



Ribosome Group

The Ribosome group consists of three groups headed by Richard Brimacombe, Paola Fucini, and Knut Nierhaus. Research topics are structure and function of ribosomes applying biochemical, cryo-electron-microscopic and crystallographic methods.

Ribosomal RNA Structure



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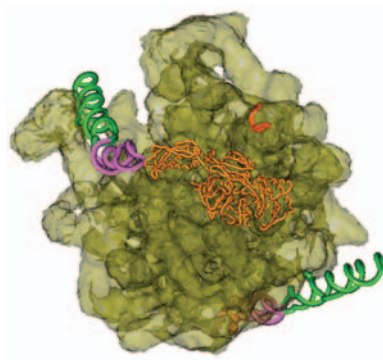
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Research report

The work of this group has for many years been concerned with the development and application of cross-linking techniques as a tool for studying the structure and function of bacterial ribosomes. During the late 'nineties significant advances were made in cryo-electron microscopy (cryo-EM), which led to the derivation of 3D structures for ribosomes and their subunits at a resolution of 10 – 15 Å. In collaboration with the cryo-EM group of Marin van Heel (Imperial College, London) we used our cross-linking data, combined with the known secondary structures of the 16S and 23S rRNA molecules, to fold the rRNA into three dimensions so as to fit these cryo-EM envelopes.

The cryo-EM structures could also be used by ribosomal crystallographers to phase their crystals, with the result that in the year 2000 crystallographically derived atomic structures for the ribosomal subunits became available for the first time. This dramatic development obviously had a profound effect on all structural research in the ribosome field. In our case we have shifted emphasis so as to study complexes between ribosomes and functional ligands which are not so far amenable to crystallography; cross-links between the ligand and the ribosome are identified, and – if possible with the help of cryo-EM data from similar complexes – are used to dock the ligand onto the crystallographic atomic structure of the corresponding ribosomal subunit.

Two such complexes are currently being studied. The first is the ribosomal complex formed with 4.5S RNA and the small protein Ffh, which is the prokaryotic equivalent of the eukaryotic signal recognition particle (SRP). The SRP recognizes proteins destined for export as they emerge newly-synthesized from the ribosome. Here a crystal structure has been determined for a fragment of the 4.5S RNA complexed with Ffh,



A cryo-EM silhouette of the E. coli 50S ribosomal subunit, showing two docked positions for 4.5S RNA based on cross-linking data. The 4.5S molecule is coloured green and magenta, the magenta portion corresponding to the crystallized fragment. The docked position at lower right corresponds to the Ffh-dependent cross-link to 23S rRNA (the Ffh molecule can just be seen as the yellow structure behind the subunit), and the other position (upper left) to an Ffh-independent cross-link to the 30S subunit. The orange structure represents elongation factor EF-G (also implicated in interactions with 4.5S RNA), and the red loop shows the position of a 10-nucleotide sequence in 23S rRNA that is identical to part of the 4.5S molecule.

and we were able to precisely identify an Ffh-dependent cross-link between 4.5S RNA and the 23S rRNA, which allowed us to dock the 4.5S /Ffh complex onto the 50S subunit at the exit site for the nascent protein chain (see Diagram). However, 4.5S RNA is suspected of having multiple functions, and we observed a second (Ffh-independent) 4.5S RNA cross-link at an entirely different location on the ribosome, on the shoulder of the 30S subunit; the functional significance of this has yet to be elucidated.

The second system concerns the “tmRNA”, a molecule which functions both as mRNA and tRNA, and which in prokaryotes “rescues” ribosomes that are stalled on a damaged mRNA molecule. The secondary structure of the tmRNA has the shape of the letter “P”, with the “stalk” of the letter being formed by the tRNA-like portion of the molecule, and the “ring” by a complex group of four pseudo-knot structures. Recent cryo-EM studies have shown that in the presence of elongation factor EF-Tu and the small protein SmpB the tRNA-like part of the tmRNA lies as expected in the subunit interface space (in a normal tRNA binding site), whereas the ring of pseudo-knots is arranged on the head of the 30S subunit. Our cross-linking studies are currently being made in the absence of the protein SmpB and our preliminary results strongly suggest that in this inactive state the ring of pseudoknots is arranged in a similar way to that in the active state (in the presence of SmpB) but that the whole “stalk” containing the tRNA-like portion is swung back onto the solvent side of the 30S subunit. In such a position, normal protein synthesis could take place but the tmRNA would be in a “stand-by” state, ready to move into action if needed.

Many of our cross-linking projects have been and are being carried out in close collaboration with the group of Professors Olga Dontsova and Alexey Bogdanov in Moscow State University (see sections on “Personnel” and “External funding”). The Brimacombe research group will dissolve in mid-2005, when the group leader (RB) goes into retirement.



General information

Selected Publications 1998 - 2003

- Choi KM & Brimacombe R** (1998). *The path of the growing peptide chain through the 23S rRNA in the 50S ribosomal subunit; a comparative cross-linking study with three different peptide families*. Nucleic Acids Res 26: 887-895
- Baranov PV, Gurvich OL, Bogdanov AA, **Brimacombe R** & Dontsova OA (1998). *New features of 23S ribosomal RNA folding: the long helix 41-42 makes a "U-turn" inside the ribosome*. RNA 4: 658-668
- Sergiev P, Dokudovskaya S, Romanova E, Topin A, Bogdanov A, **Brimacombe R** & Dontsova O (1998). *The environment of 5S rRNA in the ribosome: cross-links to the GTPase-associated area of the 23S rRNA*. Nucleic Acids Res 26: 2519-2525
- Oswald M & Brimacombe R** (1999). *The environment of 5S rRNA in the ribosome: cross-links to 23S rRNA from sites within helices II and III of the 5S molecule*. Nucleic Acids Res 27: 2283-2290
- Greuer B, Thiede B & Brimacombe R** (1999). *The cross-link from the upstream region of mRNA to ribosomal protein S7 is located in the C-terminal peptide; experimental verification of a prediction from modelling studies*. RNA 5: 1521-1525
- Mueller F, Sommer I, Baranov P, Matadeen R, Stoldt M, Wöhnert J, Görlach M, van Heel M & Brimacombe R** (2000). *The 3D arrangement of the 23S and 5S rRNA in the E. coli 50S ribosomal subunit based on a cryoelectron microscopic reconstruction at 7.5 Å resolution*. J Mol Biol 298: 35-60
- Matadeen R, Sergiev P, Leonov A, Pape T, van der Sluis E, **Mueller F, Oswald M, von Knoblauch K, Brimacombe R, Bogdanov A, van Heel M & Dontsova O** (2001). *Direct localization by cryo-electron microscopy of secondary structural elements in E. coli 23S rRNA which differ from the corresponding regions in Haloarcula marismortui*. J Mol Biol 307: 1341-1349
- Sergiev P, Leonov A, Dokudovskaya S, Shpanchenko O, Dontsova O, Bogdanov A, **Rinke-Appel J, Mueller F, Oswald M, von Knoblauch K & Brimacombe R** (2001). *Correlating the x-ray structures for halo- and thermophilic ribosomal subunits with biochemical data for the E. coli ribosome*. Cold Spring Harbor Symp Quant Biol 66: 87-100
- Rinke-Appel J, Oswald M, von Knoblauch K, Mueller F, Brimacombe R, Sergiev P, Avdeeva O, Bogdanov A & Dontsova O** (2002). *Cross-linking of 4.5S RNA to the E. coli ribosome, in the presence or absence of Ffh*. RNA 8: 612-625

External funding

Structural and functional investigations of the E. coli ribosome at quasi-atomic resolution, (DFG, Br 632/5-1;5-2, 2002-2004, 2 scientists)

Collaboration with Moscow State University/exchange of scientists (DFG, 436 RUS 113/639)

Study of structure and function of rRNA in the ribosome: a new look at the old problem". (Humboldt Foundation, Grant No. IV-3-7122-1070296, 2001/2002).

DAAD: "sur place" stipendia under the Leonhard-Euler Programm for five Russian students in Moscow, each for nine months, in the framework of our collaboration with Moscow State University (ref: 325/lin).

Theses

Kyoung-Moo Choi, *Studies on the path of the growing peptide chain through the ribosome*, PhD Thesis, Freie Universität Berlin, 1998 (funded by DFG under the special programme (Schwerpunktprogramm) RNA Biochemistry)

Ingolf Sommer, *Interactive and computational methods for molecular modelling applied to the bacterial ribosome*, PhD Thesis, Technische Universität Berlin, 1999 (funded by DFG under the special programme (Schwerpunktprogramm) RNA Biochemistry)

Visiting scientists

Regular visitors from Moscow State University :
Prof. Alexey Bogdanov
Prof. Olga Dontsova
Dr. Petr Sergiev
Dr. Andrej Leonov
Olga Avdeeva (graduate student)

Appointments, scientific honors & memberships

Member of Editorial or Advisory Board for the following Journals:

- Nucleic Acids Research
- RNA
- European Journal of Biochemistry

Ribosome crystallography



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Overview & general aim

The principal aim of the ribosome crystallography group has been for more than 20 years the development of suitable techniques for the crystallization of ribosomal particles. The steady advancement of this powerful knowledge, initially started to reap its reward three years ago (1-3), under the leadership of Dr. Francois Franceschi, with the breath-taking atomic structures of the small ribosomal subunit from *Thermus thermophilus* (4) and large ribosomal subunit from *Deinococcus radiodurans* (5). Both structures, resulting from ribosomes and crystals prepared in the group of Berlin, were solved in collaboration with the group of Prof. Ada Yonath, at the MPG für strukturelle Molekularbiologie in Hamburg and Weizmann Institute in Israel. In addition to the collaborative Berlin-Hamburg unit, only three other groups worldwide have made such major contributions to ribosome studies using the same crystallographic approach.

Atomic structures of the ribosomal subunits have been a revolutionary milestone in our understanding of the translational apparatus, initiating a new era in comprehension of the key activities associated with each ribosomal subunit: the decoding process, peptide-bond formation and factor-mediated translation regulation. In this respect, the crystallography group of Berlin, has mainly concentrated its research activity on the mode of binding and action of various antibiotics; from those targeting the large subunit (6), such as the clinically relevant macrolides (7), as well as puromycins and sparsomycins (8); to those that bind the small subunit, such as aminoglycosides, tetracyclines, pactamycin and edeine. The results obtained have shed light on the mechanism of action of these drugs and because of their importance in the pharmaceutical field have resulted in at least one patent, stipulated with the help of Garching Innovation and YEDA (the corresponding Institution for Ada Yonath's group in Israel).

After the departure of Dr Francois Franceschi in June 2002, the ribosome crystallography group has continued its research activity under the leadership of Dr. Paola Fucini, with invaluable assistance from Dr. Daniel Wilson, an experienced postdoc who entered the



group during the same period. In addition to continuing the antibiotic studies in collaboration with Prof. Yonath (9), a number of new projects were undertaken. These studies mainly focus on the next awaited breakthrough of the ribosome field: Crystallization of the complete 70S ribosome alone and in functional complexes, among which, in particular, the study of ribosomal particles stalled during the translation of the nascent polypeptide chain. The studies are made possible by the stimulating environment and advanced technical equipment available in the institute. Whenever possible, these projects are undertaken in collaboration with the appropriate expert groups, within or separate from the institute, increasing the success and speed of project completion. A more specific description of the studies that are in process in the group, and the predicted impact that they will have for the scientific community at large, is reported in the following section.

Status of the scientific achievements obtained

Crystallization of functional complexes

Currently, the ribosome crystallography group is in the unique position of being able to reproducibly prepare four different types of ribosomal crystals: *T. thermophilus* 30S, 50S, 70S and *D. radiodurans* 50S subunit. The *T. thermophilus* 30S subunit and *D. radiodurans* 50S subunit diffract to high resolution and have been used as a platform for the preparation and study of novel ribosomal complexes containing small regulatory ligands. The results obtained so far can be classified within four distinct research areas: i) The mechanism of peptide-bond formation (10), ii) Egression of the newly synthesised polypeptide (11); iii) The regulation of ribosomal activity by various protein factors: ribosome modulation factor (RMF), the GTPase Era and r-protein S1; iv) Antibiotic inhibition of translation (12).

The ribosomal tunnel and the newly synthesized nascent chain

The preparation of ribosomal complexes stalled during the act of translating a polypeptide nascent chain that are suitable for crystallography studies, is a project started during the post-doctoral studies of Dr. Fucini under the supervision of Prof. Dobson and Prof. Robinson (Cambridge). The study has led to the preparation of particles that, as yet not suitable for crystallography, have been successfully analysed by cryoEM, in collaboration with the group of Prof. David Stuart (Oxford). The results lead to the supposition that the ribosomal tunnel could play an important and active part during translation and that its role could depend on the type of protein synthesized.

The L7/L12 ribosomal stalk

The L7/L12 stalk, which is a highly dynamic ribosomal element and thus absent from the X-ray diffraction maps, is being investigated using NMR, also in collaboration with the group of Prof. Dobson. The study has allowed the structure determination of the C-terminal domain and the analysis of the dynamic of this essential ribosomal protein ON the ribosome thus demonstrating that this technique will also be applicable for study of nascent chain ribosomal complexes.

Characterization of the translational apparatus

Taking advantage of the advanced technology within the MPI for Molecular Genetics, we are collaborating with the mass spectrometry group of Lehrach department (Dr. Patrick Giavalisco in Dr. Johan Gobom group), to exhaustively characterize the composition of the 'cloud' of translational factors that surround the ribosome *in vivo*. The automated MS analysis, of more than 1000 proteins selected from 2D-IEF gels, has so far revealed two important aspects: 1) the first detailed investigation into the actual composition of the translational apparatus, and 2) the potential to utilize the same approach to prepare and analyse other macromolecular complexes. For example, during our analysis we have found at least three other macromolecular complexes: the degradosome, the chaperone apparatus and in plants, rubisco, the latter of which has been crystallized and will be further investigated as a side project.

Future orientation

The ribosome crystallography group should theoretically terminate its activities in June 2004, with the retirement of Prof. Ada Yonath. However, a request for the prolongation of this group has been submitted to the MPG. In the event that the prolongation will be granted, the Berlin group will continue on the research lines delineated over the last years, the results obtained have in fact created the ideal basis for the successful preparation of new functional ribosomal complexes suitable for x-ray crystallography. The group in addition is ready to extend its activities to also encompass the crystallographic analysis side of the projects which will continue unhindered under the leadership of Dr. Frank Schluenzen, presently principal crystallographer in the group of Prof Yonath. To this end, external funding are being organized through the participation in the 'Ribosome' FP6 Integrated Project and through pharmaceutical companies which have shown their interest to finance long-term projects for the screening and analysis of various antibiotics.

General information

Selected Publications 1998-2003

1) Weinstein S, Jahn W, Glotz C, Schlunzen F, Levin I, Janell D, Harms J, Kolln I, Hansen HA, Gluhmann M, Bennett WS, Bartels H, Bashan A, Agmon I, Kessler M, Pioletti M, Avila H, Anagnostopoulos K, Peretz M, Auerbach T, **Franceschi F** & Yonath A (1999). *Metal compounds as tools for the construction and the interpretation of medium-resolution maps of ribosomal particles*. J Struct Biol 127(2):141-51

2) Tocilj A, Schlunzen F, Janell D, Gluhmann M, Hansen HA, Harms J, Bashan A, Bartels H, Agmon I, **Franceschi F** & Yonath A (1999). *The small ribosomal subunit from Thermus thermophilus at 4.5 Å resolution: pattern fittings and the identification of a functional site*. PNAS USA 96(25):14252-7

3) Bartels H, Gluehmann M, Janell D, Schlunzen F, Tocilj A, Bashan A, Levin I, Hansen HA, Harms J, Kessler M, Pioletti M, Auerbach T, Agmon I, Avila H, Simitsopoulou M, Weinstein S, Peretz M, Bennett WS, **Franceschi F** & Yonath A (2000). *Targeting exposed RNA regions in crystals of the small ribosomal subunits at medium resolution*. Cell Mol Biol 46(5):871-82

4) Schlunzen F, Tocilj A, Zarivach R, Harms J, Gluehmann M, Janell D, Bashan A, Bartels H, Agmon I, **Franceschi F** & Yonath A (2000). *Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution*. Cell 102(5):615-23

5) Harms J, Schlunzen F, Zarivach R, Bashan A, Gat S, Agmon I, Bartels H, **Franceschi F** & Yonath A (2001). *High resolution structure of the large ribosomal subunit from a mesophilic eubacterium*. Cell 107(5):679-88

6) Schlunzen F, Zarivach R, Harms J, Bashan A, Tocilj A, **Albrecht R**, Yonath A & **Franceschi F** (2001). *Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria*. Nature 413(6858):814-21

7) Schlunzen F, Harms JM, **Franceschi F**, Hansen HA, Bartels H, Zarivach R & Yonath A (2003). *Structural basis for the antibiotic activity of ketolides and azalides*. Structure 11(3):329-38

8) Pioletti M, Schlunzen F, Harms J, Zarivach R, Gluhmann M, Avila H, Bashan A, Bartels H, Auerbach T, Jacobi C, Hartsch T, Yonath A & **Franceschi F** (2001). *Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3*. EMBO J 20(8): 1829-39

9) Bashan A, Agmon I, Zarivach R, Schlunzen F, Harms J, Berisio R, Bartels H, **Franceschi F**, Auerbach T, Hansen HA, Kossoy E, Kessler M & Yonath A (2003). *Structural basis of the ribosomal machinery for peptide bond formation, translocation, and nascent chain progression*. Mol Cell 11(1):91-102

10) Bashan A, Zarivach R, Schlunzen F, Agmon I, Harms J, Auerbach T, Baram D, Berisio R, Bartels H, Hansen HA, **Fucini P**, **Wilson D**, Peretz M, Kessler M & Yonath A (2003). *Ribosomal crystallography: Peptide bond formation and its inhibition*. Biopolymers 70(1):19-41



11) Agmon I, Auerbach T, Baram D, Bartels H, Bashan A, Berisio R, **Fucini P**, Hansen HA, Harms J, Kessler M, Peretz M, Schluenzen F, Yonath A & Zarivach R (2003). *On peptide bond formation, translocation, nascent protein progression and the regulatory properties of ribosomes*. Eur J Biochem 270(12): 2543-56

12) Berisio R, Harms J, Schluenzen F, Zarivach R, Hansen HA, **Fucini P** & Yonath A (2003). *Structural insight into the antibiotic action of telithromycin against resistant mutants*. J Bacteriol 185(14):4276-9

Thesis

M Pioletti, *Structure of functional complexes of the small ribosomal subunit*, PhD Thesis, Freie Universität Berlin, 2001

External funding

Analysis of the mode of binding and action of a new class of ribosome translation inhibitors, collaboration with an American Pharmaceutical Company, 1 scientist funded

Preparation and crystallization of Denococcus radiodurans ribosomal particles, collaboration with Prof. Ada Yonath, 1 technician funded

Co-operations

Structure determination of ribosomal complexes by X-ray crystallography, with Prof. Ada Yonath, MPI für strukturelle Molekularbiologie, Hamburg & Weizmann Institute, Israel

NMR structure determination of sub-components of the translational apparatus, with Prof. Christopher Dobson, Department of Chemistry, Cambridge University, UK

Mass Spectrometry characterization of functional ribosome complexes, with Prof. Carol Robinson, Department of Chemistry, Cambridge University, UK

Cryo-electron microscopy analysis of Escherichia coli nascent chain ribosomal complexes, with Prof. David Stuart, Wellcome Trust Centre for Human Genetics, Oxford, UK

Preparation of some ribosomal ligands used for the crystallization of ribosomal complexes, with Prof. Yokoyama, RIKEN Genomic Sciences Center, Yokohama, Japan

Cryo-electron microscopy analysis of Thermus thermophilus ribosomal complexes, with Prof. Christian Spahn, Charité, Humboldt University of Berlin

Mass Spectrometry identification of still unknown components of the translational apparatus, with Dr. Patrick Giavalisco, AG Klose, Institute of Human Genetics, Charité, Humboldt University of Berlin

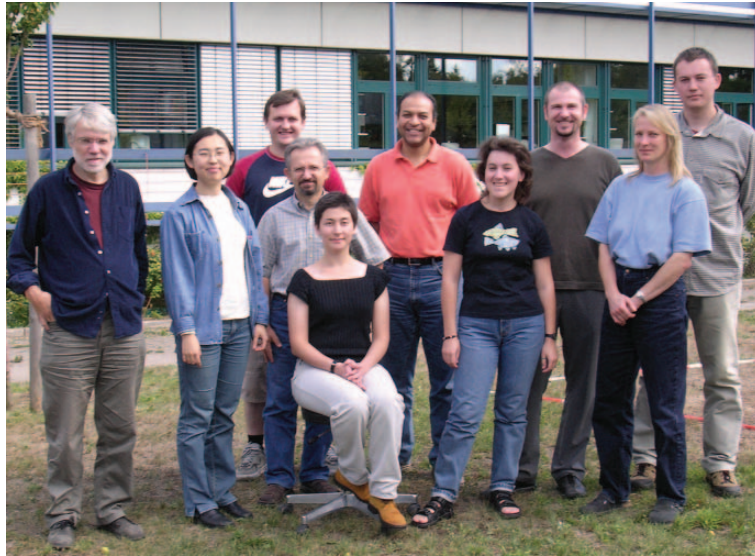
Biochemical characterization of ribosome translational inhibitors, with Dr. George Dinos, Laboratory of Biochemistry, University of Patras, Greece

Analysis of the mode of binding and action of a new class of ribosome translation inhibitors, with an American Pharmaceutical Company

Co-operation within the institute

Mass Spectrometry characterization of modified ribosomal proteins, with Dr. Johan Gobom, Dept. Lehrach

Ribosomal Function



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Introduction

During the last three decades the group follows three experimental lines that are not strictly separated:

1: 1974 we have developed a method to take apart the large subunit of *E. coli* ribosomes and reassemble them again to fully active particles (**total reconstitution**). Since then we are applying this method to study the role of single ribosomal components for assembly and function of the ribosome.

2: 1980 we detected a third tRNA binding site on *E. coli* ribosomes, which we could confirm subsequently with ribosomes from all three kingdoms (evolutionary domains). This third tRNA site was termed E site, which has been accepted by the scientific community with the advent of cryo-electron microscopy (cryo-EM) and crystal structures where the tRNA in the E site could be visualized. In the last two decades we developed and mastered an arsenal of functional assays for ribosome studies with the result that **functional studies** as well as **structural analysis of functional complexes** of the ribosome are in the focus of our work. Accordingly, I will discuss our work since 1998 in three sections: (1) reconstitution and function, (2) structural analysis of functional complexes, and (3) functional studies.



Reconstitution & function

The ribosomal protein L2 is one of the universal ribosomal proteins that is - at the same time - one of the best conserved proteins. We could demonstrate that without L2 50S subunits assemble normally, but cannot form 70S ribosomes with 30S subunits. Mutational studies revealed the importance of L2 for tRNA binding to the A and P sites as well as for peptide-bond formation. The results can be reconciled with the assumption that L2 is essential for the formation of a bridge between the subunits, and that this bridge binds tRNAs and probably translocates them from A and P to P and E sites, respectively (Diedrich et al., 2000). Structural studies are consistent with this interpretation.

Structural analysis of functional complexes

Most ribosomal proteins have multiple contacts with the rRNA *in situ* as demonstrated with crystal structures of ribosomes. It therefore prohibitively difficult to extract the primary binding site of a ribosomal protein from ribosome crystals, a site that might define the entry site of this protein into the process of ribosomal assembly. We developed a technique for the identification of the primary binding site (Stelzl et al., 2000) and demonstrated its usefulness.

The first correct tRNA localization was derived from neutron scattering studies (Nierhaus et al., 1998, together with the Stuhmann group at the GKSS, Hamburg), where even the correct angle between the tRNAs was determined to 40° as later confirmed by cryo-EM and crystal structures. Later, we cooperated with the Frank group (Albany, NY), probably the best group for cryo-EM work in translation. The result of this fruitful cooperation was a series of seven papers, the main results were: (i) we could precisely determine the tRNA positions in A, P and E sites thus leading to the first movie showing how the tRNAs are moving through the ribosome during protein synthesis (Agrawal et al., 2000). (ii) We demonstrated that a deacylated tRNA is found at the hybrid-site P/E under unfavorable buffer conditions, but at the canonical P site under *in vivo* near conditions (Agrawal et al., 1999). This observation represents the first evidence that the hybrid-site model is not an appropriate description of the elongation cycle, the central functional phase of protein synthesis. (iii) In a collaboration with Diane Taylor in Edmonton, Canada, Sean Connell came to my group for three years, and he solved one of the two most important mechanisms of resistance against tetracycline, an antibiotic widely used in medicine. The resistance is mediated by a protein Tet(O) that is a derivative of the elongation factor G and exploits the tricks of the elongation factors in order to cause resistance (Connell et al., 2003). Together with Spahn in the Frank group we determined the position of Tet(O) on the ribosome (Spahn et al., 2001). (iv) The binding of the ternary complex before accommodation of the aminoacyl-tRNA into the A site was analyzed by cryo-EM, and a kink in the anticodon arm of the L-shaped tRNA was detected that provides an important detail, namely how the tRNA anticodon of the incoming ternary complex can contact the codon at the A site, a prerequisite for decoding the genetic message (Valle et al., 2002).

Functional studies

Phosphorothioated tRNA was used to assess the contact patterns of tRNA in each of the three tRNA binding sites A, P and E. The tRNAs were analyzed during the elongation cycle, and the most important outcome was that the two tRNAs on the ribosomes had strikingly different contact patterns, but which did not change when translocated from A and P sites to the P and E sites, respectively. We concluded that the ribosomal microenvironment of the tRNAs during the translocation did not change, in other words, there seems to be a ribosomal conveyor that binds tightly two tRNAs and carries them from A+P to P+E sites (Dabrowski et al., 1998). The corresponding model for the elongation cycle, the α - ϵ model, is at the moment the most appropriate explanation of the plethora of observations concerning the ribosomal elongation cycle (Wilson &

Nierhaus, 2003). We further demonstrate with the same technique that codon-anticodon interaction is essential for tRNA-30S contacts within the 70S ribosome, in other words, codon-anticodon interaction at the P site is sensed by the ribosome and triggers tRNA-30S interactions (Schäfer et al., 2002). This observation explains a number of features of tRNA binding to ribosomes.

Norbert Polacek from Andrea Bartas's group in Vienna analysed in my group features of tertiary structure of tRNAs and rRNAs during the elongation cycle. A Pb^{2+} cleavage pattern changed periodically before and after translocation demonstrating for the first time a dynamic and mobile role for the 23S rRNA during translocation (Polacek et al., 2000).

Finally, the RelA mechanism was dissected, the central factor for the stringent response, the most important regulation circle in bacteria. We demonstrated that RelA binds with high affinity to idle ribosomes, but as soon as (p)ppGpp is made the affinity drops and RelA dissociates and will bind to another idle ribosome (Wendrich et al., 2002, together with the Marahiel group in Marburg, Germany). This observation explains why one RelA molecule per 200 per ribosomes can synthesize (p)ppGpp at levels that accurately reflect the starved ribosome population (Wendrich et al., 2002).

At the moment we prepare a manuscript describing experiments where we show that codon-anticodon interaction is essential for maintaining the reading frame thus giving another functional reason for the universal presence of the ribosomal E site. Another manuscript is ready for submission that defines the inhibition mechanism of the antibiotics edeine and pactamycin and their antagonistic relationship (together with Georg Dinos from the Kalpaxis group in Patras, Greece).

For the last three years in my scientific carrier at the MPI I identified three main topics I would like to concentrate on provided continuing support of the MPG for our work:

1: We would like to extend our experience of the elongation phase to problems of termination: (a) which mechanism frees the E site from deacylated tRNA? (b) does EF-G translocate the termination factor RRF during termination?

2: the tmRNA monster binds efficiently to the ribosomal A site if a peptidyl-tRNA is at the P site and no intact codon at the A site due to mRNA fragmentation. What are the conditions for the efficient binding?

3: We would like to isolate physiological tRNA fragments that bind to the peptidyl transferase center of 50S crystals to answer a number of unresolved questions. We also would like to prepare authentic pre- and post-translocational complex to crystallize them and to analyze the problem of translocation, one of the most challenging problems of ribosomology. This project is a collaboration with the Fucini group.

General information

Selected Publications 1998-2003

Connell SR, Trieber CA, Dinos GP, Einfeldt E, Taylor DE & Nierhaus KH (2003). *Mechanism of Tet(O)-mediated tetracycline resistance*. EMBO J 22:945-953

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Valle M, Sengupta J, Swami NK, Grassocci RA, **Burkhardt N, Nierhaus KH**, Agrawal RK & Frank J (2002). *Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process*. EMBO J 21:3557-3567



Wendrich TM, **Blaha G**, **Wilson DN**, Marahiel MA & **Nierhaus KH** (2002). *Dissection of the mechanism for the stringent factor RelA*. Mol Cell 10:779-788

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Teaching

Kurs Proteinbiosynthese, 21.2.- 10.3.2000 (three weeks full-time), Technical University of Berlin and Free University of Berlin

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Theses

Ullrich Stelzl, *In vitro Selektion der RNA-Binde-stelle von Proteinen aus statistischen RNA-Fragmenten (SERF): Charakterisierung neuer rRNA-Protein Wechselwirkungen im Escherichia coli Ribosom*, PhD Thesis, University of Vienna, 1999

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Guest scientists

Pavel Ivanov

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Dr. Jean-Hervé Alix, University of Paris, France

Dr. Tetyana Budkevich, University of Kiev

Dr. Sean Connell, University of Edmonton, Canada

Dr. Daniel Wilson, Newzealand, Humboldt Stipendiat

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