

# **How Lazy are the tRNAs on the Ribosome?**

## **New Insights for the $\alpha - \varepsilon$ Model**

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For the life, love and great friendships  
that I found in the beautiful city, Berlin

## Abstract

In this work the phosphorothioate technique is applied that allows a precise assessment of the accessibility of each individual phosphate group within RNA. The measurements were performed with deacylated tRNAs bound to the ribosomal P site under various buffer conditions, and with a tRNA in acylated and deacylated form present at the ribosome in various functional states. The buffer conditions used have led in the past to different results concerning the tRNA location with important implications to elongation cycle models and the mechanism of translocation. The results have revealed that (i) the tRNA undergoes significant conformational changes already in solution, if physiological conditions at low  $Mg^{2+}$  and polyamines were compared with conventional buffer conditions at high  $Mg^{2+}$  in the absence of polyamines. Surprisingly, the tRNA seems to adopt a more compact structure under low  $Mg^{2+}$  conditions plus polyamines as compared to high  $Mg^{2+}$  (10 and 20 mM, conventional system). (ii) If bound to the programmed P site the tRNAs seem to be forced into the same structure irrespective of the buffer system used, since tRNAs under all conditions tested demonstrate an almost identical accessibility pattern. The very same accessibility pattern under various buffer conditions indicates the same micro-environment of the tRNAs, a result that notably contrasts the significant differences in tRNA location cryo-EM has revealed under corresponding conditions. Taken together, these results represent a further support for a ribosomal carrier that binds tightly the tRNAs and moves them during translocation from the PRE to the POST state. Another experiment compared the protection pattern of a tRNA at the programmed and non-programmed P site of 70S ribosomes (i.e. in the presence or absence of mRNA). The surprising result was that in the non-programmed 70S ribosome the accessibility pattern those caused by 30S contacts were practically absent, whereas the 50S part of the accessibility pattern was almost normal. Obviously, codon-anticodon interactions at the P site are causing the 30S contacts even outside the anticodon demonstrating an influence of this interaction on the 30S-tRNA contacts in general.

In the second part the phosphorothioate results are compared with the crystal structure of *Thermus thermophilus* 70S ribosomes carrying three tRNAs. An excellent agreement is found concerning the results with accessibility patterns derived from tRNAs bound to single subunits and the tRNA contacts with both subunits within the P site of 70S ribosomes. A new and significant insight could be obtained concerning the tRNA fixation points: Not only the tRNA nucleotides important for fixation of the tRNA at the P site are universally conserved as noted earlier, but also the nucleotides of the rRNA and ribosomal proteins involved in tRNA fixation are universally or highly conserved.

Finally, a compilation of the available facts from cryo-EM, crystallographic studies of functional complexes and biochemical data do not favor the hybrid-site model, but are consistent with the  $\alpha$ - $\epsilon$  model.

## Abstrakt

In dieser Arbeit fand die Phosphorothioat-Technik Anwendung, die eine präzise Bestimmung der Erreichbarkeit mit  $I_2$  (Jod) jedes individuellen Phosphatrestes gestattet. Die Messungen wurden mit deacylierter tRNA in der P Stelle unter verschiedenen Pufferbedingungen durchgeführt, ferner mit einer tRNA in acylierter und deacylierter Form, die an Ribosomen in verschiedenen Funktionszuständen gebunden wurde. Die gewählten Pufferbedingungen, wie mittels Kryo-EM in früheren Arbeiten gezeigt werden konnte, haben die tRNA Position im Ribosom stark beeinflusst. Die Ergebnisse zeigten, dass (i) die tRNA ihre Konformation in Lösung ändert, wenn man eine tRNA in einem physiologischen Puffer (niedrige  $Mg^{2+}$  Konzentration plus Polyamine) mit der in konventionellem Puffer (hohes  $Mg^{2+}$  ohne Polyamine) vergleicht. Überraschenderweise scheint die tRNA in physiologischen Bedingungen (niedriges  $Mg^{2+}$ ) eine kompaktere Struktur anzunehmen. (ii) Wenn die tRNAs in die P Stelle gebunden wurden, scheint diese die tRNA eine definierte Konformation aufzuzwingen unabhängig von dem gewählten Puffer, da tRNA-Phosphate unter allen Bedingungen ein praktisch identisches Erreichbarkeitsmuster aufweisen. Dieses Ergebnis kontrastiert zu den signifikanten Unterschieden, die die tRNAs im Ribosom unter korrespondierenden Bedingungen einnehmen (Kryo-EM). Zusammengenommen unterstützen diese Befunde ein weiteres Mal die Annahme einer beweglichen ribosomalen Domäne, die die tRNAs fest bindet und während der Translokation vom PRE zu dem POST Zustand transportiert.

In einem weiteren Experiment wurde das Erreichbarkeitsmuster der tRNA-Phosphate in programmierter und nicht programmierter P Stelle untersucht. Das überraschende Ergebnis war, dass in nicht-programmierten 70S Ribosomen Hinweise auf 30S Kontakte praktisch fehlten, während das entsprechende 50S Muster normal vorhanden war. Augenscheinlich ist Codon-Anticodon Wechselwirkung in der P Stelle Voraussetzung für 30S Kontakte mit der tRNA auch außerhalb des Anticodons.

Im zweiten Teil wurden Phosphorothioat-Ergebnisse mit der Kristallstruktur von 70S *Thermus thermophilus* Ribosomen verglichen, die drei tRNAs tragen. Kontaktmuster (Phosphorothioat Experimente) von tRNAs, die an isolierte Untereinheiten gebunden wurden, stimmten ausgezeichnet mit den Kontakten zu den Untereinheiten einer P-Stellen tRNA innerhalb des 70S Ribosoms überein. Ein signifikantes Ergebnis konnte abgeleitet werden: nicht nur die tRNA Nukleotide, die an der Ribosomenbindung beteiligt sind, sind universell, sondern auch Nukleotide der rRNA and Aminocylreste, die an der tRNA Fixierung beteiligt sind.

Schließlich konnte gezeigt werden, dass eine Zusammenstellung der zugänglichen Daten von Kryo-EM, Kristallographie und biochemischen Daten das Hybrid-Stellen Model nicht unterstützen, aber im Einklang mit dem  $\alpha$ - $\epsilon$  Model stehen.

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## Abbreviations

AA	Acrylamide
$\alpha$ S-NTP	thioated-NTP
aa-tRNA	aminoacyl-tRNA
AcPhe-tRNA <sup>Phe</sup>	N-Acetyl-Phe-tRNA <sup>Phe</sup>
Å	Ångström
ATP	Adenosine tri-phosphate
BAA	Bis-acrylamide
BPB	Bromophenol blue
dsDNA	double strand DNA
EF-G	Elongation factor G
EF-Ts	Elongation factor thermo stable
EF-Tu	Elongation factor thermo unstable
GDP	Guanine di-phosphate
Gly	Glycine
GTP	Guanine tri-phosphate
H <sub>t</sub> M <sub>u</sub> N <sub>v</sub> SH <sub>w</sub> Spd <sub>x</sub> Spm <sub>y</sub>	H Hepes t mM
	M MgAc u mM
	N NH <sub>4</sub> Ac v mM
	SH $\beta$ -Mercapto-ethanol w mM
	Spd Spermidine x mM
	Spm Spermine y mM
HPLC	High Performance Liquid Chromatography
IF	Initiation factor
kb	kilo bases
kJ	kilo Joules
kV	kilo Volts
mA	milli ampere
$\mu$ Ci	micro Curie
MDa	megadalton
MgAc	magnesium acetate
M.W.	molecular weight
N-AcPhe-tRNA <sup>Phe</sup>	N-Acetyl-Phe-tRNA <sup>Phe</sup>
NaAc	sodium acetate
NH <sub>4</sub> Ac	ammonium acetate
nt	nucleotide(s)
NTP	Nucleoside tri-phosphate
$\Omega$	Ohm
PK	Pyruvate kinase

Phe	Phenylalanine
Poly(U)	Poly-uridine mRNA
PTF	Peptidyl transferase centre
rpm	revolutions per minute
rRNA	ribosomal RNA
SD	Shine Dalgarno sequence
30S	small ribosomal subunit
50S	large ribosomal subunit
V	Volts
v/v	volume/volume
w/v	weight/volume