Ribosomal Peptide-Bond Formation

Markus Pech[1] and Knud H. Nierhaus[1,*]
[1]Max-Planck-Institut für Molekulare Genetik, Ihnestr. 73, 14195 Berlin, Germany
*Correspondence: Nierhaus@molgen.mpg.de
DOI 10.1016/j.chembiol.2008.04.008

In this issue of *Chemistry & Biology*, Lang et al. (2008) add an important step toward a molecular understanding of ribosomal peptide-bond formation: they unravel the involvement of an essential partner of the reaction, namely the 2′-OH group of the 23S rRNA nucleotide A2451.

The advent of crystal structures of the ribosomal subunits and somewhat later of the whole ribosome has revolutionized our understanding of the ribosome (for review see Steitz, 2008). Indeed, these studies led to the phrase "Ribosome is a Ribozyme" (Cech, 2000), resulting from the fact that rRNA is the major constituent element for two fundamental processes: (1) decoding the mRNA information at the A-site decoding center on the small ribosomal subunit; and (2) peptide-bond formation at the peptidyltransferase center on the large ribosomal subunit. Although the structural principles of decoding have been resolved, the evaluation of the mechanism of peptide-bond formation is still ongoing.

A peptidyl-tRNA at the P site and an aminoacyl-tRNA at the A site define the first step in polypeptide chain elongation reaction, with both acyl residues attached to the A76 3′ position of their respective tRNA via an ester bond (Figure 1A, left panel). The second step is initiated by a nucleophilic attack by the α-amino group from the A-tRNA on the carbonyl carbon of the P-tRNA, resulting in a peptide bond between the peptidyl residue and the aminoacyl group at the A site (Figure 1A). The A site now contains a peptidyl-tRNA one aminoacyl residue longer, whereas a deacylated tRNA resides at the P site. An early model, based on chemical rates measured for related chemical reactions, suggested that the enzymatic rate acceleration is mainly achieved by the precise stereochemical alignment of the substrates, the 3′-terminal CCA ends of both tRNAs with their respective residues (physical concept: template model). Additionally, evidence for an involvement of a general acid-base catalysis was also presented (chemical concept: a transient formation of a covalent bond between enzyme and substrate; Nierhaus et al., 1980). Indeed, the first crystal structure of the large ribosomal subunit containing a transition state analog at the peptidyltransferase center (Nissen et al., 2000) confirmed biochemical evidence (Green et al., 1998) and showed that the CCA-ends of both A- and P-tRNA are specifically recognized and firmly held in place by a number of specific contacts between rRNA and tRNAs nucleotides.

Furthermore, the structure suggested that the N3 of A2451 is part of general acid-base catalysis mechanism, since it is within the hydrogen-bond distance to the α-amino group (see the neighborhood of N3 and the α-amino groups in Figure 1B, right panel). This suggestion led to number of mutational studies, culminating in a mutational study of all nucleotide residues at the peptidyltransferase center, i.e., A2451, U2506, U2585, and A2602 (Youngman et al., 2004, and references therein). The surprising result was that all bases, including A2451, are not of primary importance for peptide-bond formation, but rather for the release of the peptidyl residue during termination of protein synthesis. These results simplified our picture of the elements participating in peptide-bond formation significantly. Thus, an inner nucleotide shell of the peptidyltransferase center, comprising these four residues important for peptide release, and an outer shell, comprising the A and P loops involved in fixation of the CCA ends of A- and P-tRNA, were defined. Eventually, a participation of a general acid-base catalysis in peptide-bond formation was questioned and excluded (Sievers et al., 2004).

These conclusiones per exclusionem did not address all the players directly involved in peptide bond formation. Two important observations paved the way for a deeper understanding of this enzymatic activity: (1) the 3′-terminal 2′-OH group of the P-tRNA was shown to play an essential role for the mechanism of peptide-bond formation (Dorner et al., 2003; Weinger et al., 2004), and (2) a second 2′-OH group was identified to be crucial for peptide bond formation, namely that of A2451. Interestingly, removal of the entire nucleobase of A2451 nucleotide, leaving the sugar-phosphate backbone intact, does not significantly impair the rate of peptide-bond formation. In contrast, removal of the 2′-OH of A2451 completely abolishes activity (Figure 1B; Erlacher et al., 2005). A molecular dynamics study placed the two essential 2′-OH groups into a mechanistic context and established the important concept of a "proton shuttle": when the 3′-ester bond of the peptidyl-tRNA at the P site is cleaved, a proton is delivered from the adjacent 2′-OH group, which in turn receives a proton from the α-amino group of the aminoacyl-tRNA at the A site (Figure 1B, left panel; Trobro and Åqvist, 2005).

What remained to be clarified was the question of cooperation between the two essential 2′-OH groups during peptide-bond formation (Figure 1B, right panel). This question is now addressed by Lang et al. (2008) in this issue of *Chemistry & Biology*. In particular they investigate whether the proton donor or acceptor capacity of the 2′-O of A2451 are essential for formation of a hydrogen bridge. They report results of chemically and biochemically challenging experiments, in which they replace the 2′-OH group by –F (no proton donor, retained proton acceptor capacity), by -H and -OCH3 (neither proton donor nor acceptor capacity) and -NH_2 (with both proton donor and acceptor capacity). Next, they test kinetics of peptide-bond formation in each case. The results are unequivocal: the proton donor capacity of this group is essential, but proton acceptor capacity is

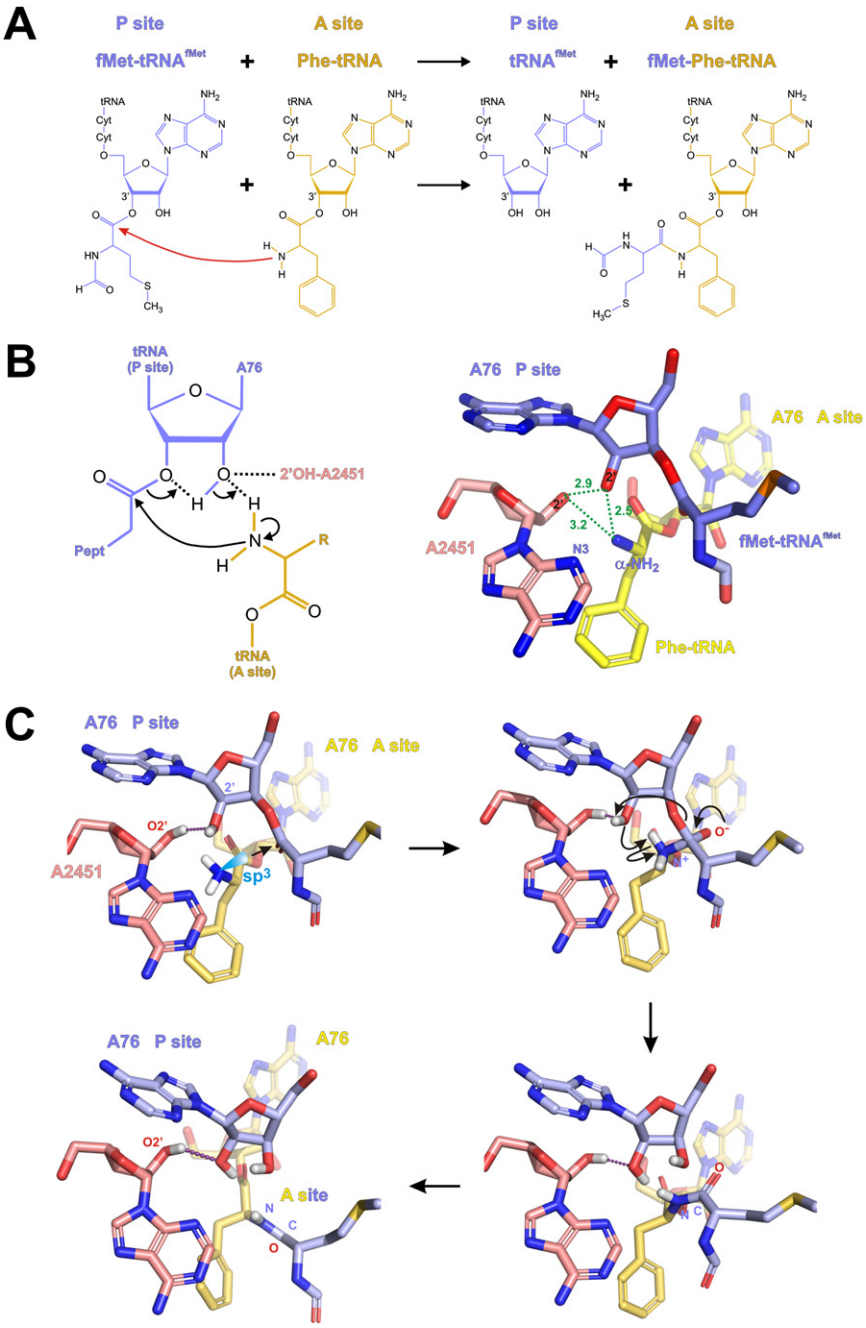

Figure 1. Peptide-Bond Formation on the Ribosome

(A) Both acyl groups of the P- and A-tRNA are linked to the ribose at the 3′ position.

(B) The main players for the mechanism. Left panel, pairwise movement of the electrons illustrating the proton shuttle. Right panel, the distances between the 2′-OH groups of A76 of the P-tRNA and A2451 and the α-amino group of the A-tRNA are given.

(C) The mechanism: (Upper left) The starting situation with an fMet-tRNA at the P site and a Phe-tRNA at the A site; only the main players are shown (see [B], right panel). The ester carbonyl carbon is attacked by the lone pair of the sp3 hybridized α-amino group (black arrow). (Upper right) The tetrahedral intermediate is formed. Note the proton shuttle from the α-amino group of the A-tRNA to the 2′-O of A76 of the P-tRNA, whereas that from the 2′-O moves to the 3′-O of the P-tRNA. The black arrows indicate the pairwise electron movement for proton shuttling. (Lower right) Configuration after the proton shuttling. (Lower left) Products after peptide-bond formation: a deacylated tRNA is at the P site and an fMet-Phe-tRNA at the A site. The H-bridge from 2′-OH of A2451 to the 2′-O of the P-tRNA is maintained throughout the reaction. Coordinates from PDB entry 1VQN (Schmeing et al., 2005), but with an f-Met residue on the P-tRNA. Modified from Figure 3 of Lang et al., (2008).

not. Based on these data the authors present a convincing model (Figure 1C), where the 2′-OH group of A2451 forms an H-bond with the 2′-OH group of A76 of the P-tRNA during the entire reaction process. This hydrogen bond bridge has two important consequences:

(1) A possible fast intramolecular transesterification of the peptidyl residue between the 2′ and 3′ oxygen of A76 of the P-tRNA is prevented, which is important since transesterification has been observed to occur in solution with rates in the range of the average rate of an amino-acid incorporation into the nascent peptide chain and might therefore interfere with peptidyl transfer reaction unless effectively prevented.

(2) The ribose of the A76 residue of the P-tRNA is kept in its RNA-unfavorable C2′-endo configuration (i.e., the C2′ atom is above the sugar plane and the C3′ below), which is important for the proton shuttle).

With these new results we reach a satisfying, albeit likely not final, description of the ribosomal mechanism of peptide-bond formation. The enzymatic reaction follows the template model, viz. a precise stereochemical alignment of the CCA-ends of both A- and P-tRNAs by the A and P loops, respectively, brings the reactants into a proper stereochemical position. The reaction itself involves a proton shuttle (Figure 1B, left panel), where a proton moves from the α-amino group of the A-tRNA to the 2′-O of the P-tRNA, which gives its own proton to the 3′-O of the P-tRNA during cleavage of the corresponding ester bond. The 2′-OH of A2451 hydrogen-bonds the 2′-O of the P-tRNA during the whole reaction preventing transesterification and holding it in the RNA-unfavorable 2′-endo configuration, which however favors the proton shuttle.

REFERENCES

Cech, T.R. (2000). Science *289*, 878–879.

Dorner, S., Panuschka, C., Schmid, W., and Barta, A. (2003). Nucleic Acids Res. *31*, 6536–6542.

Erlacher, M.D., Lang, K., Shankaran, N., Wotzel, B., Huttenhofer, A., Micura, R., Mankin, A.S., and Polacek, N. (2005). Nucleic Acids Res. *33*, 1618–1627.

Green, R., Switzer, C., and Noller, H. (1998). Science *280*, 286–289.

Lang, K., Erlacher, M., Wilson, D.N., Micura, R., and Polacek, N. (2008). Chem. Biol. *15*, this issue, 485–492.

Nierhaus, K.H., Schulze, H., and Cooperman, B.S. (1980). Biochem. Int. *1*, 185–192.

Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000). Science *289*, 920–930.

Schmeing, T.M., Huang, K.S., Strobel, S.A., and Steitz, T.A. (2005). Nature *438*, 520–524.

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

Steitz, T.A. (2008). Nat. Rev. Mol. Cell Biol. *9*, 242–253.

Trobro, S., and Åqvist, J. (2005). Proc. Natl. Acad. Sci. USA *102*, 12395–12400.

Weinger, J.S., Parnell, K.M., Dorner, S., Green, R., and Strobel, S.A. (2004). Nat. Struct. Mol. Biol. *11*, 1101–1106.

Youngman, E.M., Brunelle, J.L., Kochaniak, A.B., and Green, R. (2004). Cell *117*, 589–599.

Joining the Army of Proteasome Inhibitors

Alexei F. Kisselev[1,*]
[1]Norris Cotton Cancer Center and Department of Pharmacology and Toxicology, Dartmouth Medical School, Lebanon, NH 03756, USA
*Correspondence: alexei.f.kisselev@dartmouth.edu
DOI 10.1016/j.chembiol.2008.04.010

An article in this issue of *Chemistry & Biology* (Hines et al., 2008) and a recent study in *Nature* (Groll et al., 2008) establish three natural products as novel proteasome inhibitors. These inhibitors, discovered in an unusual way, reveal a different mechanism of proteasome inhibition and suggest new therapeutic application of its inhibitors.

Targeted degradation of proteins by the ubiquitin-proteasome pathway plays an essential role in the regulation of protein homeostasis and in the regulation of essentially every function of the living cells. The proteasome is a large, multisubunit, proteolytic complex that processively degrades ubiquitinylated proteins into small peptides. Numerous inhibitors of this degradation machine, discovered in the past 15 years, serve as excellent tools to determine proteasome involvement in a cellular or physiological process and to determine if a protein of interest is degraded by the proteasomes (Kisselev and Goldberg, 2001). Proteasome inhibitors cause selective apoptosis of malignant cells, and represent a new class of antineoplastic agents (Adams, 2004). One such inhibitor, bortezomib (VELCADE), has been approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. Three second-generation proteasome inhibitors, carfilzomib (PR-171) (Demo et al., 2007), salinosporamide A (NPI-0052) (Chauhan et al., 2005), and CEP-18770 (Piva et al., 2008), are in phase I and II clinical trials (Figure 1).

Two recent papers, one in *Nature* (Groll et al., 2008), and one in this issue of *Chemistry & Biology* (Hines et al., 2008) now report additional proteasome inhibitors. If there are so many proteasome inhibitors already available, why do these compounds deserve special attention? One of them inhibits the proteasome by a mechanism not previously described and the other suggests potential for additional therapeutic applications of these compounds. In addition, these inhibitors were discovered in an unusual way.

Groll et al. (2008) set out to investigate the mechanisms of the Syringolin A (SylA, Figures 1 and 2) virulence factor of the plant pathogen *Pseudomonas syringie*. Treatment of wheat and *Arabidopsis thaliana* with this peptide derivative leads to changes in gene expression profiles that resemble changes occurring in yeast and mammalian cells treated with proteasome inhibitors (i.e., upregulation of transcripts encoding proteasomal subunits and heat shock proteins). This observation allowed Groll et al. to hypothesize that this compound is a proteasome inhibitor. Indeed they found that it irreversibly inhibits all three types of proteasomal proteolytic sites. In order to elucidate the mode of inhibition, they solved the structure of SylA complex with the yeast 20S proteasome. This structure revealed a novel mode of inhibition whereby the hydroxy group of proteasome's catalytic threonine performs a Michael type 1,4-addition to the vinyl ketone moiety in the 14-membered ring of the inhibitor (Figure 2). This mechanism resembles mechanisms of inhibition by another class of proteasome inhibitors, peptidyl vinyl sulfones (Groll and Huber, 2004). They also found that another microbal metabolite, Glidobactin A (GlbA), inhibited the chymotrypsin- and the trypsin-like activities of the proteasome and reacted with active site threonines in a similar fashion. Both SylA and GlbA blocked proliferation and induced apoptosis of malignant cells, further confirming that they are proteasome inhibitors.

Hines et al. (2008) investigated the mechanism of neurotropic activity of marine fungal metabolite fellutamide B. It was known that treatment of cultured neurons and fibroblasts with this compound induces nerve growth factor (NGF) secretion, but the mechanism leading to this event had not been elucidated. They noticed similarities in the structures of this lipopetide aldehyde and peptide aldehyde proteasome inhibitor MG132, and tested whether it is a proteasome inhibitor. Indeed they found that fellutamide B is a very potent inhibitor of the chymotrypsin-like sites and that it also inhibits the trypsin-like and caspase-like sites,