Molecular Cell
Review

Cell PRESS

The Ribosomal Peptidyl Transferase

Malte Beringer[1] and Marina V. Rodnina[1,*]
[1] Institute of Physical Biochemistry, University of Witten/Herdecke, Witten, D-58448, Germany
*Correspondence: rodnina@uni-wh.de
DOI 10.1016/j.molcel.2007.03.015

Peptide bond formation on the ribosome takes place in an active site composed of RNA. Recent progress of structural, biochemical, and computational approaches has provided a fairly detailed picture of the catalytic mechanism of the reaction. The ribosome accelerates peptide bond formation by lowering the activation entropy of the reaction due to positioning the two substrates, ordering water in the active site, and providing an electrostatic network that stabilizes the reaction intermediates. Proton transfer during the reaction appears to be promoted by a concerted proton shuttle mechanism that involves ribose hydroxyl groups on the tRNA substrate.

The Peptidyl Transfer Reaction

Protein synthesis in the cell is performed on ribosomes, large ribonucleoprotein particles that consist of three RNA molecules and more than 50 proteins. Ribosomes are composed of two subunits, the larger of which has a sedimentation coefficient of 50S in prokaryotes (the 50S subunit) and the smaller which sediments at 30S (the 30S subunit); together they form 70S ribosomes. The ribosome is a molecular machine that selects its substrates, aminoacyl-tRNAs (aa-tRNAs), rapidly and accurately and catalyzes the synthesis of peptides from amino acids. The 30S subunit contains the decoding site, where base-pairing interactions between the mRNA codon and the tRNA anticodon determine the selection of the cognate aa-tRNA. The large ribosomal subunit contains the site of catalysis—the peptidyl transferase (PT) center—which is responsible for making peptide bonds during protein elongation and for the hydrolysis of peptidyl-tRNA (pept-tRNA) during the termination of protein synthesis.

The ribosome has three tRNA binding sites: A, P, and E sites (Figure 1). During the elongation cycle of protein synthesis, aa-tRNA is delivered to the A site of the ribosome in a ternary complex with elongation factor Tu (EF-Tu) and GTP. Following GTP hydrolysis and release from EF-Tu, aa-tRNA accommodates in the A site of the PT center and reacts with pept-tRNA bound to the P site, yielding deacylated tRNA in the P site and A site pept-tRNA that is extended by one amino acid residue. The subsequent movement of tRNAs and mRNA through the ribosome (translocation) is catalyzed by another elongation factor (EF-G in bacteria). During translocation, pept-tRNA and deacylated tRNA move to the P and E sites, respectively; a new codon is exposed in the A site for the interaction with the next aa-tRNA, and the deacylated tRNA is released from the E site.

The reaction catalyzed by the PT center of the ribosome is the aminolysis of an ester bond, with the nucleophilic α-amino group of A site aa-tRNA attacking the carbonyl carbon of the ester bond linking the peptide moiety of P site pept-tRNA. The reactivity of esters with amines intrinsically is rather high, as the uncatalyzed reaction proceeds with a rate of $\sim10^{-4}$ $M^{-1}s^{-1}$ at room temperature. The ribosome increases the rate of peptide bond formation by 10^6- to 10^7-fold, and it may catalyze the reaction in various ways, including chemical catalysis employing ribosome residues as general acids and bases, electrostatic stabilization of the zwitterionic transition state, desolvation, or simply by bringing the two substrates into close proximity and correct orientation to each other. In the last few years, a fairly consistent picture of the catalytic mechanism of peptide bond formation on the ribosome has emerged due to the progress in ribosome crystallography, as well as kinetic, biochemical, genetic, and computational approaches. In this review, we summarize these recent results and present a current model for the mechanism of peptide bond formation on the ribosome.

Enzymology of Peptide Bond Formation

The investigation of peptide bond formation started when Monro and colleagues showed that the PT active site is located on the large ribosomal subunit (Monro, 1967; Monro and Marcker, 1967). Those studies were performed with the so-called "fragment reaction" utilizing low-molecular-weight compounds as substrate analogs. An N-blocked aminoacylated oligonucleotide, such as CCA-fMet, and puromycin (Pmn; O-methyl tyrosine linked to N6-dimethyl adenosine via an amide bond) were used as substrates mimicking the aminoacylated terminus of tRNAs in the P and A sites, respectively. Unfortunately, the fragment reaction required high concentrations of alcohol, and the observed reaction rates appeared to be much slower than the rates of protein synthesis in vivo, raising the question of whether such a decidedly nonphysiological system was representative for the reaction in the cell. In particular, the possibility remained that the presence of the small subunit was necessary to induce a conformation of the active site favoring the reaction (Bashan et al., 2003).

One of the problems of the fragment reaction was the low affinity of small substrate analogs to their binding sites on the 50S subunit. To solve this problem, Steitz, Strobel,

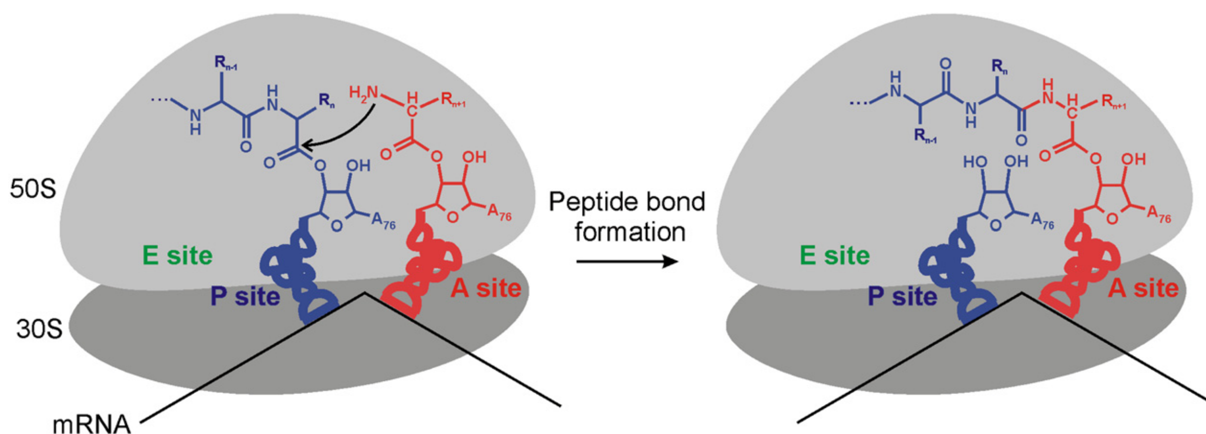

Figure 1. Schematic of Peptide Bond Formation on the Ribosome
The α-amino group of aminoacyl-tRNA in the A site (red) attacks the carbonyl carbon of the pept-tRNA in the P site (blue) to produce a new, one amino acid longer pept-tRNA in the A site and a deacylated tRNA in the P site. The 50S subunit, where the PT center is located, is shown in light gray and the 30S subunit in dark gray. A, P, and E sites of the ribosome are indicated.

and colleagues designed new, somewhat larger substrate analogs that could be used in a modified, alcohol-free version of the fragment reaction (Schmeing et al., 2002, 2005a, 2005b). Furthermore, when full-length pept-tRNA and Pmn or cytidine-Pmn (CPmn) were used, the reaction rates on isolated 50S subunits were comparable to those measured on the 70S ribosomes (Wohlgemuth et al., 2006) or observed in vivo. This strongly supports the conclusion that the 50S subunit alone possesses the full potential of catalyzing peptide bond formation (Wohlgemuth et al., 2006).

The elucidation of the catalytic mechanism of peptide bond formation required a complete reconstituted in vitro translation system in which parameters such as pH, temperature, and ionic conditions could be changed. One critical issue in such experiments is to show that the rate of product formation reflects the chemistry step, rather than substrate binding or conformational rearrangements. In a complete translation system, this condition is unlikely to be fulfilled, because the overall rate of protein synthesis is limited by aa-tRNA selection, which ensures that only the correct amino acid will be incorporated into the protein. The movement of aa-tRNA into the A site is a multistep process that requires structural rearrangements of the ribosome, EF-Tu, and aa-tRNA (reviewed in Rodnina et al. [2005b]). Binding of aa-tRNA in complex with EF-Tu·GTP to the ribosome and codon recognition results in GTP hydrolysis by EF-Tu. Aa-tRNA is released from EF-Tu·GDP and moves through the ribosome into the PT center where its 3′-terminal aminoacylated CCA end is engaged in multiple interactions with the rRNA (Kim and Green, 1999; Yusupov et al., 2001; Hansen et al., 2002; Bashan et al., 2003). The rate of accommodation of aa-tRNA in the A site is ~10 s^{-1}, and peptide bond formation follows instantaneously (Pape et al., 1998; Bieling et al., 2006). Because accommodation precedes peptide bond formation, it limits the rate of product formation as long as it is slower than peptidyl transfer (Pape et al., 1998). Thus, studying the catalytic mechanism of peptide bond formation is possible only when peptidyl transfer is uncoupled from accommodation.

One possibility to overcome the accommodation problem is to decrease the rate of the chemistry reaction such that it becomes significantly slower than the tRNA accommodation step and thus amenable for biochemical analysis. This was accomplished by replacing the reactive nucleophilic α-NH_2 group by the much less reactive OH group (Bieling et al., 2006). The OH derivatives bind to the PT center in the same way as unmodified substrates (Schmeing et al., 2005b), and the reaction with Pmn-OH exhibited the same activation enthalpy as with unmodified Pmn (Table 1), suggesting that the change of the nucleophilic group did not considerably alter the reaction pathway. The hydroxyl derivative of Phe-tRNA (OH-tRNA) was fully reactive with fMet-tRNA in the P site, but, because of the decreased nucleophilicity of the attacking group, the rate of reaction was much lower, 10^{-3} s^{-1}, i.e., far below the rate of accommodation.

For general acid-base catalysis to occur in an aqueous environment at physiological conditions, the pK_a values of the catalytic groups have to be close to neutrality in order to efficiently abstract or donate a proton during the reaction. Measuring the reaction rate at different pH values in the range between 6 and 9 revealed that the rate of the reaction between hydroxy-Phe-$tRNA^{Phe}$ and fMet-tRNA was not affected by pH changes (Table 2). This result indicates that catalysis by the PT center intrinsically is independent of pH and argues against an involvement of ionizing groups of the ribosome in the catalytic mechanism of the PT reaction (Bieling et al., 2006). Furthermore, peptide bond formation between full-length pept-tRNA and unmodified aa-tRNAs did not show any pH dependence (Bieling et al., 2006), and—although the rate-limiting accommodation step probably would have masked

Cell PRESS

Table 1. Activation Parameters of Uncatalyzed and Catalyzed Peptide Bond Formation

Amine	Ester	Rate	Activation Parameters (kcal/mol)		
			$\Delta G^{\neq}$	$\Delta H^{\neq}$	$T\Delta S^{\neq}$
Uncatalyzed					
Tris	fGly-ethylene glycol	10^{-4} ($M^{-1}s^{-1}$)	22.2	9.1	−13.1
Tris	fMet-tRNA^{fMet}	10^{-4} ($M^{-1}s^{-1}$)	22.7	16.2	−6.5
Catalyzed by 70S Ribosomes					
Pmn[a]	fMetPhe-tRNA^{Phe}	10^{3} ($M^{-1}s^{-1}$)	14.0	16.0	2.0
Pmn[b]	fMetPhe-tRNA^{Phe}	5 (s^{-1})	16.5	17.2	0.7
OH-Pmn[b]	fMet-tRNA^{fMet}	6×10^{-3} (s^{-1})	20.5	16.8	−3.7

fGly, N-formyl glycine; fMet, N-formyl methionine.
[a] Measured at limiting concentrations of Pmn; the rate obtained at these conditions (k_{cat}/K_M) is comparable to the second-order reaction of model substrates in solution (25°C) (Sievers et al., 2004).
[b] Measured at saturating concentration of Pmn or OH-Pmn (k_{cat} conditions) (25°C) (Rodnina et al., 2005a).

part of a potential pH effect—these results are consistent with a small, if any, influence of ionizing groups of the ribosome on the reaction. Taking into account the ionization of the α-NH_2 group of aa-tRNA (pK_a = 8) and a measured reaction rate of 6 s^{-1} observed at pH 6, the maximum rate of peptidyl transfer with unmodified full-length tRNA can be estimated to be intrinsically very high, >300 s^{-1} (Bieling et al., 2006).

Another possibility to separate accommodation and peptidyl transfer steps is to use substrate analogs that bind to the A site rapidly and do not require accommodation. In such experiments, 70S ribosomes programmed with natural mRNA and carrying initiator fMet-tRNA or pept-tRNA in the P site were reacted with Pmn as A site substrate analog (Katunin et al., 2002). The reaction rate was not limited by Pmn binding (Katunin et al., 2002; Sievers et al., 2004), and the kinetics of the catalytic step could be monitored by quench flow. The maximum rate of peptide bond formation, measured with pept-tRNA as P site substrate, was about 50 s^{-1} at pH values >8 (Katunin et al., 2002; Youngman et al., 2004). The rate of reaction varied within a factor of 50–100 depending on the length of the peptidyl moiety of the P site tRNA, the C-terminal amino acid of the peptide, or the identity of the tRNA in the P site: the reaction rate was close to 1 s^{-1} with fMet-tRNA^{fMet} in the P site, 10–20 s^{-1} with di- and tripeptidyl-tRNAs fMetPhe-tRNA^{Phe} and fMetPhePhe-tRNA^{Phe} (Katunin et al., 2002; Brunelle et al., 2006), and 50 s^{-1} with fMetAlaAsnMetPheAla-tRNA^{Ala} (Katunin et al., 2002).

Table 2. Inhibition of Peptide Bond Formation by Protonation of Ribosomal Groups

A Site Substrate	Rate Decrease	Reference
Pmn	150-fold	Katunin et al., 2002
OH-Pmn	150-fold	Katunin et al., 2002
C-Pmn	None	Brunelle et al., 2006
aa-tRNA	None	Bieling et al., 2006
OH-tRNA	None	Bieling et al., 2006

In contrast to full-length aminoacyl-tRNA, pronounced pH dependence was observed for peptide bond formation with the minimal A site substrate, Pmn (Table 2). Protonation of a ribosomal group with a pK_a around 7.5 reduced the rate of reaction about 150-fold (Katunin et al., 2002; Brunelle et al., 2006). This effect may in principle indicate an involvement of a ribosome residue as general base in reaction, although the magnitude of inhibition by protonation of a group with pK_a of 7.5 is much less than expected for an essential base. Alternatively, the pH effect may reflect a conformational rearrangement of active-site residues that impairs catalysis and does not take place with full-length aa-tRNA as A site substrate (Katunin et al., 2002; Beringer et al., 2005; Bieling et al., 2006). This conclusion is corroborated by the observation that the reaction between C-Pmn in the A site and pept-tRNA in the P site was not influenced by the ionization of ribosomal groups (Brunelle et al., 2006), suggesting that the presence of the cytidine residue, which mimics C75 of the A site tRNA and presumably its interaction with G2553, was sufficient to induce and stabilize the active conformation of the PT center. Generally, the reaction with full-length tRNA seems to be more robust than the reaction with Pmn, indicating the importance of more remote interactions for positioning the tRNA in the A site. Even when base pairs between C74 or C75 of P site tRNA with rRNA residues G2252 or G2251, respectively, were disrupted by mutagenesis, there was no effect on the rate of peptide bond formation with aa-tRNA as A site substrate (Feinberg and Joseph, 2006).

Crucial information about the mechanism of catalysis can be obtained by comparing the thermodynamic properties of the catalyzed and uncatalyzed reactions. Enzymes that employ general acid-base catalysis act by lowering the activation enthalpy of the reaction. If the ribosome acted as such a chemical catalyst, then the rate enhancement produced by the ribosome should result from

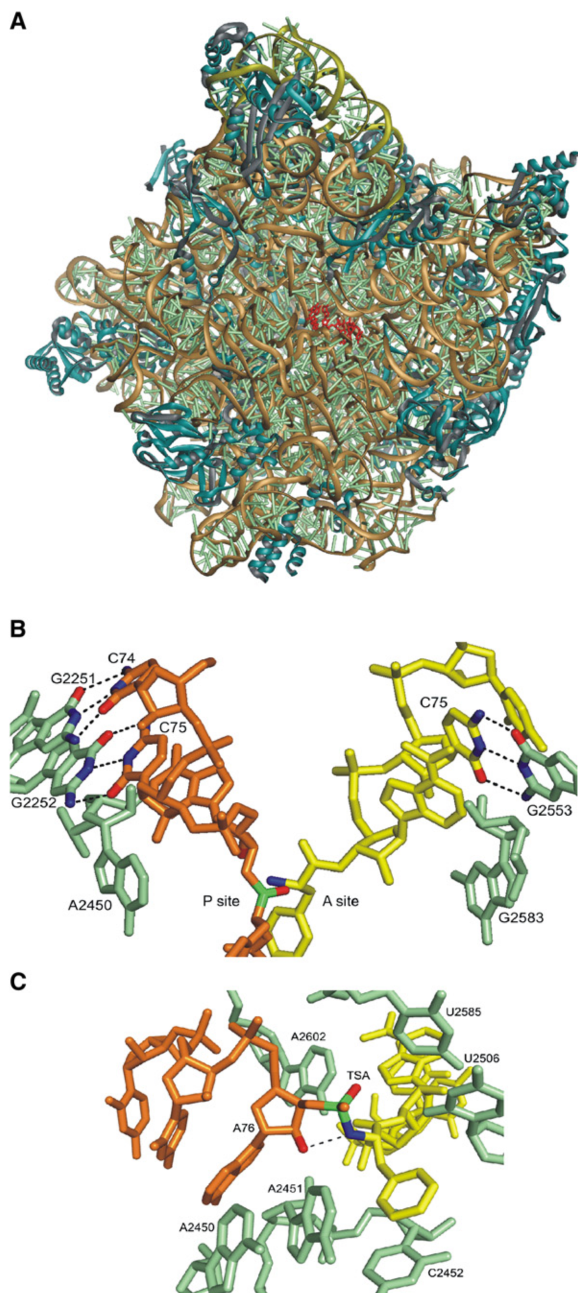

Figure 2. Structure of the Peptidyl Transferase Center

(A) Crystal structure of the 50S subunit from *H. marismortui* with a transition state analog (red) bound to the active site (Protein Data Bank ID code 1VQP; Schmeing et al. [2005a]). Ribosomal proteins are blue, the 23S rRNA backbone is brown, the 5S rRNA backbone is olive, and rRNA bases are pale green. The figure was generated with Pymol (http://www.pymol.org).

(B) Substrate binding to the active site. Base pairs formed between cytosine residues of the tRNA analogs in the A site (yellow) and P site (orange) with 23S rRNA bases (pale green) are indicated (Protein Data Bank ID code 1VQN; Schmeing et al. [2005b]). The α-amino group of the A site substrate (blue) is positioned for the attack on the carbonyl carbon of the ester linking the peptide moiety of the P site substrate (green). Inner shell nucleotides are omitted for clarity.

(C) Transition state analog (TSA) bound to the PT center (Protein Data Bank ID code 1VQP; Schmeing et al. [2005a]). The hydrogen bond between the nucleophilic nitrogen (blue) and the 2′-OH of P site A76 is indicated.

a reduction in the enthalpy of activation. If, on the other hand, the ribosome used other mechanisms of catalysis, such as substrate positioning in the active site, desolvation, or electrostatic shielding, then the rate enhancement produced by the ribosome should be largely entropic in origin. Comparing the rate of the ribosome-catalyzed reaction with a second-order model reaction in solution revealed that the acceleration is achieved by a major lowering of the entropy of activation (Sievers et al., 2004) (Table 1), whereas the enthalpy of activation was practically unchanged (Rodnina et al., 2005a). Consistent with the pH independence of the reaction with full-length tRNA, these findings suggest that general acid-base catalysis by ribosomal residues does not play a significant role in peptidyl transfer on the ribosome.

Some details of the reaction pathway, and particularly the exact difference between the reactions on the ribosome and in solution, are still not understood. Measurements of kinetic isotope effects of the reaction on the 50S subunit (Seila et al., 2005) suggested that the ribosome may promote peptide bond formation by a mechanism that differs in detail from the uncatalyzed aminolysis reaction in solution (Jencks and Gilchrist, 1968); the reaction characteristics on the 50S subunit are most consistent with the model in which the formation of the tetrahedral intermediate is the first irreversible step of the reaction and deprotonation of the amine is occurring concurrently with intermediate formation (Seila et al., 2005). A very low Brønsted coefficient (~0.2) suggests little charge development on the amine in the transition state (Seila et al., 2005). On the other hand, molecular dynamics simulation suggested a late transition state for C-N bond formation and strong H bonding between the nucleophile and the P site O2′ before the proton is transferred, which implies that the geometry and charge distribution of the rate-limiting transition state may be similar to that of the high energy intermediate, irrespective of whether it actually is formed before or after the intermediate (Trobro and Åqvist, 2006).

Structure of the Active Site

50S subunits are composed of two rRNA molecules, 23S rRNA and 5S rRNA, and more than 30 proteins (Figure 2A). Based on crosslinking studies, the PT center was identified in domain V of 23S rRNA with its interacting proteins (Noller, 1991). Biochemical studies showed that 50S subunits largely depleted of proteins retained PT activity (Noller et al., 1992). High-resolution crystal structures of the 50S subunit from *Haloarcula marismortui* revealed that the PT center is composed of RNA only, with no protein within 15 Å of the active site (Ban et al., 2000). Crystal structures of 50S subunits from *Deinococcus radiodurans*

(Harms et al., 2001; Bashan et al., 2003) and of 70S ribosomes from *Escherichia coli* (Schuwirth et al., 2005) and *Thermus thermophilus* (Yusupov et al., 2001; Korostelev et al., 2006; Selmer et al., 2006) confirmed this overall composition of the 50S subunit, with the only difference that the flexible N-terminal tail of ribosomal protein L27 (which does not have an ortholog in *H. marismortui*) may contact the 3′ terminus of the P site tRNA (Selmer et al., 2006). The deletion of as few as three amino acids at the N terminus of L27 leads to an impaired PT activity of *E. coli* ribosomes (Maguire et al., 2005). However, other organisms have ribosomes without protein L27 or any protein groups at the place where the N terminus of L27 is expected to be in *T. thermophilus* ribosomes, suggesting that L27 is not part of an evolutionary conserved mechanism that is expected to employ identical residues in all organisms. Given the high degree of sequence conservation of rRNA, in particular at the PT center (Noller and Woese, 1981; Gutell et al., 1985; Ban et al., 2000), the active site for the PT reaction is likely to consist of RNA in all organisms.

Prior to the PT reaction, the tRNA substrates have to bind their respective sites on the ribosome (Figure 2B). The acceptor arms of the A and P site tRNAs are located in a cleft of the 50S interface side (Yusupov et al., 2001). Their universally conserved CCA ends are oriented and held in place by interactions with residues of 23S rRNA near the active site. The CCA ends of a full-length tRNA substrate or smaller substrate analogs are bound to the P site in essentially the same way (Schmeing et al., 2005b; Selmer et al., 2006). In the P site, C74 and C75 of the tRNA are base paired to G2251 and G2252 of the P loop of 23S rRNA (Samaha et al., 1995; Nissen et al., 2000; Schmeing et al., 2005a). The CCA end of the A site tRNA is fixed by base pairing of C75 with G2553 of the A loop of 23S rRNA (Kim and Green, 1999; Nissen et al., 2000; Bashan et al., 2003; Schmeing et al., 2005a). The 3′-terminal A76 of both A and P site tRNAs forms interactions with residues G2583 and A2450, respectively. The conserved 23S rRNA bases A2451, U2506, U2585, C2452, and A2602 are located at the core of the PT center (Nissen et al., 2000; Bashan et al., 2003; Schmeing et al., 2005a, 2005b) (Figure 2C). No ordered metal ion was seen in the immediate vicinity of the site of reaction.

High-resolution crystal structures of several ribosomal complexes have provided insight into the prereaction and postreaction states, and the use of transition state analogs has revealed the conformation of the active site during peptidyl transfer (Table 3). Additionally, structures of ribosomes from different organisms can now be compared, yielding information about the conservation of active-site residues. In general, the rRNA backbone in the PT center is found in very similar conformations in 50S subunits from *H. marismortui* with various ligands (Ban et al., 2000; Nissen et al., 2000; Hansen et al., 2002; Schmeing et al., 2002, 2005a, 2005b), 50S from *D. radiodurans* (Harms et al., 2001; Bashan et al., 2003), 70S ribosomes from *T. thermophilus* with a P site tRNA (Yusupov et al., 2001; Korostelev et al., 2006; Selmer et al., 2006), or vacant *E. coli* 70S ribosomes (Schuwirth et al., 2005). However, small-scale reorientations do occur, and the movement of some particularly flexible nucleotides may have functional implications for the catalytic mechanism (see below).

In order to visualize the prereaction state, Steitz and colleagues solved the crystal structure of a ribosomal complex containing an aa-tRNA analog (CC-hydroxypuromycin, CChPmn) as A site substrate in combination with a P site substrate analog (Schmeing et al., 2005b) (Table 3). CChPmn represents a tRNA-like CCA end linked to an aminoacyl-like tyrosine in which the α-NH_2 group is replaced by an OH group. Because the OH group does not attack the P site electrophile as efficiently as an α-NH_2 group would do, this approach allowed avoidance of product formation during crystallization (Schmeing et al., 2005b). In this model for the prereaction state, the nucleophilic group (OH) forms hydrogen bonds with both N3 of A2451 and the 2′-OH of A76 of the P site substrate. A somewhat different neighborhood of the nucleophilic α-NH_2 group was reported for a substrate analog attached to a short RNA hairpin bound to *D. radiodurans* 50S subunits (Bashan et al., 2003). The reason for this discrepancy is not clear, and its solution awaits high-resolution structures of subunits with, preferably, full-size aminoacyl- and pept-tRNAs.

The crystal structures of *H. marismortui* 50S subunits complexed with different transition state analogs revealed that the reaction proceeds through a tetrahedral intermediate with S chirality. The oxyanion of the tetrahedral intermediate is stabilized by a water molecule that is positioned by nucleotides A2602 and U2584 (Schmeing et al., 2005a) (Figure 2C). The only atom within hydrogen-bonding distance of the α-NH_2 group mimic is the 2′-OH of A76 of the P site portion of the transition state analog (Schmeing et al., 2005a). N3 of A2451, which is within hydrogen-bonding distance in the prereaction state, seems to lose this interaction with the attacking group during the course of the reaction. The transition state analogs are held in place and stabilized by an intricate H bond network composed of water molecules and ribosomal bases (Schmeing et al., 2005a; Trobro and Åqvist, 2006).

After peptidyl transfer, the growing peptide chain is esterified to the A site tRNA. In the crystal structure of a ribosome-product complex that represents the state directly after the reaction (Schmeing et al., 2002), the peptide moiety points toward the exit tunnel without making specific contacts with the ribosome. The tRNA molecules are present in a very similar position and conformation as before peptide bond formation, and only some bases of 23S rRNA have adopted a different conformation compared to prereaction state, especially U2585. This structure indicates that peptide bond formation and the movement of the tRNA 3′ termini during hybrid state formation (see below) are not directly coupled (Schmeing et al., 2002).

Table 3. High-Resolution Crystal Structures of Ribosome-Substrate Complexes

Structures with Substrate Analogs

Subunit	A Site Substrate	P Site Substrate	Protein Data Bank ID	Reference
50S *Hma*	Tyr-minihelix		1FG0	Nissen et al., 2000
70S *Tth*	$tRNA^{Phe}$	$tRNA^{fMet}$	1GIX (30S)	Yusupov et al., 2001
			1GIY (50S)	
50S *Hma*		CCA-pcb	1M90	Hansen et al., 2002
50S *Dra*	ACCPmn		1NJO	Bashan et al., 2003
	Acceptor stem mimic		1NJM	
50S *Hma*	ChPmn	CCA-pcb	1VQ6	Schmeing et al., 2005b
	CChPmn	CCA-pcb	1VQN	
70S *Tth*	$tRNA^{Phe}$ (30S only)	$tRNA^{fMet}$	2J00 (30S)	Selmer et al., 2006
			2J01 (50S)	
			2J02 (30S)	
			2J03 (50S)	
70S *Tth*		$tRNA^{Phe}$	2I1C (30S)	Korostelev et al., 2006
			1VS9 (50S)	

Structures with Transition State Analogs

Subunit	Protein Data Bank ID	Reference
50S *Hma*	1FFZ	Nissen et al., 2000
	1FFK	
50S *Hma*	1VQ4	Schmeing et al., 2005a
	1VQ5	
	1VQP	
	1VQM	
	1VQL	
50S *Hma*	1VQ7	Schmeing et al., 2005b

Structure with Product Analog

Subunit	A Site Substrate	P Site Substrate	Protein Data Bank ID	Reference
50S *Hma*	CC-Pmn-pcb	CCA	1KQS	Schmeing et al., 2002

Hma, *H. marismortui*; *Tth*, *T. thermophilus*; *Dra*, *D. radiodurans*.

Probing the Catalytic Mechanism—Effect of Base Substitutions

The results of the structural work provided the basis to probe the role of active-site residues for the catalysis of peptide bond formation. Mutation of any residue in the core of the active site leads to lethal phenotypes in *E. coli* (Muth et al., 2000; Polacek et al., 2001; Thompson et al., 2001), which makes it impossible to express pure populations of mutant ribosomes. In some instances, the problem can be circumvented by expressing a mixture of mutant and wild-type ribosomes, measuring kinetics on a mixed population of ribosomes, and then deconvoluting the contribution of the wild-type and mutant ribosomes to the observed time course of product formation (Katunin et al., 2002). However, only after techniques had been developed to introduce affinity tags into rRNA by which it was possible to purify mutant ribosomes, more systematic studies could be undertaken aimed at assessing the specific role of each of the active-site residues (Leonov et al., 2003; Hesslein et al., 2004; Youngman et al., 2004). A number of rRNA bases at the active site were mutated, and the effects of replacements were examined in vivo and in vitro.

N3 of the conserved base A2451 is located within 3–4 Å of the reactive α-NH_2 group, giving rise to the suggestion that A2451 might have a critical role during the formation of the tetrahedral intermediate (Nissen et al., 2000). However, spontaneous mutation of A2451U in rat mitochondrial rRNA conferred chloramphenicol resistance to cells in culture (Kearsey and Craig, 1981), suggesting that in some organisms the mutation was not lethal and thus unlikely to block the PT reaction. *E. coli* ribosomes carrying

the A2451 mutations accumulated in polysomes in vivo and were active in peptide synthesis in vitro (Polacek et al., 2001; Thompson et al., 2001). The Pmn reaction on A2451U mutant ribosomes was slowed down by a factor of 150 only (Katunin et al., 2002; Youngman et al., 2004). Compared to the 10^6–10^7 rate acceleration by the ribosome, this effect is too small for the replacement of an essential or important residue. Additionally, the same mutation had very little effect on peptide bond formation with full-size aa-tRNA, which argues against a catalytic function of A2451 in the PT mechanism (Youngman et al., 2004). Further analysis of A2451U and G2447A mutations (the latter residue forming a fundamental part of the charge relay system postulated to bring about the essential function of A2451) in two organisms, *E. coli* and *M. smegmatis*, strongly argues against a critical role of A2451 in peptide bond formation (Beringer et al., 2003, 2005; Youngman et al., 2004). Rather, the A2451U mutation alters the structure of the PT center and seems to function as a pivot point in stabilizing the ordered structure of the active site (Beringer et al., 2005). However, the 2′-OH of the ribose moiety of A2451 seems to be important, as it may take part in the intricate hydrogen bond network in the active site and interact directly with the critical 2′-OH group of the P site tRNA (Trobro and Åqvist, 2005, 2006). Consistently with this suggestion, substitution of the 2′-OH of A2451 by hydrogen led to an impaired PT activity (Erlacher et al., 2006).

Further mutagenesis in the so-called "inner shell" of the PT center (positions U2506, U2585, and A2602) showed that all but one of the nine mutations tested exhibited drastic reductions (30- to 9400-fold compared to wild-type ribosomes) in the rate of peptide bond formation with Pmn as A site and fMetPhe-tRNA as P site substrates (Youngman et al., 2004. Unexpectedly, U2506 seemed to be the most critical residue (4700- to 9400-fold reduction). These results indicated that the effect of A2451 mutations on peptide bond formation was not specific for that base, again arguing against a role of A2451 in general acid-base catalysis. In striking contrast to the effects observed with Pmn, no large deficiency in the rate of peptidyl transfer was found for any of the A2451, U2506, U2585, and A2602 mutants when full-size aa-tRNA or CPmn was used as an A site substrate, suggesting that none of the rRNA bases in the PT center take part in chemical catalysis (Youngman et al., 2004; Brunelle et al., 2006). Rather, the effects of mutations on cell viability were attributed to an impaired peptide release during the termination step of protein synthesis (Polacek et al., 2003; Youngman et al., 2004).

The Importance of the 2′-OH of A76 of the P Site tRNA

The only group found within hydrogen-bonding distance of the attacking nucleophile in the transition state analogs is the 2′-OH of A76 of pept-tRNA in the P site (Schmeing et al., 2005a, 2005b). The presence of the 2′-OH is crucial for the reaction, both on isolated 50S subunits (Krayevsky and Kukhanova, 1979) and 70S ribosomes (Weinger et al., 2004). Hydrogen bonding between the 2′-OH of A76 and the nucleophilic α-NH_2 group may help to position the nucleophile. Alternatively, the 2′-OH could act as a general acid providing a proton for the leaving group, the 3′-O of A76, and as a general base to deprotonate the nucleophile in the catalysis of the PT reaction (Weinger et al., 2004). For the 2′-OH to participate in proton transfer, its pK_a would need to be reduced toward neutrality. However, the large rate effect of the 2′-OH replacements—substitutions by hydrogen (2′-deoxy) or fluor (2′-fluoro) reduced the ribosome activity by six orders of magnitude (Weinger et al., 2004)—is inconsistent with the limited effect of pH changes on peptide bond formation with Pmn (a factor of 150), C-Pmn (no pH dependence), or aa-tRNA (no pH dependence) (Katunin et al., 2002; Bieling et al., 2006; Brunelle et al., 2006). Alternatively, by analogy to group I intron splicing (Shan and Herschlag, 1999; Adams et al., 2004), it was proposed that the 2′-OH may coordinate a catalytic metal ion, such as Mg^{2+} or K^+ (Weinger et al., 2004), in line with earlier suggestions (Barta and Halama, 1996). However, extensive studies aiming at the identification of metal binding sites in the 50S crystal structures did not reveal Mg^{2+} ions or monovalent metal ions in the PT center that could directly promote catalysis or any other groups in the vicinity of the 2′-OH of the P site tRNA that would be able to shift its pK_a (Schmeing et al., 2005a).

One attractive role for the 2′-OH of A76 of the P site tRNA is to take part in a proton shuttle that bridges the attacking α-NH_2 group and the leaving 3′ oxygen, and there are several catalytic pathways that can be envisaged. The attack of the α-NH_2 group on the ester carbon may result in a six-membered transition state, where the 2′-OH group donates its proton to the adjacent 3′ oxygen while simultaneously receiving one of the amino protons. Such a scenario would not require a pK_a shift of the 2′-OH group, owing to the concerted nature of the bond-forming and -breaking events. The structural details of the putative transition state and the involvement of an ordered water molecule in the proton shuttle remain to be tested (Schmeing et al., 2005a; Sharma et al., 2005; Trobro and Åqvist, 2005, 2006; Rangelov et al., 2006). The residues in the active site may provide a stable, preorganized hydrogen bond network that stabilizes the transition state (Trobro and Åqvist, 2005). The PT center can thus be viewed as a rather rigid environment of preorganized dipoles that do not need to undergo major rearrangements during the reaction. The most favorable mechanism would not involve any general acid-base catalysis by ribosomal groups. Rather, the catalytic effect is mainly associated with lowering the free energy of solvent reorganization (Trobro and Åqvist, 2005). Interestingly, the contribution of substrate alignment or proximity at the active site was estimated to be quite small, about 3.4 kcal/mol (Sharma et al., 2005; Trobro and Åqvist, 2006), compared to the 8.5 kcal/mol reduction of the free energy of activation on the ribosome (Sievers et al., 2004). In fact, activation entropies were reduced to similar extents in the reaction

with prebound (k_{cat} conditions) and with free (k_{cat}/K_M conditions) substrates (Sievers et al., 2004), indicating that the effect is not associated with substrate binding. The activation entropy is a macroscopic quantity that includes a number of microscopic contributions derived from contributions by both the reactants and the ribosomal environment, and activation entropies are in many cases dominated by solvent reorganization effects (Sharma et al. [2005], Trobro and Åqvist [2005], [2006], and references therein).

Conformational Rearrangements at the Active Site: A Potential Role for Induced Fit

The structure of the PT center has long been known to be sensitive to monovalent cation concentrations and to changes of pH (Maden and Monro, 1968). Inner shell residue A2451 appears to have a pivot point role in reorganizing the active site (Beringer et al., 2005). The accessibility of A2451 to chemical modification changes in response to alterations in pH, depending on ionic conditions, temperature, and the organism from which the ribosomes were obtained (Bayfield et al., 2001; Muth et al., 2001; Xiong et al., 2001; Beringer et al., 2005). Interestingly, the apparent pK_a of the ionizing group involved in the rearrangement was close to 7.5. However, A2451 itself is unlikely to ionize with a pK_a close to neutrality. Rather, the ionization of the base pair A2453-C2499, located ~10 Å away from the reaction center, at pH values below neutrality could induce rearrangements of inner shell nucleotides, including A2451 (Bayfield et al., 2004). Such rearrangements may result in a conformation of the active site in which the PT reaction is disfavored, unless interactions of ribosomal residues with remote regions of the tRNA molecules hold the reaction partners in place (Katunin et al., 2002; Bieling et al., 2006; Brunelle et al., 2006).

Recently, Steitz and colleagues solved the structures of 50S subunits in complexes with A site substrate analogs of different lengths (Schmeing et al., 2005b). The smallest A site substrate contained the terminal C75 and A76 of the tRNA and the aminoacyl moiety. The substrate was bound to the 50S subunit through the typical A site binding contacts, and the conformation of active site residues in the complex was similar to that with an empty A site. The P site substrate, CCA-pcb, a CCA oligonucleotide linked to an analog of the growing peptide chain, exhibited a conformation that is unfavorable for the PT reaction, as the carbonyl oxygen was pointing toward the α-NH_2 group of the A site substrate mimic and the atoms that are to react were about 4 Å apart. Binding of an A site substrate that additionally included C74 induced a conformational change in the PT center (Schmeing et al., 2005b). The stacking of the CCA bases positioned the substrate closer to the center of the active site and involved rearrangements of residues G2583, U2506, and U2585. These rearrangements resulted in a conformation of the active site in which the carbonyl carbon of pept-tRNA was more appropriately oriented for the attack by the nucleophile, implying a faster reaction. In addition to peptidyl transfer, the hydrolysis of pept-tRNA during the termination of protein synthesis may be facilitated by induced fit as well.

Which Other Movements May Be Involved?

Three inner shell residues of 23S rRNA seem to be particularly flexible. MD simulations indicate that U2506 can adopt energetically equivalent conformational orientations allowing it to be flexible without the need for induced fit (Trobro and Åqvist, 2006). U2585 is seen in different conformations depending on the length of the A site substrate (see above) but also seems to change position during the progression of the reaction (Schmeing et al., 2002). Residue A2602, which lies between the A and P sites in the active site, can adopt different orientations and shows weak electron density in the crystal structures. A2602 is a critical residue for the hydrolytic activity of the active site during protein release (Polacek et al., 2003; Youngman et al., 2004). However, it is not clear whether the flexibility of any of these residues is functionally important for either peptidyl transfer or for the hydrolysis of pept-tRNA.

Compared to the structure of the *H. marismortui* 50S complexes containing oligonucleotide substrate analogs (Schmeing et al., 2005a, 2005b), the structure of *T. thermophilus* 70S ribosomes with tRNA bound to the P site showed significant movement of critical nucleotides in the catalytic site (Korostelev et al., 2006), while the positions of most nucleotides in the surrounding structure were superimposable. A2451 moved toward the O3′ position of ribose 76, coupled to a movement of the noncanonical A2450-C2063 base pair and A76 of the P site tRNA toward each other, bringing the A-C pair into hydrogen bonding distance of the 2′-OH group of A76. The authors suggested that the A-C pair, which is expected to have a pK_a in the range of 6.0–6.5 (Cai and Tinoco, 1996), may act as a proton donor/acceptor during the PT reaction. If the pK_a of the A-C pair were shifted by the ribosome environment, this value could be compatible with the observed pH rate dependence of the Pmn reaction showing an apparent pK_a of 7.5. The relevance of the A2450-C2063 movement remains unclear, and it was not observed in the high-resolution crystal structure of a very similar *T. thermophilus* 70S ribosome complex solved by the Ramakrishnan group (Selmer et al., 2006). As described above, ionizing groups of the ribosome contribute little if at all to catalysis (Katunin et al., 2002; Youngman et al., 2004; Bieling et al., 2006; Brunelle et al., 2006). Replacement of the A2450-C2063 pair by an isosteric but uncharged G-U wobble pair results a 200-fold or more decrease in the rate of peptide bond formation with either aa-tRNA or Pmn as A site substrates; the effect of the replacement on the pH rate profile could not be assessed due to impaired binding of Pmn to the A site (Hesslein et al., 2004). The effect of mutation was most probably attributable to a gross structural change in the PT center rather than to a specific catalytic effect (Hesslein et al., 2004). This argues against an important role of the ionization of the A2450-C2063 base pair in the PT reaction but does

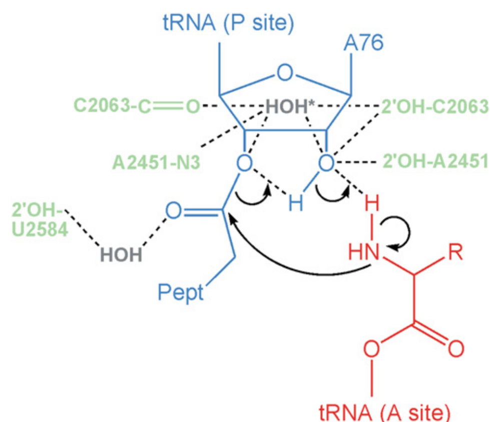

Figure 3. Concerted Proton Shuttle Mechanism of Peptide Bond Formation
Pept-tRNA (P site) and aminoacyl-tRNA (A site) are blue and red, respectively, ribosome residues are pale green, and ordered water molecules are gray. The attack of the α-NH_2 group on the ester carbonyl carbon results in a six-membered transition state in which the 2′-OH group of the A site A76 ribose moiety donates its proton to the adjacent leaving 3′ oxygen and simultaneously receives a proton from the amino group (Schmeing et al., 2005a; Trobro and Åqvist, 2005). Ribosomal residues are not involved in chemical catalysis but are part of the H bond network that stabilizes the transition state.

not exclude the possibility that it is involved in the reaction in other ways, e.g., by providing additional stabilizing interactions for the P site tRNA.

After peptide bond formation, the 3′ ends of both A and P site tRNA can move spontaneously from their initial binding sites on the 50S subunit to the P and E sites, respectively, forming the so-called hybrid states (Moazed and Noller, 1989). Hybrid states are characterized by a very slow reaction of A/P-bound pept-tRNA with Pmn (Semenkov et al., 1992a, 1992b; Sharma et al., 2004). This low but significant reactivity of pept-tRNA in the A/P state indicates that the active site can accommodate a different arrangement of the substrate and stresses the notion that the PT center may be pliable to some extent. A rotational movement of the 3′ ends of the tRNAs was suggested to accompany peptide bond formation (Bashan et al., 2003), although the mechanism by which this movement would contribute to the catalysis of enzymatic reaction is unclear.

The Mechanism of Peptide Bond Formation

The combined evidence supports the idea that peptide bond formation on the ribosome is driven by a favorable entropy change. The A and P site substrates are precisely aligned in the active center by interactions of their CCA sequences and of the nucleophilic α-amino group with residues of 23S rRNA in the active site. The most favorable catalytic pathway involves a six-membered transition state (Figure 3) in which proton shuttling occurs via the 2′-OH of A76 of the P site tRNA. The reaction does not involve chemical catalysis by ribosomal groups but may be modulated by conformational changes at the active site which can be induced by protonation. In addition to placing the reactive groups into close proximity and precise orientation relative to each other, the ribosome appears to work by providing an electrostatic environment that reduces the free energy of forming the highly polar transition state, shielding the reaction against bulk water, helping the proton shuttle forming the leaving group, or a combination of these effects. With this preorganized network, the ribosome avoids the extensive solvent reorganization that is inevitable in the corresponding reaction in solution, resulting in significantly more favorable entropy of activation of the reaction on the ribosome.

ACKNOWLEDGMENTS

Work in our laboratory was supported by the Deutsche Forschungsgemeinschaft, the Alfried Krupp von Bohlen und Halbach-Stiftung, and the Fonds der Chemischen Industrie.

REFERENCES

Adams, P.L., Stahley, M.R., Kosek, A.B., Wang, J., and Strobel, S.A. (2004). Crystal structure of a self-splicing group I intron with both exons. Nature *430*, 45–50.

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 Å resolution. Science *289*, 905–920.

Barta, A., and Halama, I. (1996). The elusive peptidyl transferase—RNA or protein? In Ribosomal RNA and Group I Introns, R. Green and R. Schroeder, eds. (Austin, TX: R.G. Landes Company), pp. 35–54.

Bashan, A., Agmon, I., Zarivach, R., Schluenzen, F., Harms, J., Berisio, R., Bartels, H., Franceschi, F., Auerbach, T., Hansen, H.A., et al. (2003). Structural basis of the ribosomal machinery for peptide bond formation, translocation, and nascent chain progression. Mol. Cell *11*, 91–102.

Bayfield, M.A., Dahlberg, A.E., Schulmeister, U., Dorner, S., and Barta, A. (2001). A conformational change in the ribosomal peptidyl transferase center upon active/inactive transition. Proc. Natl. Acad. Sci. USA *98*, 10096–10101.

Bayfield, M.A., Thompson, J., and Dahlberg, A.E. (2004). The A2453-C2499 wobble base pair in *Escherichia coli* 23S ribosomal RNA is responsible for pH sensitivity of the peptidyltransferase active site conformation. Nucleic Acids Res. *32*, 5512–5518.

Beringer, M., Adio, S., Wintermeyer, W., and Rodnina, M.V. (2003). The G2447A mutation does not affect ionization of a ribosomal group taking part in peptide bond formation. RNA *9*, 919–922.

Beringer, M., Bruell, C., Xiong, L., Pfister, P., Bieling, P., Katunin, V.I., Mankin, A.S., Bottger, E.C., and Rodnina, M.V. (2005). Essential mechanisms in the catalysis of peptide bond formation on the ribosome. J. Biol. Chem. *280*, 36065–36072.

Bieling, P., Beringer, M., Adio, S., and Rodnina, M.V. (2006). Peptide bond formation does not involve acid-base catalysis by ribosomal residues. Nat. Struct. Mol. Biol. *13*, 423–428.

Brunelle, J.L., Youngman, E.M., Sharma, D., and Green, R. (2006). The interaction between C75 of tRNA and the A loop of the ribosome stimulates peptidyl transferase activity. RNA *12*, 33–39.

Cai, Z., and Tinoco, I., Jr. (1996). Solution structure of loop A from the hairpin ribozyme from tobacco ringspot virus satellite. Biochemistry *35*, 6026–6036.

Erlacher, M.D., Lang, K., Wotzel, B., Rieder, R., Micura, R., and Polacek, N. (2006). Efficient ribosomal peptidyl transfer critically relies

on the presence of the ribose 2′-OH at A2451 of 23S rRNA. J. Am. Chem. Soc. *128*, 4453–4459.

Feinberg, J.S., and Joseph, S. (2006). A conserved base-pair between tRNA and 23S rRNA in the peptidyl transferase center is important for peptide release. J. Mol. Biol. *364*, 1010–1020.

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.

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

Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F., and Yonath, A. (2001). High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. Cell *107*, 679–688.

Hesslein, A.E., Katunin, V.I., Beringer, M., Kosek, A.B., Rodnina, M.V., and Strobel, S.A. (2004). Exploration of the conserved $A^{+}C$ wobble pair within the ribosomal peptidyl transferase center using affinity purified mutant ribosomes. Nucleic Acids Res. *32*, 3760–3770.

Jencks, W.P., and Gilchrist, M. (1968). Nonlinear structure-reactivity correlations. The reactivity of nucleophilic reagents toward esters. J. Am. Chem. Soc. *90*, 2622–2637.

Katunin, V.I., Muth, G.W., Strobel, S.A., Wintermeyer, W., and Rodnina, M.V. (2002). Important contribution to catalysis of peptide bond formation by a single ionizing group within the ribosome. Mol. Cell *10*, 339–346.

Kearsey, S.E., and Craig, I.W. (1981). Altered ribosomal RNA genes in mitochondria from mammalian cells with chloramphenicol resistance. Nature *290*, 607–608.

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

Korostelev, A., Trakhanov, S., Laurberg, M., and Noller, H.F. (2006). Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. Cell *126*, 1065–1077.

Krayevsky, A.A., and Kukhanova, M.K. (1979). The peptidyltransferase center of ribosomes. Prog. Nucleic Acid Res. Mol. Biol. *23*, 1–51.

Leonov, A.A., Sergiev, P.V., Bogdanov, A.A., Brimacombe, R., and Dontsova, O.A. (2003). Affinity purification of ribosomes with a lethal G2655C mutation in 23 S rRNA that affects the translocation. J. Biol. Chem. *278*, 25664–25670.

Maden, B.E., and Monro, R.E. (1968). Ribosome-catalyzed peptidyl transfer. Effects of cations and pH value. Eur. J. Biochem. *6*, 309–316.

Maguire, B.A., Beniaminov, A.D., Ramu, H., Mankin, A.S., and Zimmermann, R.A. (2005). A protein component at the heart of an RNA machine: the importance of protein L27 for the function of the bacterial ribosome. Mol. Cell *20*, 427–435.

Moazed, D., and Noller, H.F. (1989). Intermediate states in the movement of transfer RNA in the ribosome. Nature *342*, 142–148.

Monro, R.E. (1967). Catalysis of peptide bond formation by 50 S ribosomal subunits from *Escherichia coli*. J. Mol. Biol. *26*, 147–151.

Monro, R.E., and Marcker, K.A. (1967). Ribosome-catalysed reaction of puromycin with a formylmethionine-containing oligonucleotide. J. Mol. Biol. *25*, 347–350.

Muth, G.W., Ortoleva-Donnelly, L., and Strobel, S.A. (2000). A single adenosine with a neutral pKa in the ribosomal peptidyl transferase center. Science *289*, 947–950.

Muth, G.W., Chen, L., Kosek, A.B., and Strobel, S.A. (2001). pH-dependent conformational flexibility within the ribosomal peptidyl transferase center. RNA *7*, 1403–1415.

Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000). The structural basis of ribosome activity in peptide bond synthesis. Science *289*, 920–930.

Noller, H.F. (1991). Ribosomal RNA and translation. Annu. Rev. Biochem. *60*, 191–227.

Noller, H.F., and Woese, C.R. (1981). Secondary structure of 16S ribosomal RNA. Science *212*, 403–411.

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

Pape, T., Wintermeyer, W., and Rodnina, M.V. (1998). Complete kinetic mechanism of elongation factor Tu-dependent binding of aminoacyl-tRNA to the A site of the *E. coli* ribosome. EMBO J. *17*, 7490–7497.

Polacek, N., Gaynor, M., Yassin, A., and Mankin, A.S. (2001). Ribosomal peptidyl transferase can withstand mutations at the putative catalytic nucleotide. Nature *411*, 498–501.

Polacek, N., Gomez, M.J., Ito, K., Xiong, L., Nakamura, Y., and Mankin, A. (2003). The critical role of the universally conserved A2602 of 23S ribosomal RNA in the release of the nascent peptide during translation termination. Mol. Cell *11*, 103–112.

Rangelov, M.A., Vayssilov, G.N., Yomtova, V.M., and Petkov, D.D. (2006). The syn-oriented 2′-OH provides a favorable proton transfer geometry in 1,2-diol monoester aminolysis: implications for the ribosome mechanism. J. Am. Chem. Soc. *128*, 4964–4965.

Rodnina, M.V., Beringer, M., and Bieling, P. (2005a). Ten remarks on peptide bond formation on the ribosome. Biochem. Soc. Trans. *33*, 493–498.

Rodnina, M.V., Gromadski, K.B., Kothe, U., and Wieden, H.J. (2005b). Recognition and selection of tRNA in translation. FEBS Lett. *579*, 938–942.

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.

Schmeing, T.M., Seila, A.C., Hansen, J.L., Freeborn, B., Soukup, J.K., Scaringe, S.A., Strobel, S.A., Moore, P.B., and Steitz, T.A. (2002). A pre-translocational intermediate in protein synthesis observed in crystals of enzymatically active 50S subunits. Nat. Struct. Biol. *9*, 225–230.

Schmeing, T.M., Huang, K.S., Kitchen, D.E., Strobel, S.A., and Steitz, T.A. (2005a). Structural insights into the roles of water and the 2′ hydroxyl of the P site tRNA in the peptidyl transferase reaction. Mol. Cell *20*, 437–448.

Schmeing, T.M., Huang, K.S., Strobel, S.A., and Steitz, T.A. (2005b). An induced-fit mechanism to promote peptide bond formation and exclude hydrolysis of peptidyl-tRNA. Nature *438*, 520–524.

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 Å resolution. Science *310*, 827–834.

Seila, A.C., Okuda, K., Nunez, S., Seila, A.F., and Strobel, S.A. (2005). Kinetic isotope effect analysis of the ribosomal peptidyl transferase reaction. Biochemistry *44*, 4018–4027.

Selmer, M., Dunham, C.M., Murphy, F.V., 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*, 1935–1942.

Semenkov, Y., Shapkina, T., Makhno, V., and Kirillov, S. (1992a). Puromycin reaction for the A site-bound peptidyl-tRNA. FEBS Lett. *296*, 207–210.

Semenkov, Y.P., Shapkina, T.G., and Kirillov, S.V. (1992b). Puromycin reaction of the A-site bound peptidyl-tRNA. Biochimie *74*, 411–417.

Shan, S.O., and Herschlag, D. (1999). Probing the role of metal ions in RNA catalysis: kinetic and thermodynamic characterization of a metal ion interaction with the 2′-moiety of the guanosine nucleophile in the *Tetrahymena* group I ribozyme. Biochemistry *38*, 10958–10975.

Sharma, D., Southworth, D.R., and Green, R. (2004). EF-G-independent reactivity of a pre-translocation-state ribosome complex with

Cell PRESS

the aminoacyl tRNA substrate puromycin supports an intermediate (hybrid) state of tRNA binding. RNA *10*, 102–113.

Sharma, P.K., Xiang, Y., Kato, M., and Warshel, A. (2005). What are the roles of substrate-assisted catalysis and proximity effects in peptide bond formation by the ribosome? Biochemistry *44*, 11307–11314.

Sievers, A., Beringer, M., Rodnina, M.V., and Wolfenden, R. (2004). The ribosome as an entropy trap. Proc. Natl. Acad. Sci. USA *101*, 7897–7901.

Thompson, J., Kim, D.F., O'Connor, M., Lieberman, K.R., Bayfield, M.A., Gregory, S.T., Green, R., Noller, H.F., and Dahlberg, A.E. (2001). Analysis of mutations at residues A2451 and G2447 of 23S rRNA in the peptidyltransferase active site of the 50S ribosomal subunit. Proc. Natl. Acad. Sci. USA *98*, 9002–9007.

Trobro, S., and Åqvist, J. (2005). Mechanism of peptide bond synthesis on the ribosome. Proc. Natl. Acad. Sci. USA *102*, 12395–12400.

Trobro, S., and Åqvist, J. (2006). Analysis of predictions for the catalytic mechanism of ribosomal peptidyl transfer. Biochemistry *45*, 7049–7056.

Weinger, J.S., Parnell, K.M., Dorner, S., Green, R., and Strobel, S.A. (2004). Substrate-assisted catalysis of peptide bond formation by the ribosome. Nat. Struct. Mol. Biol. *11*, 1101–1106.

Wohlgemuth, I., Beringer, M., and Rodnina, M.V. (2006). Rapid peptide bond formation on isolated 50S ribosomal subunits. EMBO Rep. *7*, 669–703.

Xiong, L., Polacek, N., Sander, P., Bottger, E.C., and Mankin, A. (2001). pKa of adenine 2451 in the ribosomal peptidyl transferase center remains elusive. RNA *7*, 1365–1369.

Youngman, E.M., Brunelle, J.L., Kochaniak, A.B., and Green, R. (2004). The active site of the ribosome is composed of two layers of conserved nucleotides with distinct roles in peptide bond formation and peptide release. Cell *117*, 589–599.

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 5.5 Å resolution. Science *292*, 883–896.