]

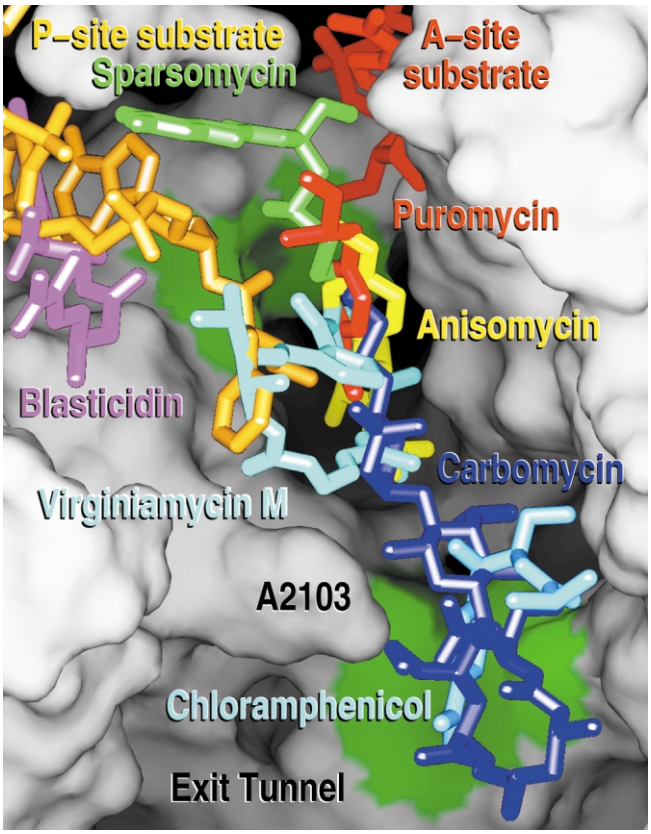

Fig. 6. The positions of seven antibiotics and A-site (red) plus P-site (yellow) substrates bound to the peptidyl transferase centre. The ribosome has been split open to reveal the lumen of the exit tunnel and adjacent regions of the peptidyl transferase site. Ribosomal components are depicted as a continuous surface that is coloured green at two positions where splayed out bases provide hydrophobic binding sites for small molecules. Seven independently determined co-crystal structures have been aligned by superimposing the 23S rRNA in each complex. The positions of sparsomycin (green), puromycin (red), blasticidin S (pink), chloramphenicol (light blue), carbomycin (dark blue), anisomycin (yellow) and virginiamycin M (light blue) are shown. The sites to which each of these antibiotics bind are all different, but there is extensive overlap. Reprinted, with permission, from Ref. [31].

although some differences in the orientation of the macrolide rings are seen.

Another group of structurally diverse antibiotics bind to either the A-site or the P-site and appear to act by blocking the binding of either the A-site or P-site tRNA, or both, which is consistent with their functioning as competitive inhibitors of peptide-bond formation [29,31] (Fig. 6). Anisomycin bound to *H. marismortui* large subunit [31] and chloramphenicol bound to *D. radiodurans* 50S subunit [29] are both located in a hydrophobic crevice formed by two splayed out bases that provides the binding site for the tyrosine side chain of the A-site substrate analogue. At mM concentration, chloramphenicol binds to a second hydrophobic crevice in *H. marismortui* subunits where macrolide antibiotics also bind [31]. Virginiamycin M occupies portions of both the A-site and P-site, whereas blastocydin S exploits another strategy by mimicking C74 and C75 of the P-site bound tRNA and base-pairing with the P-loop.

The structures of these antibiotic complexes with the 50S ribosomal subunit can provide the starting point for structure-based drug design of novel antibiotics that exploit these many and varied small molecule-binding sites in the peptidyl transferase centre. Using this structural information, it might be possible to design novel antibiotics that will be effective against presently known antibiotic-resistance mutations that arise in the 50S subunit.

Evolution

The existence of a peptidyl transferase centre that consists entirely of RNA as well as the very high level of sequence conservation around the peptidyl transferase centre and the 30S interface implies that the first ribosome was composed entirely of RNA. The fact that eubacteria share only 20 large subunit proteins with eukaryotes and archaea lends further support to the hypothesis that ribosomal proteins were late additions to the ribosome. The RNA within a 30–40 Å radius of the peptidyl transferase centre is not only highly conserved among all three kingdoms, but contains almost no globular protein domains and is largely penetrated only by protein tail extensions. *In vitro* evolution of RNA oligonucleotides can produce small RNA molecules that catalyze reactions related to peptidyl transferase reaction and bind analogues of peptide-synthesis intermediates [33,34], suggesting that the appearance of a small RNA capable of catalysing peptidyl transferase was a plausible first step in the evolution of the ribosome. Presumably, the first peptides synthesized by such a primordial peptide synthesizing RNA might have been random copolymers. Possibly the production of even random sequence, basic peptides reminiscent of the ribosomal protein tails might have been useful in stabilizing the structures of ribozymes in the RNA world.

The nature of the steps involved in the evolution of a simple peptide-synthesizing RNA domain into two subunits of RNA that are capable of messenger-directed protein synthesis is less obvious at this time. We can look forward to future experiments that might illuminate the development of messenger-directed synthesis.

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