Inserm

cnrs

EMBO
excellence in life sciences

université de BORDEAUX

11th Bordeaux RNA Club Symposium

JUNE 27-28 2019

IECB Auditorium
2, rue Robert Escarpit
33600 Pessac, France

Institut Européen de Chimie et Biologie
I E C B

RNA CLUB
Bordeaux

Organized jointly with

APTAMERS in Bordeaux

June 28-29, 2019
Bordeaux, France
www.aptamers-in-bordeaux.com

Program

Thursday, June 27, 2018 | IECB auditorium

8:20 – 8:40 Registration

8:40 – 8:50 Opening remarks (Y. Hashem)

8:50 – 10:35 1st Session - Ribosome and translation (Chair: Y. Hashem)

8:50 – 9:30 **Christine Clayton** *(invited speaker)*

Mechanisms of selective translation stimulation and suppression by the multiple eIF4E isoforms of *Trypanosoma brucei*

9:30 – 9:50 **Philippe Giegé**

Composition and architecture of Arabidopsis mitochondrial ribosome

9:50 – 10:10 **Hakim Mireau**

The translational landscape of plant mitochondria and control by PPR proteins

10:10 – 10:25 **Natalia Korniy**

Modulation of HIV-1 Gag/Gag-Pol Frameshifting by tRNA abundance

10:25 – 10:35 *Sponsor talk (NEB)*

10:35 – 10:55 Coffee break

10:55 – 13:10 2nd Session - Ribosome and translation (Chair: F. Darfeuille)

10:55 – 11:35 **Sarah Woodson** *(invited speaker)*

The dynamics of Hfq-small RNA interactions and mRNA targeting

11:35 – 11:55 **Grégory Boël**

ABC-F type translation factors modulate the stereochemistry of the peptidyl transferase center in the ribosome and are necessary for bacteria to adapt to their environment

11:55 – 12:10 **Victoriia Murina**

Antibiotic resistant ABCFs: straight from structure to mechanism?

12:10 – 12:30 **Daniel Wilson**

Structures of antibiotic-resistance ABCF proteins in complex with the ribosome

12:30 – 13:10 **Zoya Ignatova** *(invited speaker)*

Alterations of translation (components) with age

13:10 – 15:00 Lunch break and poster session

15:00 – 16:40 3rd Session - RNA Biology (Chair: A. Innis)

15:00 – 15:40 **Alessandro Vannini** *(invited speaker)*

Watching (class III) gene transcription in HD

15:40 – 16:00 **Suparna Sanyal**

Bimodal effect of RNA on aggregation of the tumor suppressor protein p53

16:00 – 16:20 **Dmitri Ermolenko**

mRNAs and lncRNAs intrinsically form secondary structures with short end-to-end distances

16:20 – 16:40 **Sanjeev Shukla**

Interplay between epigenetics, alternative splicing, and cancer metabolism

16:40 – 17:00 Coffee break

17:00 – 18:30 4th Session - RNA Biology (Chair: C. Mackereth)

17:00 – 17:40 **Eduardo Eyras** *(invited speaker)*

Long read transcriptomics - beyond model organisms

17:40 – 18:00 **Grégoire de Bisschop**

Unwinding of HIV RNA dimers by DDX3 questions its role during infection

18:00 – 18:15 **Lina Hamouche**

Dynamics of RNase Y membrane localization in *Bacillus subtilis*

18:15 – 18:30 **Jorg Morf**

Spatial transcriptome organization in the nucleus

20:00 – Dinner in Bordeaux for invited and selected speakers

Friday, June 28, 2018 | Cité du Vin, joined session

8:20 – 10:30 5th Session - Aptamers (Chair: C. Mackereth)

8:20 – 8:40 **James Attwater**

Emergence of cooperativity during *in vitro* selection of a self-synthesising ribozyme

8:40 – 9:00 **Florian Bernard**

Full-length analysis of *Caenorhabditis elegans* transcriptome using Nanopore sequencing technology

9:00 – 9:20 **Josef Wachtveitl**

Structure guided fluorescence labeling reveals a two-step binding mechanism of neomycin to its RNA aptamer

9:20 – 9:40 **P. Johnson**

Fine-tuning the binding affinity of a structure-switching aptamer using dangling nucleotides

9:40 – 10:30 **Judy Lieberman** *(invited speaker)*

Aptamer delivery of siRNAs to knockdown cancer

10:30 – 11:10 Coffee break

11:10 – 12:30 6th Session - Aptamers (Chair: L. Azéma)

11:10 – 11:30 **Murat Sunbul**

Genetically encoded light-up aptamers for live-cell super-resolution RNA imaging

11:30 – 11:50 **Arghya Sett**

Light-up aptasensors - A tool for the detection of RNA hairpins and small molecules

11:50 – 12:10 **Farah Bouheda**

Evolution of efficient RNA-based fluorescent probes using ultrahigh-throughput functional screening

12:10 – 12:30 **Olga Puchta**

High-throughput screening of fluorescent RNA aptamers

12:30 Departure

Oral communications

Mechanisms of selective translation stimulation and suppression by the multiple eIF4E isoforms of *Trypanosoma brucei*

Franziska Egler, Larissa Nasciamento, Renata Santana, Osvaldo de Melo Neto & Christine Clayton

ZMBH, Heidelberg University, Germany

Trypanosoma brucei has six isoforms of the cap-binding translation initiation factor eIF4E, and five eiF4Gs (PMID: 29077018), which potentially allows for differential mRNA target selection in order to fine-tune translation. EIF4E3 and EIF4E4 appear to be general initiation factors and EIF4E2 is dispensable; we are investigating EIF4E1, EIF4E5, and EIF4E6.

EIF4E1 interacts with 4EIP (4E-interacting protein) and not with any EIF4G; EIF4E1 and 4EIP functionally resemble mammalian 4E-HP and GIGYF2, respectively. 4EIP suppresses translation and provokes mRNA degradation. 4E-IP and EIF4E1 are dispensable in slender "bloodstream forms", which multiply in mammals, but 4EIP is required for translation suppression in the growth-arrested "stumpy" bloodstream form (PMID: 30124912). Meanwhile EIF4E1, but not 4EIP, is required for survival of "procyclic" forms, which grow in Tsetse. New results suggest that the EIF4E1-4EIP complex recruits the CAF1-NOT deadenylation complex and a cystosolic terminal uridylyltransferase 3 (TUT3). Tethered EIF4E1 is suppressive only when 4EIP is present. We are investigating whether it can, without an eIF4G, activate translation in procyclic forms, and how it and 4EIP select target mRNAs.

EIF4E3, 4, 5 and 6 all stimulate expression when tethered. They interact with different EIF4G homologues, and each is essential in at least one life-cycle stage, indicating that each has a distinct role. We have found that EIF4E6 interacts specifically, not only with EIF4G5, but also with a previously characterized stimulatory complex containing MKT1, PBP1, XAC1 and LSM12. The MKT complex is recruited to mRNAs via sequence-specific RNA-binding proteins (PMID: 24470144), offering a novel mechanism for specific translation activation by the EIF4E6-EIF4G5 complex.

Composition and architecture of Arabidopsis mitochondrial ribosome

Florent Waltz[1,3], Hakim Mireau[2], Yaser Hashem[3] & Philippe Giegé[1]

1. Institut de biologie de moléculaire des plantes, UPR2357 du CNRS, Université de Strasbourg, 12 rue du général Zimmer, F-67084 Strasbourg, France
2. Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, RD10, 78026 Versailles Cedex, France
3. Institut Européen de Chimie et Biologie, INSERM U1212, CNRS UMR5320, Université de Bordeaux, 2 rue R. Escarpit, F-33600 Pessac, France

Mitochondria are responsible for energy production through aerobic respiration and represent the powerhouse of eukaryotic cells. Their metabolism and gene expression processes combine bacterial-like features and traits that evolved in eukaryotes. Among mitochondrial gene expression processes, translation remains the most elusive. In plants, while numerous pentatricopeptide repeat (PPR) proteins are involved in all steps of gene expression, their function in mitochondrial translation remains unclear. We present the biochemical characterization of Arabidopsis mitochondrial ribosomes and identify their protein subunit composition. Complementary biochemical approaches identify 19 plant specific mitoribosome proteins, among which 10 are PPR proteins. The knock out mutations of ribosomal PPR (rPPR) genes result in distinct macroscopic phenotypes including lethality or severe growth delays. The molecular analysis of rppr1 mutants using ribosome profiling as well as the analysis of mitochondrial protein levels reveal that rPPR1 is a generic translation factor, which is a novel function for PPR proteins. Finally, single particle cryo-electron microscopy reveals the unique structural architecture of Arabidopsis mitoribosomes, characterized by a very large small ribosomal subunit, larger than the large subunit, bearing an additional RNA domain grafted on the head. Overall, results show that Arabidopsis mitoribosomes are substantially divergent from bacterial and other eukaryote mitoribosomes, both in terms of structure and of protein content. This contributes to unravel the diversity of translation systems across eukaryotes.

Reference: Waltz, F, Nguyen, T, Arrived, M, Bochler, A, Chicher, J, Hammann, P, Kuhn, L, Quadrado, M, Mireau, H, Hashem, Y. and Giegé, P. (2018) Small is big in Arabidopsis mitochondrial ribosome. Nature plants, 2019.

The translational landscape of plant mitochondria and control by PPR proteins

Trung Tan N'Guyen, Noelya Planchard, Pierre Bertin, Martine Quadrado, Céline Dargel-Graffin, Isabelle Hatin, Olivier Namy & Hakim Mireau

Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, RD10, 78026 Versailles Cedex, France

Translation in plant mitochondria is a complex process that is still poorly understood at the molecular level. Growing evidence indicates though that mitochondrial translation differs from its bacterial counterpart in many key aspects. To better understand how mitochondrial translation is orchestrated and regulated in plants, we have used the ribosome profiling technology to generate genome-wide views of the mitochondrial translatome in different plant genotypes. This approach led us to reveal that most plant mitochondrial ribosome footprints measure 27 and 28 bases. Quantification of ribosome footprints along transcripts revealed that mRNAs have highly divergent ribosome densities, suggesting a tight control of translation initiation or elongation in plant mitochondria. To better understand the basis of this control, we identified and have been characterizing mitochondria-targeted pentatricopeptide repeat (PPR) proteins specifically involved in translation. I will present our most recent advances on the characterization of these PPR proteins.

Modulation of HIV-1 Gag/Gag-Pol Frameshifting by tRNA abundance

Natalia Korniy[1], Akanksha Goyal[1], Markus Hoffmann[2], Ekaterina Samatova[1], Frank Peske[1], Stefan Pöhlmann[2,3] & Marina V. Rodnina[1]

1. Department of Physical Biochemistry, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
2. Infection Biology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany
3. Faculty of Biology and Psychology, University of Göttingen, Wilhelm-Weber-Str. 2, 37073 Göttingen, Germany

The hallmark of translation at the gag-pol overlap of human immunodeficiency virus type 1 (HIV-1) is –1 frameshifting (–1FS) event. –1FS allows for the production of two different proteins with the same N-terminus but different C-termini by changing the open reading frame established by the ribosome in the beginning of translation. The function of –1FS in HIV-1 is to maintain the ratio between viral enzymes (–1-frame product, Gag-Pol) and structural proteins (0-frame product, Gag). The dysregulation of frameshifting is detrimental for viral particle formation, propagation and infectivity of HIV-1. Here we show that –1FS at the gag-pol mRNA proceeds via two alternative mechanisms and the –1FS efficiency is modulated by the availability of the specific Leu-tRNA$^{Leu(UAA)}$ isoacceptor reading the second codon of the slippery site, UUA. This tRNA is rare in CD4+ T-lymphocytes, which are the primary targets of HIV-1 infection in humans. Depending on the availability of Leu-tRNA$^{Leu(UAA)}$, a constant Gag to Gag-Pol ratio is achieved by switching between the two frameshifting mechanisms. At high Leu-tRNA$^{Leu(UAA)}$ concentration frameshifting is suppressed, offering options for new approaches in antiviral drug development. HIV-1 contains a second potential slippery site downstream of the first one, which is normally inefficient, but can also support –1FS when altered by a compensatory resistance mutation in response to current antiviral drug therapy. Together these different regimes allow the virus to maintain a constant –1-frameshifting efficiency thus ensuring successful virus propagation.

The dynamics of Hfq-small RNA interactions and mRNA targeting

Ewelina M. Malecka-Grajek[1], Subrata Panja[1], Boyang Hua[2], Andrew Santiago-Frangos[1], Steven Hardwick[3], Ben Luisi[3], Kathrin S. Fröhlich[4] & Sarah A. Woodson[1]

1. T. C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218, United States
2. Department of Biophysics and Biophysical Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, United States
3. Department of Biochemistry, University of Cambridge, CB2 1GA Cambridge, United Kingdom
4. Department of Biology I, Microbiology, Ludwig Maximilian University Munich, 82152 Martinsried, Germany

Bacterial small non-coding RNA (sRNA) regulates the response to stress and other external signals, allowing bacteria to adapt to a range of environments. Many sRNAs base pair with complementary sites in target mRNAs, inhibiting or stimulating translation. The recognition of mRNA targets is aided by the chaperone Hfq that facilitates the interactions of sRNAs with their target mRNAs, which is the main layer of posttranscriptional regulation in many bacterial species. Hfq has distinct RNA binding surfaces that can recognize ARN, 3' U-rich, or internal AU-rich sequence motifs. It is not fully understood how Hfq's multiple binding surfaces accelerate sRNA-mRNA annealing and how the annealing process depends on the RNA orientation. We developed a TIRF-based single-molecule fluorescence platform to visualize Hfq-mediated sRNA-mRNA annealing process in real time. Using this platform, distinct events which take place before and during the formation of the Hfq-mRNA-sRNA ternary complex can be observed. Moreover, our assay allows for a direct observation of the helix formation initiation and shows that sRNA-mRNA annealing can be achieved via multiple pathways. Surprisingly, the lifetimes of the annealed complexes strongly depend on the orientation of the sRNA on Hfq, explaining why certain classes of sRNAs regulate their targets more efficiently than others.

Moreover, like many RNA binding proteins, Hfq possesses an unstructured C-terminal extension. Using a combination of We showed that acidic residues at the C-terminus of *E. coli* Hfq mimic nucleic acids to displace double-stranded RNA from the arginine patch, increasing turnover and inducing kinetic competition between sRNAs. *De novo* modeling, biophysical assays and genetic reporter assays show how the C-terminal domain of Hfq increases the stringency and speed of RNA matchmaking, which is tuned differently among Hfq proteins from various bacteria. Together, the results show how the physical properties of the Hfq chaperone flexibly match sRNA with their regulatory targets.

ABC-F type translation factors modulate the stereochemistry of the peptidyl transferase center in the ribosome and are necessary for bacteria to adapt to their environment

Gregory Boël[1], Farès Ousalem[1], Amin Omairi-Nasser[1] & Shikha Singh [2]

1. CNRS UMR 8261, Université de Paris, Institut de Biologie Physico-Chimique, Paris, France
2. Department of Biological Sciences, Columbia University, Columbus, USA

In response to metabolic conditions, protein synthesis is controlled in a variety of ways that often involve the interaction of effector proteins with the ribosome. However, the physiological functions and mechanisms of action of many of these translation regulatory factors remain ambiguous. We have reported that Escherichia coli EttA, the most prevalent member of ATP Binding Cassette F (ABC-F) protein sub-family is a translation factor that gates entry of the ribosome into the translation elongation cycle and modulates the stereochemistry of the peptidyl transferase center (PTC). Recent studies have confirmed that modulation of the stereochemistry of the PTC in the ribosome coupled to changes in its global conformation and P-site tRNA binding geometry is a common function of the ABC-F proteins. We are now investigating the physiological response of the cell to the deletion of the ettA gene. We have shown that EttA is important for bacteria to maintain efficient growth during adaptation to media that are energetically challenging. EttA is important for bacteria to adapt their metabolism to specific carbon sources. Proteomic and transcriptomic approaches have allowed us to identify which proteins have their synthesis affected by EttA during the physiological response to the stress. We have validated some of them by fluorescent reporter chromosomal fusion. We have investigated the functions of the other three E. coli ABC-F paralogs (Uup, YbiT, and YheS) using genetic, biochemical, and biophysical methods. These studies show that all four E. coli ABC-Fs interact with ribosomes, but that knockouts of the corresponding genes produce different phenotypes. We used single-molecule fluorescence resonance energy transfer (smFRET) to demonstrate that the ATP-bound conformations of all four paralogs stabilize the global state (GS) 1 ribosome conformation, although the bound complexes exhibit distinct GS1 stabilities. Finally, we used cryogenic electron microscopy (cryo-EM) to determine near-atomic-resolution structures of ATP-bound forms of EttA and YbiT complexed with ribosomes. Since EttA is involved in energy regulation we are studying orthologs in an organism that uses drastically different energy sources like the photosynthetic cyanobacterium Synechocystis. Our results suggest that two of the ABC-Fs are necessary to protect the bacteria against excess of light and that both of the ABC-F proteins interact with the ribosome. Preliminary results suggest that the two ABC-F proteins are involved in the photosystem recovery after exposure to high light intensity.

Antibiotic resistant ABCFs: straight from structure to mechanism?

Victoriia Murina

Department of Molecular Biology, Umea University, Umea, Sweden

Translation is one of main processes in all living organisms, therefore there is no surprise majority of known antibiotics target ribosomes, the machinery of translation. While translation on ribosome is straightforward and precise process of deciphering information from mRNA to protein; to respond to different growth phases, stress conditions and environment changes, the process of translation is assisted by numerous translation factors. Among them are recently confirmed ABCF translational factors, which for decades were believed to be part of unidentified ABC transporters. Antibiotic resistant ABCFs were identified in clinical isolates where they confirm resistance to broad specter of antibiotic classes: oxazolidinones, macrolides, lincosamides, pleuromutilins and streptogramins, phenicols and tetracyclines. Some of these antibiotics are considered as last resort antibiotics for human use, motivating us to continue the study and shed some perspectives on counteracting them. At the moment we are focused on characterization of distribution of ARE ABCFs, the connection between each protein class and its antibiotic resistance with a goal of making it possible to predict antibiotic resistance of newly identified ABCFs based on their sequences. Our other studies include tackling of the mechanism of ABCFs antibiotic resistance using pathogenic bacteria as model organisms for structural and biochemical assays.

Structures of antibiotic-resistance ABCF proteins in complex with the ribosome

Daniel Wilson

Institute for Biochemistry and Molecular Biology, University of Hamburg Martin-Luther-King-Platz 6 20146, Hamburg, Germany

The antibiotic resistance (ARE) ATP-binding cassette (ABC) proteins of subfamily F can act as target protection proteins by causing egress of antibiotics from ribosomes [1]. Previously, we used cryo-electron microscopy (cryo-EM) to determine the structure of the *Bacillus subtilis* ARE-ABCF protein VmlR in complex with *B. subtilis* 70S ribosomes [2]. The structure revealed that VmlR binds within the ribosomal E-site and has a long α-helical interdomain linker that distorts the P-site tRNA to reach into the ribosomal peptidyl transferase centre. In conjunction with mutagenesis data, our structure suggests that VmlR confers resistance to virginiamycin M (streptogramin A), lincomycin (a lincosamide) and tiamulin (a pleuromutilin) using an allosteric mechanism, rather than via a direct steric clash. We have now determined cryo-EM structures of ABCF proteins in clinically relevant Gram-positive bacteria, including VgaALC bound to the *Staphylococcus aureus* ribosome, LsaA bound to the *Enterococcus faecalis* ribosome and Lmo0919 bound to the Listeria monocytogenes ribosome. A comparative analysis of these structures will be presented providing structural and functional insight into the mechanism of action of these ARE-ABCF proteins.

References: [1] Sharkey, L.K.R., and O'Neill, A.J., 2018. Antibiotic resistance ABC-F proteins: bringing target protection into the limelight. ACS Infect. Dis. 4, 239–246. [2] Crowe-McAuliffe, C.T., et al., 2018. Structural basis for antibiotic resistance mediated by the *Bacillus subtilis* ABCF ATPase VmlR. Proc. Natl. Acad Sci. U.S.A. 115, 8978–8983.

Invited speaker

Alterations of translation (components) with age

Zoya Ignatova

Institute of Biochemistry & Molecular Biology, University of Hamburg, Germany

Spontaneous recoding events occur virtually at any codon but at very low frequency and are commonly assumed to increase as the cell ages. Leveraging a model undergoing an enhanced translational frameshifting, we study the frequency of frameshifting in different tissues and aging. Frameshifting frequency varies among different tissues with age and strongly depends on steady state concentration of mRNA. Furthermore, we use quantitative proteomics and quantify ribosomal protein pool from three brain tissues and one metabolically active tissues in juvenile, adult and middle-aged mice groups.

Invited speaker

Watching (class III) gene transcription in HD

Alessandro Vannini

The Institute of Cancer research (ICR), London, United kingdom

RNA polymerase (Pol) III transcribes essential non-coding RNAs, such as the entire pool of tRNAs, the 5s rRNA and the U6 spliceosomal snRNA. Pol III activity is finely regulated in a cell-cycle and tissue dependent manner, but these layers of regulation are often lost in human diseases, such as cancer and neurodegenerative pathologies. Using an integrated structural biology approach we aim at understanding the molecular mechanism underlying Pol III transcription and its regulation in health and disease.

Bimodal effect of RNA on aggregation of the tumor suppressor protein p53

Suparna Sanyal

Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden

Inactivation of the tumor suppressor protein p53 causes cancer. Other than mutations, p53 can also be inactivated by aggregation although the cellular factors causing it are not known. P53 is a primarily DNA binding protein, but has been shown to interact with RNA in cancer cells. We asked the question whether RNA interaction can promote p53 aggregation and inactivation. For that, we have tested RNAs of varying length, sequence and source for their role in p53 aggregation using the core domain of p53 (p53c) as the study system. Our results show that RNA influences aggregation of p53c in a bimodal fashion. At a low RNA to protein ratio (~1:50) large amorphous aggregates of p53c are readily formed. Conversely, at a high RNA to protein ratio (~1:10) the amorphous aggregation of p53C is clearly suppressed. Instead, amyloid p53C oligomers are formed, which act as 'seeds' for nucleating de novo aggregation of p53. The fact that p53c aggregation / disaggregation is independent of RNA sequence, type, or source, RNA most likely acts as a physical agent causing phase separation in a concentration dependent manner. Our data show that RNA induced bimodal modulation of p53c aggregation may have important implications in vivo.

References: [1] PS. Kovachev, D. Banerjee, LP. Rangel, J. Eriksson, MM. Pedrote, MMDC. Martins-Dinis, K. Edwards, Y. Cordeiro, JL. Silva, and S. Sanyal, Journal of Biological Chemistry, 292(22) 2017 Jun 2;:9345-9357. [2] M. Vieter and S. Sanyal, Cancers (Basel), 10(9), 2nd ed., Prentice Hall, Englewood Cliffs, NJ, 1991.

mRNAs and lncRNAs intrinsically form secondary structures with short end-to-end distances

Wan-Jung C. Lai, Mohammad Kayedkhordeh, Erica V. Cornell, Elie Farah, Stanislav Bellaousov, Robert Rietmeijer, Enea Salsi, David H. Mathews & Dmitri N. Ermolenko

Department of Biochemistry & Biophysics and Center for RNA Biology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642, USA

The 5' and 3' termini of RNA play important roles in many cellular processes. Using Förster resonance energy transfer (FRET), we show that mRNAs and lncRNAs have an intrinsic propensity to fold in the absence of proteins into structures in which the 5' end and 3' end are ≤ 7 nm apart irrespective of mRNA length. Computational estimates suggest that the inherent proximity of the ends is a universal property of most mRNA and lncRNA sequences. Only guanosine-depleted RNA sequences with low sequence complexity are unstructured and exhibit end-to-end distances expected for the random coil conformation of RNA. While the biological implications remain to be explored, short end-to-end distances could facilitate the binding of protein factors that regulate translation initiation by bridging mRNA 5' and 3' ends. Furthermore, our studies provide the basis for measuring, computing and manipulating end-to-end distances and secondary structure in RNA in research and biotechnology.

Interplay between epigenetics, alternative splicing, and cancer metabolism

Sanjeev Shukla

Indian Institute of Science Education and Research Bhopal, Dept of Biology, Bhopal Bypass Road, Bhauri, Bhopal-462066, India

The cancer cells thrive on glucose by converting it to lactate at the end of glycolysis. The phenomenon is known as aerobic glycolysis or Warburg effect and promotes the growth of the cancer cells. The alternative spliced isoform Pyruvate kinase M2 (PKM2) contributes to the Warburg effect by promoting aerobic glycolysis whereas PKM1 isoform promotes oxidative phosphorylation. The PKM gene contains two mutually exclusive exons, exon 9 and 10 which are alternatively included in the final transcript to give rise to PKM1 and PKM2 isoform respectively. In this study, we report that the intragenic DNA methylation-mediated binding of BORIS (Brother of regulator of imprinted sites) at the alternative exon of Pyruvate Kinase (PKM) is associated with cancer-specific splicing that promotes Warburg effect and breast cancer progression. Interestingly, inhibition of DNA methylation or BORIS depletion or CRISPR/Cas9-mediated deletion of BORIS binding site leads to splicing switch from cancer-specific PKM2 to normal PKM1 isoform. This results in the reversal of Warburg effect and inhibition of breast cancer cell growth, which may serve as a useful approach to inhibit the growth of breast cancer cells. In another story, we find that the PAK2-catenin-cMyc axis contributes to the overexpression of PKM2 and thereby an increased Warburg effect in oral cancer. Together these results suggest a crucial interplay between epigenetics, alternative splicing, and metabolism, which contributes to the tumorigenesis.

Invited speaker

Long read transcriptomics - beyond model organisms

Eduardo Eyras

JCSMR - Australian National University

New long-read sequencing technologies provide an unprecedented opportunity to study transcriptomes from any sample and species. However, analyses so far rely on a genome sequence or use hybrid approaches with short-reads, limiting the scope of the technology. The lack of dedicated tools hinders the full deployment of long-read technologies for the systematic and cost-effective interrogation of transcriptomes from any sample and species. This is crucial for non-model organisms, which are relevant to ecology, agriculture, and medicine, but also for model organisms, as available genome sequences do not always include inter-individual differences. We have developed a new method, RATTLE, for the reference-free reconstruction of transcriptomes from Oxford Nanopore cDNA/RNA sequencing reads. RATTLE uses similarity based clustering to reconstruct genes and alternative transcript isoforms, and to perform error correction from long reads without the need of a genome reference. Using experimental and simulated data and comparing to other methods, we show that RATTLE improves the mapping of long reads to the reference and delineates more precisely exon-intron structures. RATTLE closes the existing technological gaps to build and interpret transcriptomes using long-read sequencing without relying on a genome sequence or additional technologies.

Unwinding of HIV RNA dimers by DDX3 questions its role during infection

Grégoire De Bisschop, Bruno Sargueil & Nathalie Chamond

CITCOM - UMR 8038 Université Paris Descartes Faculté de Pharmacie de Paris 4 avenue de l'Observatoire 75006 Paris, France

Like most retroviruses, HIV-1 virus carries two RNA molecules (genomic RNA or gRNA) that are assembled as dimers through non covalent interactions. HIV-1 gRNA can be either targeted to newly assembling viral particles or to ribosomes where it yield Gag and Gag- Pol proteins. Why some copies of this RNA are packaged into virions whereas other are translated remains unclear. It has been proposed that monomers are efficiently translated while dimers are selected for encapsidation. Thus, dimerization of gRNA is a critical step during infection that must be finely tuned. Several cellular factors are known to be involved in HIV viral cycle regulation; among them, the DEAD-box helicase DDX3 is thought to be hijacked by the virus to assist viral RNA export and translation. We discovered that DDX3 has the ability to unwind stable HIV dimers with an unprecedented efficiency. Here, we investigate DDX3 interaction with HIV RNA and analyze the structural rearrangement triggered by DDX3 by time-resolved SHAPE structure probing. This allows us to follow the molecular events going from the RNA dimers to monomers. We are currently assessing the effect of this structural rearrangement on viral translation.

Dynamics of RNase Y membrane localization in *Bacillus subtilis*

Lina Hamouche, Cyrille Billaudeau, Anna Rocca, Arnaud Chastanet, Saravuth Ngo, Soumaya Laalami & Harald Putzer

Laboratoire de Microbiologie et de Génétique Moléculaires, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, Université Paul Sabatier, Toulouse, France

Metabolic instability of mRNA is an essential process, fundamental to the control of gene expression in all organisms. In bacteria, mRNA turnover is controlled most efficiently at the initiating step of the degradation process. Studies of *Escherichia coli* and *Bacillus subtilis*, prototypical Gram negative and Gram positive bacteria separated by 3 billion years of evolution, have revealed the major and very disparate enzymes involved in the initiation of mRNA degradation (*E. coli* RNase E and *B. subtilis* RNase Y). Both endoribonucleases are localized at the cell membrane which amounts to a subcellular compartmentalization of mRNA decay. We used total internal reflection fluorescence microscopy (TIRFm) and single particle tracking (SPT) tools to visualize RNase Y, and analyze its localization and dynamics in living cells. We find that RNase Y rapidly diffuses at the membrane in the form of very dynamic short-lived foci. We classified the dynamic behavior of these ribonuclease foci into three different classes of movement, one of them likely corresponding to the enzyme bound to its substrate. The formation of foci is not dependent on the transcription of RNA substrates. However, the kinetic parameters of RNase Y foci are altered following transcription arrest. Our data suggest that RNase Y foci do not constitute the primary active form of the nuclease. This contrasts with the role of similar RNase E foci in *E. coli* (Strahl et al., 2015). Recently, a complex of three small proteins, YaaT, YlbF and YmcA (Y-complex) has been shown to bind to RNase Y and alter its activity in vivo (Deloughery et al., 2018). We show that Y-complex mutations have differential effects on certain mRNA substrates by probably modulating the assembly status and dynamics of RNase Y at the membrane.

Spatial transcriptome organization in the nucleus

Jorg Morf

Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN, UK

Spatial transcriptomics aims to understand how the ensemble of RNA molecules in tissues and cells is organized in 3D space. We developed Proximity RNA-seq, a ligation-free method which identifies co-localization preferences for pairs and groups of nuclear-retained and nascent RNAs in cell nuclei. Proximity RNA-seq is based on massive-throughput RNA barcoding of sub-nuclear particles in water-in-oil emulsion droplets, followed by sequencing. We show a bipartite organization of the nuclear transcriptome in which RNAs in different compartments are positioned at distinct distances from nucleoli. The compartments correlate with transcript families, tissue specificity and extent of alternative splicing. Furthermore, the simultaneous detection of multiple RNAs in proximity to each other distinguishes RNA-dense from sparse compartments. Finally, the integration of proximity measurements at the DNA and RNA level identifies transcriptionally active genomic regions surrounding nucleoli close to compact chromatin and with faster RNA polymerase II elongation. The localization of transcriptional output by Proximity RNA-seq reveals new dimensions in the spatial organization of genome function in the nucleus. Application of Proximity RNA-seq will facilitate study of the spatial organization of transcripts in the nucleus, including non-coding RNAs, and its functional relevance.

Emergence of cooperativity during in vitro selection of a self-synthesizing ribozyme

James Attwater, Aditya Raguram, Alexey Morgunov, Edoardo Gianni, Philipp Holliger

MRC Laboratory of Molecular Biology, Cambridge, UK

In vitro evolution can reveal surprising capabilities of individual RNA molecules. Recently, during such an experiment to develop ribozymes that assemble other RNAs, we isolated the first RNA molecule able to build a copy of itself from small building blocks, assembling itself in stages from RNA trinucleotide 'triplets'. Unusually, this species emerged as a cooperative RNA heterodimer from the in vitro selection pool, an underexplored possibility in SELEX experiments. I will describe its synthetic capabilities, the behaviour of the complex RNA pools that led to its emergence, and the implications for the inception of RNA self-replication (both modern and primordial).

Full-length analysis of *Caenorhabditis elegans* transcriptome using Nanopore sequencing technology

Florian Bernard[1,2], Delphine Dargere[1], Oded Rechavi[2] & Denis Dupuy[1]

1. Acides Nucléiques : Régulations Naturelle et Artificielle, Université de Bordeaux, INSERM U1212, CNRS UMR5320, Pessac, France
2. Sagol School of Neuroscience - Tel Aviv University, Israel

A recent meta-analysis of alternative exon usage in *Caenorhabditis elegans* based on publicly available RNA-seq dataset (Tourasse et al., Genome Research, 2017) refined our comprehension of *C. elegans* transcriptome, especially regarding the splicing quantitative aspects of alternative splicing in messenger RNAs. However, Next-Generation Sequencing technologies (NGS) like Illumina technology are proving to be limited to fully characterize one's transcriptome. PCR-based sequencing methods are known to introduce amplification bias affecting the overall distribution of mRNAs detected in one experiment and short-reads are not suited to accurately predict the frequency of isoforms derived from multiple alternative splicing events. In this study, we are exploiting the new possibilities offered by Oxford Nanopore Technology (ONT) to overcome those limitations. Nanopore-based sequencing allow to directly sequence nucleic acids without any prior amplification step and generates long-reads covering up to the full-length of the molecule. Hence, we are aiming to further characterize *C. elegans* transcriptome by providing a more accurate measure of isoforms ratios, a better comprehension of exons associations during alternative splicing and by characterizing differentially transspliced mRNAs. To do so, we analyzed two different populations of mRNAs: a library of poly(A) mRNAs representing the whole-animal transcriptome and a library of SL1-enriched mRNAs. Those libraries were sequenced using an ONT MinION device and analyzed using a combination of tools recommended for long-reads analysis and in-house python scripts. We assessed the efficiency of three different sequencing kits commercialized by ONT that are recommended for transcriptomics. Our results suggest that direct cDNA sequencing is most suited for transcriptome analysis in *C. elegans*, in regard to the quantity of data generated while preserving the quality of the dataset. The two libraries were compared together at the level of both genes and isoforms. We are reporting a set of non-SL1 genes that are found highly expressed in poly(A) libraries but not detected in SL1-enriched libraries. Additionally, we are also showing that alternatives promoters can lead to populations of isoforms exhibiting different trans-splicing status.

Structure guided fluorescence labeling reveals a two-step binding mechanism of neomycin to its RNA aptamer

Henrik Gustmann[1], Anna-Lena Johanna Segler[2], Dnyaneshwar B. Gophane[2], Andreas J. Reuss[1], Markus Braun[1], Julia E. Weigand[3], Snorri Th. Sigurdsson[2] & Josef Wachtveitl[1]

1. Institute of Physical and Theoretical Chemistry, Goethe-University, Frankfurt am Main, Germany.
2. Science Institute, University of Iceland, Reykjavik, Iceland.
3. Department of Biology, Technical University Darmstadt, Germany.

The understanding of RNA dynamics on a molecular level relies on suitable spectroscopic reporter groups. A small and very rigid cytidine analogue was introduced as DNA (Ç) or RNA label ($Ç_m$), either as spin label or in the reduced version as fluorophore ($Ç_{(m)}^f$).[1-3] This label class stands out, because its rigid incorporation suppresses conformational ambiguity and allows a direct comparison between EPR and fluorescence methods.

In an initial study, we compared the photophysical properties of the fluorescent, methoxy group protected, RNA-label $Ç_m^f$ to the well-established non-fluorescent spin-label $Ç_m$ and clarified the quenching mechanism.[4] Quantum chemical calculations support the experimental finding that the $Ç_m$ emission is quenched via a fast (≤ 1 ps) internal conversion into a non-fluorescent, spectroscopically dark, doublet state. This dark state is located on the nitroxide moiety of the label. The quenching process also could be interpreted as a fast internal Dexter energy transfer. The full spectroscopic characterization of $Ç_m$ and $Ç_m^f$ provides an expanded view on the photochemistry of fluorophore-nitroxide-compounds in general.

As a next step, $Ç_m^f$ was incorporated into RNA model sequences for hybridization studies.[5] It turned out, that the $Ç_m^f$ emission sensitively reports local changes of its microenvironment. Fluorescence lifetime or quantum yield measurements could discriminate between labelled single and double strands. Furthermore, both observables are affected by the neighboring bases of the $Ç_m^f$ label. Thus, a discrimination of pyrimidine and purine neighbors is possible. Fluorescence anisotropy indicates a very rigid incorporation of $Ç_m^f$ into the RNA strands. Overall, it was shown that $Ç_m^f$ is ideally suited for kinetic studies of hybridization or ligand binding.

Consequently, the neomycin binding aptamer (N1) was labelled with the fluorophore $Ç_m^f$ at four different positions adjacent to the binding pocket.[5] Steady state emission experiments confirm the conformational selection mechanism previously proposed in NMR studies.[6,7] Furthermore, fluorescence stopped flow measurements demonstrate a very fast ligand binding to the aptamer, which is best described by a two-step model, consisting of an unspecific ligand binding to the preformed aptamer as a first step and a subsequent step accompanied by the formation of specific H-bonds and minor conformational adjustments.

Currently, we test RNA-systems with $Ç_m^f$ as a FRET-donor in combination with tC_{nitro} as acceptor.[8,9] The rigidity of the $Ç_m^f$ should be useful to gain distance and orientation information within RNA structures. Currently this is tested on RNA model sequences, where different singly labelled strands were combined to gain a variety of donor-acceptor distances within the RNA duplexes. A clear distance dependence is observed while an orientation dependence on the other hand seems to be averaged out due to the flexibility of the RNA. Subsequently, FRET and PELDOR measurements on identically labelled N1 aptamers are planned to gain more information on ligand binding and conformational dynamics of this aptamer.

References:[1] N. Barhate et al. (2007), *Angew Chem Int Ed*, 46, 2655; [2] P. Cekan et al. (2009), *Nucleic Acids Res*, 37, 3990; [3] C. Höbartner et al. (2012), *J Org Chem*, 77, 7749; [4] H. Gustmann et al. (2017), *Phys Chem Chem Phys*, 19, 26255; [5] H. Gustmann et al. (2018), *Nucleic Acids Res, 47, 15*; [6] E. Duchardt-Ferner et al. (2010), *Angew Chem Int Ed*, 49, 6216; [7] E. Duchardt-Ferner et al. (2016), *Angew Chem Int Ed*, 55, 1527; [8] K. Börjesson et al. (2009), *J Am Chem Soc*, 131, 4288; [9] S. Preus et al. (2010), *J Phys Chem B*, 114, 1050.

Fine-tuning the binding affinity of a structure-switching aptamer using dangling nucleotides

Sladjana Slavkovic, Philip E. Johnson

Department of Chemistry, York University, Toronto M3J 1P3, Ontario, Canada

Altering the binding affinity of biomolecules in a designed manner is an important, but difficult to achieve goal. We have used a structure-switching or ligand-induced folding construct of the cocaine-binding aptamer to increase the binding affinity of the aptamer by introducing dangling nucleotides into the sequence. Depending on the identity of the terminal base pair and the identity of a 5' or 3' dangling nucleotide the introduction of a dangling nucleotide can stabilise (or destabilize) the structure with a known ΔG value. For structure-switching aptamers, the unbound state is unfolded, or loosely folded, and the aptamer folds with ligand binding. The incorporation of the dangling nucleotide reduces the needed free energy supplied by ligand binding to fold the aptamer, resulting in a tighter observed binding affinity. We obtain a linear relationship between the predicted ΔG of stabilization by the dangling nucleotide and the resulting increase in binding affinity. We believe this method of introducing dangling nucleotides is a general method for increasing the affinity of structure-switching aptamers.

Aptamer delivery of siRNAs to knockdown cancer

Ying Zhang, Judy Lieberman

Program in Cellular and Molecular Medicine, Boston Children's Hospital, Harvard Medical School, Boston, USA

Immune checkpoint blocking antibodies have revolutionized cancer therapy. They can restore immune surveillance and durably control some cancers. However, for most cancers only a minority of patients responds. An important obstacle to immune therapy is that many tumors are not recognized as "foreign" by the immune system. Tumor cells have many ways to evade immune control. Here we describe a way to induce gene silencing in vivo in epithelial cancers that uses RNA aptamers that bind with high affinity to a cell surface receptor to deliver covalently linked siRNAs into cells that express the recognized receptor. EpCAM, the first described tumor antigen, is highly expressed on epithelial cancers and their especially malignant subpopulation of cancer stem cells. EpCAM aptamer-siRNA chimeras (AsiCs) that use a high affinity EpCAM aptamer that recognizes both mouse and human EpCAM selectively bind to and knock down gene expression in EpCAM+ breast tumor grafts, but not normal tissue, suppress tumor initiation and inhibit tumor growth in vivo. Recently we have used EpCAM-targeted gene knockdown in the tumor to make immunologically "cold" aggressive Her2+ and triple negative breast tumors visible to the immune system and to counteract tumor strategies of immune evasion and immune suppression. In particular EpCAM-AsiCs were used to induce tumor neoantigen expression to render immunologically ignored tumors visible and to activate anti-tumor functional immunity by knocking down immune evasion genes expressed by the tumor. AsiC cocktails targeting multiple genes enhanced immune responses to the tumor and increased tumor regression. By targeting the tumor, rather than activating immune cells nonspecifically, tumor-targeted immune therapy should have few side effects.

Genetically encoded light-up aptamers for live-cell super-resolution RNA imaging

Murat Sunbul

Heidelberg University, Institute of Pharmacy and Molecular Biotechnology (IPMB), Heidelberg, Germany

In light of numerous diverse roles of RNA in processes such as transcription, translation, catalysis and gene regulation at the cellular level, there is an urgent need to establish a versatile, easy-to-use technology for specific, genetically encoded fluorescence labeling of RNA, especially for live-cell imaging applications. To this end, we developed two orthogonal light-up aptamers which bind to small, cell-permeable, bright and photostable fluorophores with distinct spectral colors for super-resolution RNA imaging in live cells using STORM (stochastic optical reconstruction microscopy), SIM (structured illumination microscopy) and STED (stimulated emission depletion) microscopy. The first aptamer, named SiRA, binds to silicon rhodamines (SiRs) which are photostable, NIR-emitting fluorogenic dyes. 50-nucleotide SiRA aptamer was generated via SELEX and binds to SiR with nanomolar affinity (KD ~400 nM). SiRs change their open-closed equilibrium between the non-colored spirolactone and the fluorescent zwitterion in response to their environment. SiRA aptamer preferentially binds to the open-form resulting in a significant fluorescence increase upon interaction. Remarkably, SiRA is resistant to photobleaching and constitutes the brightest far-red light-up aptamer system known. SiRA allowed us to visualize the expression of RNAs in bacteria in no-wash live-cell imaging experiments. We also reported the first super-resolution STED microscopy images of aptamer-based, fluorescently labeled mRNA in live cells.[1] The second aptamer, named SRB-2, binds to sulforhodamine B (SR) fluorophore with high specificity and affinity. To convert this aptamer/fluorophore pair into a fluorescence light-up system, SR fluorophore was conjugated to dinitroaniline (DN) which diminishes the fluorescence of SR via contact quenching. SR-DN is essentially non-fluorescent in solution; however, upon binding to SRB-2 (KD ~1.4 μM), the fluorescence intensity increases >100-fold. We used this method to image abundant RNAs in live bacteria.[2] Then, we determined the binding constants of various structurally different rhodamine-based dyes to SRB-2. The obtained structure-activity relationships allowed us to rationally design of a novel, bright, orange fluorescent turn-on probe (TMR-DN) with low background fluorescence and high affinity to SRB-2 (KD ~35 nM). The utility of SRB-2/TMR-DN was demonstrated by imaging mRNAs in bacteria and mammalian cells.[3] To further improve the properties of the SRB-2/TMR-DN complex, we created a library of SRB-2 mutants and selected aptamers that bind to TMR-decorated beads. Using SELEX, we discovered a mutant (SRB-3) that binds to TMR-DN with higher affinity (KD ~20 nM) and is 50% brighter than SRB-2. After rationally improving the folding features of SRB-3, the resulting SRB-4 allowed imaging low copy number mRNAs in bacteria and high copy number mRNAs in mammalian cells using SIM and STORM with very high resolution.

References: [1] Wirth, R.; Gao, P.; Nienhaus, G. U.; Sunbul, M.; Jäschke, A., SiRA: A Silicon Rhodamine-Binding Aptamer for Live-Cell Super-Resolution RNA Imaging. J. Am. Chem. Soc. 2019, 141, 7562. [2] Sunbul, M. and Jäschke A.: Contact-mediated quenching allows RNA imaging in bacteria with a fluorophore-binding aptamer. Angew. Chem. Int. Ed. 2013, 52, 13401. [3]. Sunbul, M. and Jäschke, A.: SRB-2: a promiscuous rainbow aptamer for live-cell RNA imaging. Nucleic Acids Res. 2018, 46 (18), e110

Light-up Aptasensors- A tool for the detection of RNA hairpins and small molecules

Arghya Sett[1], Lorena Zara[2, 3], Eric Dauss [1,2] & Jean-Jacques Toulmé[1, 2]

1. ARNA Laboratory, INSERM U1212, CNRS UMR5320, University of Bordeaux, Bordeaux,France
2. Novaptech, Pessac, France
3. Département de Pharmacochimie Moléculaire, CNRS UMR5063, Université Grenoble Alpes St Martin d'Hères, France.

The pair comprising a conditionally non-fluorescent dye and its cognate nucleic acid aptamer that activates the dye's fluorescence upon binding emerged as a promising tool for fluorescence based biosensing and molecular imaging. A number of RNA light-up aptamers have been developed already against some fluorogenic dyes (MG= Malachite green, DFHBI=Z-4-(3,5-difluoro-4-hydroxybenzylidene)-2-methyl-1-(2,2,2-trifluoroethyl)-1H-imidazol-5(4H)-one, TO= Thiazole orange). In this study we have converted a MG specific light-up aptamer into a structure-switching aptasensor malaswitch. We destabilized the stem-loop structure of the native aptamer and we introduced an apical loop which can form kissing interaction with an RNA hairpin, exclusively in presence of MG. The ternary MG-malaswitch-RNA hairpin complex is much more stable than the binary MG-malaswitch one. The malaswitch-MG combination allows the detection of RNA hairpins and was applied to the specific quantification of microRNA precursors. On the basis of this malaswitch, we developed light-up aptamers responding to the presence of small molecules like adenosine and theophylline. This approach of 'light up aptasensor' holds an immense potential for small molecule detection and sensing other biomolecules causing various diseases and disorders.

Evolution of efficient RNA-based fluorescent probes using ultrahigh- throughput functional screening

Farah Bouhedda[1], Tkhe Kyong Fam[2], Mayeul Collot[2], Alexis Autour[1], Stefano Marzi[1], Andrey Klymchenko[2] & Michael Ryckelynck[2]

1. Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR 9002, F-67000 Strasbourg, France
2. Nanochemistry and Bioimaging group, Laboratoire de Bioimagerie et Pathologies, CNRS UMR 7021, Université de Strasbourg, 67401 Illkirch, France

Cells have to adapt to their ever-changing environment. To do so, they evolved sophisticated regulatory networks involving several types of regulators such as non-coding RNAs. However, it is known that gene expression can significantly vary from one cell to another even within isogenic populations [1]. Therefore, proper characterization of regulatory networks requires being able to monitor target RNAs over the time, on the same population and with a single-cell resolution. These two conditions can be fulfilled by using fluorescence microscopy imaging and genetically-encoded fluorogenic molecules.
Our group is specialized in the development of fluorogenic modules made of a light- up RNA aptamer able to specifically interact with a pro-fluorescent dye (fluorogen) and activate its fluorescence [2]. The small size of these RNA aptamers strongly contrasts with approaches like MS2-GFP that require the use of tandem repeats of bulky protein-binding sites. The development efficiency of these RNAs is dramatically improved by using ultrahigh- throughput functional screening technologies like droplet-based microfluidics [3,4]. Indeed, this technology makes possible to screen, in a single experiment, millions of mutants individualized and in vitro expressed within picoliter water-in-oil droplets with an exquisite control over droplet dispersity and composition.
In this talk, I will present how we used this innovative evolutionary technology to develop a new generation of bright and photostable orange emitting fluorogenic module. This new tool will allow exploring RNA-mediated gene expression regulation in cells both in a dynamic way and with a single-cell resolution.

References: [1]. Locke, J.C.; Elowitz, M.B. Nature reviews. Microbiology 2009, 7, 383-392. [2]. Bouhedda, F.; Autour, A.; Ryckelynck, M. International journal of molecular sciences 2017, 19. [3]. Autour, A.; Westhof, E.; Ryckelynck, M. Nucleic acids research 2016, 44, 2491-2500. [4]. Autour, A.; Jeng, S.C.; Cawte, A.; Abdolahzadeh, A.; Galli, A.; Panchapakesan, S.S.; Rueda, D.; Ryckelynck, M.; Unrau, P.J. Nature communications 2018, 9.

High-throughput screening of fluorescent RNA aptamers

Olga Puchta[1], Grzegorz Sobczyk[1], Graeme Whyte[2], Janusz Bujnicki[3] & Grzegorz Kudla[1]

1. Human Genetics Unit, MRC Institue of Genetics & Molecular Medicine, University of Edinburgh, Edinburgh, Scotland
2. Institute of Biological Chemistry, Biophysics and Bioengineering, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, Scotland
3. Laboratory Of Bioinformatics And Protein Engineering, International Institute of Molecular and Cell Biology, Warsaw, Poland

The discovery of Green Fluorescent Protein few decades ago has revolutionized the way we do molecular biology research. Today we are witnessing a similar process in the RNA world with the development and utilization of various RNA mimics of the GFP such as Spinach and Broccoli, which bind different chemical fluorophores with high affinity and induce their fluorescence. They can be fused to RNAs expressed in the cell and used for investigation of their localization and stability in vivo. Here we show how we repurposed commercially available gene expression microarray for high-throughput screening of fluorescent RNAs. We designed a library of all possible single and double mutants of the Broccoli RNA aptamer and a fraction of mutants of Spinach RNA, fused to a different sequence complementary to a specific probe on the microarray. We used microscope imagining to measure fluorescence of each mutant from library in various conditions: ranges of magnesium, potassium or fluorophore (DFHBI) concentrations, pH and temperature. Collected data allowed us to recapitulate 2D structure of Broccoli - different from prediction by free energy minimization, identify crucial positions involved in forming of G-quadruplexes containing structure (responsible for fluorophore binding) and mutants with shifted emission spectrum. We also noticed interesting lack of correlation between affinity to DFHBI and brightness of the mutants. Among other possibilities such approach will potentially provide a set of well characterized aptamers which can serve as fluorescent intracellular sensors of pH or ions concentration.

Posters

My Nutz Test - An iGEM Strasbourg Project

Yasmine Amrani[1], Julianne Bord-Contu[1], Léna Coudray[1], Lorine Debande[1], Alexandre Gilardet[2], Claire Husser[1], Ines Jmel Boyer[1], Kateryna Len[1], Julie Lapouges[1], Jessica Medina Sanchez1[1], Lorraine Pinot[2], Malo Sanglier[2], Rebecca Vale[1], Bryan Virlouvet[1], Lisa Welker[1], Hubert Becker[3], Luc Bonnefond[4], Carine Meignin[5] & Michael Ryckelynck[6]

1. Université de Strasbourg, Strasbourg, France
2. Ecole Supérieure de Biotechnologies de Strasbourg (ESBS), Illkirch, France
3. Université de Strasbourg, GMGM, CNRS UMR 7156, 67000 Strasbourg, France
4. Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, Illkirch, France
5. Institut de Biologie Moléculaire et Cellulaire, RIDI, CNRS UPR9022, Strasbourg, France
6. Institut de Biologie Moléculaire et Cellulaire, ARN, CNRS UPR9002, Strasbourg, France

Our team is working on the development of a detection kit for allergens, which does not need antibodies. It should be fast and portable for easy on-site use, as well as versatile in order to adapt to any allergen. Our detection kit is based on a triple hybrid system implemented in E. coli. Flexibility will be provided thanks to an aptazyme, which can be interchanged for specific allergens. The aptazyme switch is an RNA molecule whose price for synthesis is continuously decreasing with the breakthroughs in molecular biology. Therefore, it allows the development of a cheap and easy-to-use test. Two constructs will be tested. In both, an aptazyme links an MS2 protein to a PP7 protein. In the first one, MS2 is linked to a domain recognizing a promoter upstream of a reporter gene repressor whereas PP7 is linked to the RNA polymerase, thus recruiting it. If the allergen is present in the food, the aptazyme will undergo cleavage, which will block the synthesis of this constitutive repressor. This repressor usually inhibits the transcription of a reporter gene, so that the presence of the allergen leads to the expression of this reporter. In the second construct, MS2 and PP7 are linked to LexA DNA binding domains which themselves bind to a LexA operator. When both are linked to the operator, it inhibits the transcription of a reporter gene. When the aptazyme is cleaved, it deactivates this repression, allowing the expression of the reporter. First, our aim is bringing a proof of concept to demonstrate the feasibility of our approach, then adapt it to specific food allergens with new aptazymes based on known sequences of aptamers that recognize allergens.

Initiation translation complex of *Staphylococcus aureus* ribosome

Margarita Belinite[1,4], Iskander Khusainov[2], Stefano Marzi[3], Pascale Romby[3], Marat Yusupov[1], and Yaser Hashem[4]

1. Institut de Génétique et de Biologie Moléculaire et Cellulaire, Université de Strasbourg, INSERM U964, CNRS UMR7104, Illkirch Graffenstaden, France
2. EMBL Heidelberg – Germany
3. Architecture et Réactivité de l'ARN, Université de Strasbourg, CNRS UPR 9002, Strasbourg, France
4. Institut europeen de chimie et biologie (IECB) – INSERM U1212 – CNRS UMR5320 - 2 rue Robert Escarpit 33600, Pessac, France

Staphylococcus aureus (*S. aureus*) is a pathogenic bacteria that causes a range of illnesses such as pneumonia, is often the cause of wound infections following surgery and has a high resistance to most commonly used antibiotics. Several antibiotics inhibit protein synthesis, specifically affecting initiation stage, a dynamic and rate-limiting step that should be strictly regulated. There is a certain number of peculiarities in the structure of ribosomes and translation factors as well as in translation regulation in different species. For instance, in *S. aureus* S1 protein is almost twice shorter than in gram-negative bacteria and, moreover, was not found to be associated with the ribosome during normal cell growth. Also, there are staphylococcus specific mRNAs such as spa mRNA that due to its structure can easily bind to the ribosome without the help of proteins such as S1. Here, we show the cryo-electron microscopy structures of initiation complexes with a constituent small ribosomal subunit (30S), three specialized translation initiation factors: IF1, IF2 and IF3, spa mRNA and fMettRNA. Our structures represent could pave the way for new therapeutic developments in the design of new antibiotics against this bacteria.

Interacting networks of ribosomal RNA expansion segments from kinetoplastids

Anthony Bochler, Aline Rimoldi Ribeiro, Jailson Brito Querido, Quentin Vicens & Yaser Hashem

Institut europeen de chimie et biologie (IECB) – INSERM U1212 - CNRS 5320– 2 rue Robert Escarpit 33600, Pessac, France

Although ribosomes from different eukaryotes possess a conserved core, they differ in their outer-most layer, which is composed of structured RNA regions called 'expansion segments' (ESs). Expansion segments have been shown to play a critical role during ribosome biogenesis, as well as during translation initiation, because of their position close to the mRNA entry and exit sites. In kinetoplastid parasites such as *Trypanosoma brucei* and *Trypanosoma cruz*i -which cause African sleeping sickness and Chagas disease-, ESs are typically larger than in other eukaryotes. Here, we used sequence alignments, three-dimensional modeling and cryo-electron microscopy in order to revise and extend the pool of all the current existing structures of ribosomes from kinetoplastids. Through this comparative approach, we were able to systematize the contribution of each ES and its subdomains into an inter- acting network at the surface of the ribosome, which reinforces the view that ESs are not merely random additions at the periphery of the catalytic center. This ES network provides a framework for understanding kinetoplastid-specific translation regulation and biogenesis. In addition, our work is a foundation for further biochemical and structural studies that could lead to the development of drugs that would be specific to these devastating parasites, thereby avoiding harmful side effects on their host.

Prebiotic peptide bond formation by the proto-ribosome: a missing link between RNA and protein dominated world

Tanaya Bose, Gil Fridkin, Nikita Dinger, Anat Bashan & Ada Yonath

Department of Structural Biology, Weizmann Institute of Science 76100, Rehovot, Israel

High-resolution structures of ribosomes from various organisms determined by us and elsewhere, highlight that the peptidyl transferase center (PTC) is located in the core of a pseudo symmetrical rRNA sub-region, within the otherwise asymmetrical particle. The nucleotide sequence of this region is highly conserved among all domains of life, hinting that its key role was maintained throughout evolution. Hence, it may imply that it is a remnant of an early origin of life entity and its pseudo 2-fold symmetry may imply a dimeric origin. These characteristics, in addition to the findings that RNA can create itself and can possess catalytic activities led to the “proto-ribosome” concept. Namely that this entity represents the origin of the ribosome, and perhaps also the origin of life, which existed in the RNA dominated world and is the missing link to the protein dominated contemporary world. During contemporary protein synthesis, peptide bond formation is performed in the large ribosomal subunit (LSU) at the PTC, which consists solely of ribosomal RNA(rRNA). The PTC hosts the 3' tRNA ends of both the A-tRNA and the P-tRNA, at proper stereochemistry that provides the geometrical requirements for substrate assisted catalysis. Isolated LSU can catalyze peptide bond formation in vitro without the presence of the small ribosomal subunit (SSU). Moreover, isolated LSU can catalyze peptide bond formation of the fragment reaction, that consists of only the 3'ends of the tRNA molecules, namely the amino acylated CCA and puromycin, a 3'end tRNA analog. This reaction is used to assay the peptidyl transferase activity of the entire ribosome as well as the LSU. The proto-ribosome hypothesis states that there was an RNA entity, capable of self-folding, dimerizing, accommodating substrates, catalyzing chemical reactions like peptide bond formation and releasing the product. Thus, we first designed several constructs resembling this conserved region and tested their ability to dimerize. Some of them were found to homo dimerize and we monitored their ability of forming peptide bond. On this way we achieved few constructs that could perform the peptide bond reaction. The formation of the peptide bond was monitored using MALDI.

Start codon accessibility dictates toxin translation efficiency in *Helicobacter pylori*

Delphine Bronesky, Simon Bonabal, Sandrine Chabas, Tiphenn Desroziers, Sara Masachis & Fabien Darfeuille

INSERM U1212, CNRS UMR5320, Univ. Bordeaux, ARNA Laboratory, 146 rue Leo Saignat, F-33076 Bordeaux, France

In bacteria, translation initiation is a major checkpoint for controlling the fidelity and efficiency of protein synthesis. This process starts with the formation of a pre-initiation complex containing the small ribosomal subunit, initiation factors, the translation initiation region (TIR) of an mRNA encompassing the Shine Dalgarno (SD) sequence and the AUG start codon. During the characterization of a new toxin antitoxin system (aapA1/IsoA1)1 in the human gastric pathogen *Helicobacter pylori*, we serendipitously discovered that the synthesis of the AapA1 toxin could be maintained despite the use of an AUU triplet as initiator codon. This result was really surprising since only two bacterial genes, pcnB and infC, were known to begin with AUU. First, by using in vitro translation and toeprinting assays we confirmed that the mutation was indeed acting at the translation initiation step. A closer inspection of the cloverleaf-like structure of the AapA1 toxin-encoded mRNA revealed that the AUG start codon was trapped into a stable RNA hairpin and that its replacement by an AUU triplet would remove one G-C base pair in this hairpin. This led us to hypothesize that the AUU codon was rendering the TIR more accessible to the ribosome and that, this non canonical start codon would greatly increase translation initiation efficiency. Our hypothesis was further confirmed by the identification of several suppressor mutations that were able to restore the stability of the hairpin sequestering the start codon. In particular, one mutation changing a G-U wobble pair into a more stable G-C pair was sufficient to inhibit translation initiation despite the presence of the AUU as a start codon. Altogether, our results revealed that SD sequence accessibility was not the only determinant regulating toxin translation initiation. Start codon accessibility is clearly a limiting factor to keep toxin synthesis as low as possible. Further work is ongoing to target this regulatory stem-loop by small ligand to artificially turn on the expression of this toxin.

Reference: 1. Arnion et al., Mechanistic insights into type I toxin antitoxin systems in *Helicobacter pylori*: the importance of mRNA folding in controlling toxin expression. Nucleic Acids Research (2017) 45, 8, 5, 4782–4795, https://doi.org/10.1093/nar/gkw1343

Exploring the landscape of DDX3X post-translational modifications

Mehdi Sakita, Karim Ben Ali Kacem, Bruno Sargueil & Nathalie Chamond

Cibles Thérapeutiques et conception de médicaments – Université Paris Descartes - CNRS UMR8038, Paris 5, France

DDX3X is an RNA helicase belonging to the DEAD-box helicase family. It presents ATPase and helicase activities allowing the remodeling of RNA structures and/or the structural rearrangement of RNPs. This multifunctional protein is involved in numerous aspects of the RNA metabolism such as transcription, splicing, export or degradation. In addition, DDX3X is a physiological regulator of gene expression although its role in translation can be positive or negative depending on the protein or system considered. DDX3X has also been associated with several pathologies such as cancer progression and viral infections. DDX3X presents numerous potential or demonstrated post-translational modifications (PTM) such as phosphorylation, acetylation, sumoylation and arginine methylation. PTM allow for the dynamic regulation of proteins activity, localization and/or stability and could favor the multiple roles of DDX3X in the cell. Most of the reports indicating the presence of PTM on DDX3 arise from global proteomic analyses and only DDX3X phosphorylation has been investigated functionally. In this context, to explore the landscape of DDX3X PTM in cells, we combined cell fractionation and affinity purifications approaches to purify endogenous DDX3X from different cellular compartments; and 2D electrophoresis and western-blot and/or mass spectrometry approaches to identify DDX3X PTM. We are currently assessing the spatiotemporal regulation of these isoforms under pathophysiological conditions and will present our latest results.

Characterization of the ochratoxin A-binding aptamer and binding to its ligand

Zachary Churcher[1], Sladjana Slavkovic[1], Aron Shoara[1], Richard Manderville[2] & Philip Johnson[1]

1. Department of Chemistry [York University - Toronto] – Canada
2. Departments of Chemistry and Toxicology [University of Guelph- Guelph] – Canada

NMR spectroscopy, isothermal titration calorimetry (ITC), and UV-Vis spectroscopy are being used to investigate the interaction between the ochratoxin-A-binding aptamer (OTA) and its ligand ochratoxin-A. Ochratoxin A is a mycotoxin produced by certain types of Penicillium and Apsergillus fungi. Found in grain, pork and a number of other sources, ochratoxin-A is one of the most abundant food contaminating mycotoxins. Ochratoxin- A is a strong neurotoxin thought to deplete dopamine levels in brain and cause oxidative damage to DNA. The OTA aptamer is predicted to be a monomolecular antiparallel G- quadruplex with a two G-tetrad core, and two tails extending from the same face of the quadruplex. Initial NMR data shows the quadruplex requires the ligand to form the correct conformation but remains in the same conformation even if the ligand falls away from the aptamer. NOESY data suggests that the two tails of the aptamer may be forming a double helix structure extending off one of the faces of the quadruplex. This hybrid-quadruplex motif has been seen recently in the literature in other aptamers. Initial ITC data shows the binding of the ligand and the aptamer to have a binding affinity (Kd) of 6 μM, binding at a 1:1 stochiometric ratio, which is also supported by titration NMR data. Thermal analysis using UV-Vis spectroscopy shows a melting temperature of 55◦C for the unbound aptamer, and 50◦C for the ligand-bound complex. These data are being used to determine the structure of the aptamer-ligand complex, and to understand the interaction between these two molecules.

Post-transcriptional inactivation of toxin-antitoxin systems via exonucleolytic degradation of protective stem-loops

Sara Masachis[1], Nicolas J. Tourasse[1], Sandrine Chabas[1], Fanny Boissier[1], Hélène Arnion[2], Isabelle Iost[1] & Fabien Darfeuille[1]

1. University of Bordeaux, INSERM U1212, CNRS UMR 5320, ARNA Laboratory, F-33000 Bordeaux, France.
2. INSERM UMR 850, University of Limoges, Faculty of Pharmacy, 2 rue du Dr Marcland, F-87025, Limoges, France

Controlling mRNA decay is a key determinant of gene expression regulation in bacteria. This is particularly true for toxin-antitoxin (TA) systems, where fine-tuning of toxin expression is vital for the bacterium. We previously reported the characterization of a new family of type I TA systems hosted on the chromosome of the major human pathogen *Helicobacter pylori* [1]. Here, we show that antitoxin expression is essential for cell survival. Indeed, the antitoxin specifically binds to the translationally active form of the toxin-encoding mRNA thereby inhibiting its translation. To identify suppressor mutations allowing survival in absence of antitoxin, we deep sequenced the TA locus of suppressors strains in which the antitoxin promoter has been inactivated [2]. While most mutations map to the coding region, around 30% are located in the 5' and 3' untranslated regions of the toxin mRNA. Surprisingly, some of these mutations lead to a strong destabilization of the active mRNA. Indeed we show that a single nucleotide substitution is sufficient to induce a complete degradation by the 3'-to-5' PNPase exoribonuclease. Similarly, mutations located at the 5' end of the mRNA induce its degradation by the 5'-to-3' exoribonuclease J (RNase J). A structural analysis demonstrates that these mutations are disrupting base pairing of two highly stable hairpins located at both ends of the toxin mRNA, promoting its degradation by the two major exoribonucleases. In conclusion, our results reveal the importance of two protective stem-loops to ensure the functional stability of the active mRNA in absence of the antitoxin.

References: [1]. Arnion H, et al., Mechanistic insights into type I toxin-antitoxin systems in Helicobacter pylori: the importance of mRNA folding in controlling toxin expression. Nucleic Acids Res. 2017 May 5;45(8):4782-4795. [2]. Masachis S, et al., A genetic selection reveals functional metastable structures embedded in a toxin-encoding mRNA. bioRxiv 615682; doi: https://doi.org/10.1101/615682.

Time Travel with Elongation Factor – Tu (EF-Tu)

Arindam De Tarafder, Soneya Majumdar & Suparna Sanyal

Department of Cell and Molecular Biology, Uppsala University, Box-596, BMC, 75124, Uppsala, Sweden

Elongation factor Tu (EF-Tu) is a highly essential translation factor responsible for delivering aminoacyl tRNAs to the ribosome for every codon on the mRNA. EF-Tu, as the name suggests, is not thermostable in common mesophilic bacteria. But, how would it be billions of years ago when extreme temperatures prevailed throughout our planet? We seek an answer to this question by studying ancestral sequence reconstructed EF-Tus spanning 500 million to 3.5 billion years of evolutionary age with structure and function.
We have cloned, expressed and purified nodal EF-Tus 262, 168, and 170 originally resurrected by Eric Gaucher and colleagues using Ancient Sequence Reconstruction tool [1]. In order to achieve structural insight, we have solved the crystal structures of the ancient EF-Tus at high resolution (~2˚A) with X-ray crystallography. Further, we characterized the 262 EF-Tu, ~1.2 billion years old, for the rate and accuracy of dipeptide formation using a fully reconstituted bacterial translation system. Precise determination of the Michealis-Menten parameters show that 262 EF-Tu has similar catalytic efficiency or specificity (kcat/KM) of dipeptide formation on the ribosomes from both mesophilic bacteria *Escherichia coli* and thermophilic bacteria *Thermus thermophilus*. In comparison, EF-Tus from *E. coli* and *T. thermophilus* (Tt EF-Tu) show two to three fold higher specificity to- wards their respective ribosomes. The specificity constants of 262 EF-Tu and Tt EF-Tu do not change by increasing the temperature to 50°C. Our results provide strong support to the theory that ancient enzymes are more promiscuous than the modern enzymes [2]. We also show that high promiscuity of this ancient EF-Tu comes with the cost of reduction in the catalytic constant (kcat). In summary, characterization of such an abundant and conserved translation factor provides vital clues of molecular evolution and thermal adaptability of the translation machinery in bacteria.

References: [1] Zhou, Y. et al., 2012. Reconstitution of translation from *Thermus thermophilus* reveals a minimal set of components sufficient for protein synthesis at high temperatures and functional conservation of modern and ancient translation components. Nucleic Acids Research, 40(16), pp.7932–7945. [2] Jensen, R.A., 2003. Enzyme Recruitment in Evolution of New Function. Annual Review of Microbiology 30, 409–425.

Cryo Electron Tomography study of protein biogenesis at the Endoplasmic Reticulum membrane

Juliette Fedry[1], Stefan Pfeffer[2], Floor Peters[1], Bertrand Kleizen[1], Ineke Braakman[1] & Friedrich Förster[1]

1. Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
2. Ruprecht Karls University Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

Approximately one third of the cellular proteins utilizes the secretory pathway for entrance into the endoplasmic reticulum (ER). The native core translocon associated with ER ribosomes comprises the protein-conducting channel Sec61, which enables substrate translocation, and additional complexes involved in nascent chain processing (TRanslocon Associated Protein complex TRAP and OligoSaccharyl-Transferase OST). However, our understanding of how this complex handles the nascent chain at the ER membrane is currently restricted to puzzle pieces, mostly due to the difficulty to reconstitute this system from purified components. In order to provide an integrative structural insight into fundamental cellular mechanisms such as the insertion of nascent proteins into or their translocation across the ER membrane, their maturation and assembly to oligomeric complexes, we reproduce the biogenesis of different proteins in vitro, using ER derived microsomes. The sample is then imaged by Cryo-Electron Tomography. Candidate proteins include glycosylated and multi pass membrane proteins as well as oligomeric complexes. In the tomograms of actively processing microsomes, membrane-bound ribosomes are localized by template matching and subtomogram averaging is used to visualize the processing of the nascent chain under close-to-native conditions.

Synthesis of 7-substituted-7-deazaadenosine triphosphates as building blocks for enzymatic construction of modified RNA

Vojtěch Havlíček[1,2], Michal Hocek[1,2]

1. Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic.
2. Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12843 Prague 2, Czech Republic.

Modified RNAs and DNAs play a very important role as a tool used to explore and to study biological functions and structure of natural nucleic acids. They can also be used as binding aptamers for therapeutic purposes. [1] In our group, a new method for the preparation of 5-substituted pyrimidine and 7-substituted 7-deazapurine modified nucleoside triphosphates was developed. A small series of nucleoside triphosphates (ATP, UTP, CTP, and GTP) bearing methyl, ethynyl, phenyl, benzofuryl and dibenzofuryl groups was prepared. Cross-coupling reactions on nucleosides followed by chemical phosphorylation to triphosphates were used. Another approach is to subject halogenated nucleotide (nucleoside triphosphate) straight to the cross-coupling reaction. These compounds were used for enzymatic synthesis of modified RNAs by T7 RNA polymerase. [2,3] Based on these results, we prepared a small library of modified ATPs (7-modified 7-deazapurine nucleoside triphosphates) that will be used for enzymatic synthesis of modified RNAs. The synthesis started from 6-chloro-7-deazapurine, from which 7-iodotubercidine triphosphate was prepared in six steps. Applying Sonogarhira and Suzuki-Miyamura crosscoupling reactions, 7-iodoturcidine was derivatized to give a small library of modified ATPs. This library will be used to study the enzymatic preparation of RNA strands.

References: [1] Breaker, R. R. Nature 2004, 432, 838–845. [2] Milisavljevič, N.; Perlíková, P.; Pohl, R.; Hocek, M. Org. Biomol. Chem. 2018, 16, 5800–5807. [3] Perlíková, P.; Rylová, G.; Nauš, P.; Elbert, T.; Tloušťová, E.; Bourderioux, A.; Slavětínská Poštová, L.; Motyka, K.; Doležal, D.; Znojek, P.; Nová, A.; Harvanová, M.; Džubák, P.; Šiller, M.; Hlaváč, J. Hajdúch, M.; Hocek, M. Mol. Cancer Ther. 2016, 15, 922–937.

Ornithine capture by a translating ribosome controls bacterial polyamine synthesis

Alba Herrero del Valle, Britta Seip, Iñaki Cervera-Marzal, Guénaël Sacheau, A. Carolin Seefeldt & C. Axel Innis

Institut europeen de chimie et biologie (IECB) – INSERM U1212 – 2 rue Robert Escarpit 33600, Pessac, France

Polyamines are essential metabolites that play an important role in cell growth, stress adaptation, and microbial virulence. In order to survive and multiply within a human host, pathogenic bacteria adjust the expression and activity of polyamine biosynthetic enzymes in response to different environmental stresses and metabolic cues. Here, we show that ornithine capture by the ribosome and the nascent peptide SpeFL controls bacterial polyamine synthesis by inducing the expression of the ornithine decarboxylase SpeF, via a mechanism involving ribosome stalling and transcription antitermination. In addition, we present the cryo-EM structure of an *Escherichia coli* (*E. coli*) ribosome stalled during translation of speFL in the presence of ornithine. The structure shows how the ribosome and the SpeFL sensor domain form a highly selective binding pocket that accommodates a single ornithine molecule but excludes near-cognate ligands. Ornithine pre-associates with the ribosome and is then held in place by the sensor domain, leading to the compaction of the SpeFL effector domain and blocking the action of release factor RF1. Thus, our study not only reveals basic strategies by which nascent peptides assist the ribosome in detecting specific metabolites, but also provides a framework for assessing how ornithine promotes virulence in several human pathogens.

New method for unbiased quantification of riboswitch transcriptional activity

Piotr Machtel, Anna Wasilewska, Julian Zacharjasz & Kamilla Bakowska-Zywicka

Functional Transcriptomics Team, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

In bacteria, the expression of significant fraction of genes is controlled by premature transcription termination. An interesting example of such mechanism are riboswitches; regulatory sequences, usually located within the 5′ untranslated region (5′UTR) of certain genes, able to bind small cellular compounds, therefore evoking regulatory effect. In vast majority of riboswitches, interaction of ligand-binding domain with a given ligand (metabolite) causes structural rearrangements, resulting in the formation of termination hairpin and consequently, premature transcription termination. Terminated as well as read-through tran- scripts are detectable by standard experimental procedures (like northern blot or real-time PCR). However, an absolute quantification cannot be easily achieved by currently available methods. This fact was an inspiration for creation of a new method for direct identification and absolute quantification of transcription termination events, as a result of riboswitch activity. The first step in the developed protocol requires a site-directed RNaseH-induced cleavage (covering transcription termination site), resulting in separation of two transcript's populations: 5′ part – corresponding to the sum of terminated and read-through transcripts and 3′ part, corresponding to full-length transcripts alone. The cleavage efficiency is then determined by primers flanking the cleavage site. An absolute concentration of terminated and read-through transcripts is quantified using the droplet digital PCR (ddPCR) technology. As it is shown, utilization of our protocol allows for precise and unbiased quantitative analysis.

Functional and physiological characterization of two ABC-F (EttA and MsrD) proteins involved in translation

Farès Ousalem, Thomas Oiffer, Corentin Fostier, Anna Liponska & Grégory Boël

CNRS, Université de Paris UMR 8261, Institut de Biologie Physico-Chimique, Paris, France

The superfamily of the ATP binding cassette (ABC) protein is comprised of ATPase enzymes, which are, for the most, involved in transmembrane transport. Within this superfamily however, some protein families have other functions unrelated to transport like the ABCF family. One member of this family, the energy sensing translational throttle A (EttA) gates ribosome entry into the elongation. A model of EttA function suggests that the protein maintains translation initiation complexes in a hibernating state when the ADP to ATP ratio is high and allows the translation to restart when ATP cellular concentration increases. Since protein synthesis uses more than 30% of total cellular energy, this system should allow the bacteria to save energy. As expected, the strain deleted of the ettA gene has a fitness deficient phenotype during conditions when the intracellular ATP concentration is low. Now we are characterizing the physiologic response mediated by EttA with the goal to understand the implication of EttA on cellular physiology.
Phenotype screening coupled with transcriptomic and proteomic analysis has shown that deletion of ettA impairs bacteria adaptation. Loss of ettA reduces bacteria motility by de- creasing the expression of all the genes involved in the flagella apparatus. We have shown in vivo that the EttA protein is important for bacteria growing in high salt conditions. The EttA protein also regulates the synthesis of certain enzymes involved in the metabolism of the Krebs cycle. Accordingly, the growth of the strain lacking the ettA gene is reduced on some carbon sources that are assimilated directly by the Krebs cycle. These results advocate for a specific action of EttA towards certain mRNA.
Some proteins in the ABC-F family confer antibiotic resistance that inhibits protein synthesis. We also start to develop an *Escherichia coli* model to study the molecular mechanism of ABC-F involved in antibiotic resistance

Structure and function of the DNA recombination apparatus involved in bacterial transformation

Thomas Perry, Chiara Rapisarda & Rémi Fronzes

Structure and function of bacterial nano-machines, Institut Européen de Chimie et Biologie 2, Rue Robert Escarpit - 33607 PESSAC - France

Homologous recombination (HR) is a reaction essential for maintaining genomic integrity and for generating genetic diversity. HR is a highly conserved mechanism, which allows the exchange of nucleotide sequences between two DNA molecules. This process is involved in the natural genetic transformation which is widely distributed in bacteria, more than 80 bacterial species have been reported to be naturally transformable. But natural genetic transformation was originally discovered in Streptococcus pneumoniae (Sp) in response to environmental signals, and ever since the pneumococcus has served as a paradigm for this important phenomenon. Transformation requires dedicated cytosolic proteins, some of which have been characterized only recently, for the processing of internalized single-stranded DNA (ssDNA) fragments into recombination products. But many steps of this process remain unknown. It's in this context that my investigation takes place, in fact my research project aims to study, by cryo-microscopy, the interaction between the recombinase S. pneumoniae RecASp and the DNA. RecA, ATP and single-stranded DNA (ssDNA) form an helical filament that binds to double-stranded DNA (dsDNA), searches for homology, and then catalyzes the exchange of the complementary strand, producing a new heteroduplex. Here, we report a structure for the RecASp-ssDNA and the RecASp-dsDNA complexes at 3.9Å and 3.8Å resolution respectively, using electron cryo-microscopy. RecASp encases ssDNA and dsDNA in a helical filament with a rise of 15.38Å and 14.97Å respectively, and with a twist of 58°. We show also, using these structures, why RecASp catalyzes ATP-dependent strand-exchange up to 3-fold more efficiently than Escherichia coli RecA protein.

Methylation of the GGQ motif of the bacterial class-I release factors enhances accuracy in translation termination

Shreya Pundir, Xueliang Ge & Suparna Sanyal[1]

Department of cell and molecular biology, Uppsala University, Box-596, BMC, Uppsala, 75124, Sweden

Accurate translation termination in prokaryotes is achieved by correct reading of the stop codons by the class-I release factors, RF1 and RF2. Both RF1 and RF2 recognize UAA, whereas UAG is specific for RF1 and UGA is specific for RF2. Both RF1 and RF2 possess a conserved peptide release motif 250GGQ252 (Gly-Gly-Gln); the Q residue is post- translationally methylated by the methyl transferase enzyme prmC. It is known that GGQ methylated RFs are faster in peptide release [1, 2], but additional roles of GGQ methylation is unknown.
Using a fluorescence based in-vitro peptide release assay, we have quantitatively characterized unmethylated and methylated RF1 and RF2 (referred to as mRF1 and mRF2, respectively) for accuracy in translation termination. The Michaelis-Menten parameters, namely the turnover number (kcat) and the catalytic efficiency (kcat/KM) of the release factor variants for peptide release on cognate and near-cognate codons are determined. Further, accuracy of stop codon recognition is estimated from (kcat/KM)cognate/(kcat/KM)near- cognate value. Our quantitative analyses demonstrate that mRF1 and mRF2 have two- to four-fold higher accuracy than their unmethylated variants. We also find that the accuracy of termination is independent of free Mg2+ concentration, but depends on the pH of the reaction. We hypothesize that the GGQ methylation may have an impact on the dynamics of the open/closed conformation of RF1 and RF2 on the ribosome. The methylation based accuracy modulation of the release factors can be a fine tool for regulating translational termination in limited resource condition.

References: [1] Indrisiunaite, G., Pavlov, M. Y., Heurgué-Hamard, V., & Ehrenberg, M. (2015). On the pH dependence of class-1 RF-dependent termination of mRNA translation. Journal of Molecular Biology, 427(9), 1848–1860. [2] Pierson, W. E., Hoffer, E. D., Keedy, H. E., Simms, C. L., Dunham, C. M., & Zaher, H. S. (2016). Uniformity of Peptide Release Is Maintained by Methylation of Release Factors. Cell Reports, 17(1), 11–18.

Understanding late cytoplasmic pre-40S ribosome maturation

Finaritra Raolijaona, Stéphane Thore & Sébastien Fribourg

ARNA laboratory, INSERM U1212 - CNRS UMR5320, University of Bordeaux, Pessac, France

Ribosomes are large macromolecular complexes universally responsible of mRNA translation into proteins. Ribosome assembly is a highly orchestrated process, which requires a multitude of biogenesis factors to ensure ribosomal correct RNA cleavage, modification and folding as well as ribosomal proteins incorporation. In human, dysfunction in ribosome assembly has been related to a group of diseases generally known as Ribosomopathies besides alteration in the biogenesis could promote cancer cells formation. Cytoplasmic maturation of the small ribosomal subunit is associated with sequential release of assembly factors and concomitant maturation of the pre-ribosomal RNA. During the final maturation of the small subunit, the pre-18S rRNA is cleaved off by the endonuclease Nob1 in a step where its binding partner Pno1 plays an important role. However clear mechanism of the stepwise process is not fully understood. In order to better understand these late steps of cytoplasmic pre-40S maturation, we first aim to redefine the endonuclease domain then study its binding mode with hPno1 using different tools such sequence analysis, structure prediction and biochemical experiments. Our findings further support the recently published cryo-EM structure of the human pre-40S.

Analysis of Ligand-Induced Folding and Aptachain Self-Assembly in The Cocaine-Binding Aptamer

Aron A. Shoara[1], Logan W. Donaldson[2], and Philip E. Johnson[1]

1. Department of Chemistry and Centre for Research on Biomolecular Interactions, York University, Toronto, ON, M3J 1P3 – Canada
2. Department of Biology, York University, Toronto, ON, M3J 1P3 – Canada

The cocaine-binding aptamer has become a widely employed model system for the development of aptamer-based biosensors for two main reasons: (i) it has a structure-switching mechanism that depends on the length of one stem, and (ii) it binds ligands, such as qui- nine, tighter than its originally selected cocaine ligand. This work presents progress on two aspects of the cocaine-binding aptamer. First, we present the dynamics of the free and ligand-bound of two constructs of the cocaine-binding aptamer linked to stilbene employing fluorescence decay kinetics and fluorescence anisotropy of stilbene conjugates. Conventional fluorescence sensing of aptamers depends on emission measurements by comparing signals in the presence and absence of analytes. However, conventional techniques do not provide information on whether an aptamer is undergoing a structure-switching binding mechanism. Photoisomerization reaction mechanisms are powerful means in the development of aptamer sensors, where the absorption or emission of light is converted to detectable electrical signals. We utilized the cis-trans photoisomerization and fluorescence anisotropy of 4-acetamido- 4-isothiocyanato-2,2-stilbenedisulfonic acid (SITS) conjugated with two constructs of the cocaine-binding aptamer. We compared the dynamics of the free and ligand-bound MN19- SITS and MN4-SITS aptamers employing cocaine and quinine as ligands. By comparing the kinetics of MN19-SITS and MN4-SITS, we demonstrate that cocaine and quinine induce a structural switching folding mechanism for MN19-SITS but not MN4-SITS. Additionally, we show the optimization of aptachain. This is where the cocaine-binding aptamer is split into two overlapping strands and assembles into an extended oligomer complex upon ligand binding. We use size-exclusion chromatography and UV thermal melts to show that the quinine-bound oligos form a larger assembly of aptachain units than in the absence of ligand. We propose that splitting aptamers into overlapping strands that form oligomers in the presence of a ligand will be generally applicable to aptamers and prove useful in a variety of biotechnology applications. Finally, we think that the change in the fluorescence decay kinetics of MN19-SITS is a powerful and sensitive tool to study the ligand-induced folding mechanism of this aptamer as well as other aptamers that also have a ligand-induced structure-switching mechanism.

Structural characterization of assembly intermediates of eukaryotic ribosome biogenesis

Marlène Vayssières, Magali Blaud & Nicolas Leulliot

Laboratoire Cibles Thérapeutiques et conception de médicaments - UMR8038, CNRS – Université Paris Descartes - Paris, France

The ribosome is the cell's huge ribonucleic machinery in charge of synthesizing proteins from the mRNA. In yeast, the ribosome is composed of three ribosomal RNAs (rRNAs), the 25S, 5.8S and 18S, associated to 79 proteins. The different steps of ribosome biogenesis, including rRNA transcription, processing, and ribosomal protein assembly require the coordinated action of over 200 non ribosomal proteins, also named assembly factors. Assembly of the ribosomal proteins onto the rRNA is highly coordinated with rRNA processing. In- deed, the maturation of ribosomes is a multistep and dynamic process in which the protein complexes are remodeled and recruited following specific maturation events, thereby pro- viding several quality control checkpoints. One of the most striking proofreading steps is the last pre-18S rRNA cleavage, mediated by the endonuclease Nob1 [1]. The maturation of the pre-rRNA 20S into mature 18S rRNA seems to occur in a 80S-like particle composed of a pre-40S subunit bound to a mature 60S subunit, in a translation-like cycle [2, 3]. The assembly factor Rio1 is one of the protein needed to the 20S pre-rRNA processing, found in the 80S-like particle [4]. We solve the structure by cryo-EM at 3.2°A, of 80S-like particle with a Rio1 mutant as bait, in order to better understand this translation-like checkpoint.

References: [1] Fatica, Tollervey, et Dlakić, « PIN Domain of Nob1p Is Required for D-Site Cleavage in 20S Pre-RRNA »; Pertschy et al., « RNA Helicase Prp43 and Its Co-Factor Pfa1 Promote 20 to 18 S RRNA Processing Catalyzed by the Endonuclease Nob1 ». [2] Lebaron et al., « Proofreading of Pre-40S Ribosome Maturation by a Translation Initiation Factor and 60S Subunits ». [3] Strunk et al., « A Translation-like Cycle Is a Quality Control Checkpoint for Maturing 40S Ribosome Subunits ». [4] Ferreira-Cerca et al., « Dominant Rio1 kinase/ATPase catalytic mutant induces trapping of late pre-40S biogenesis factors in 80S-like ribosomes ».

Participants

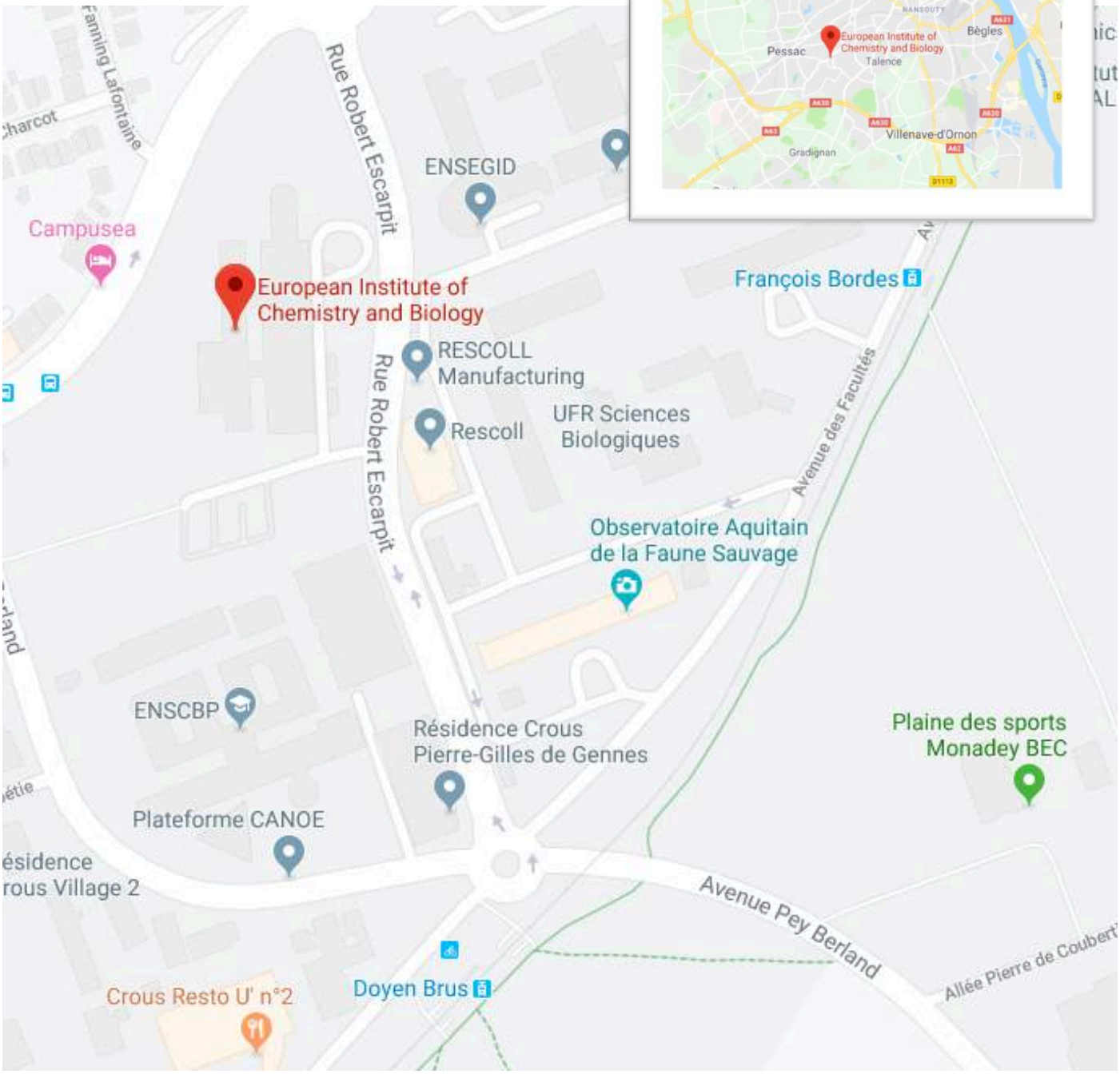
Shujaat Ali (Bordeaux, France)
Yasmine Amrani (Strasbourg, France)
Marie-Line Andreola (Bordeaux, France)
Mikayel Aznauryan (Pessac, France)
Philippe Barthelemy (Bordeaux, France)
Margarita Belinite (Pessac, France)
Florian Bernard (Pessac, France)
Anthony Bochler (Pessac, France)
Gregory Boël (Paris, France)
Fanny Boisier (Bordeaux, France)
Tanaya Bose (Rehovot, Israel)
Delphine Bronesky (Bordeaux, France)
Sandrine Chabas (Bordeaux, France)
Nathalie Chamond (Paris, France)
Zachary Churcher (Toronto, Canada)
Christine Clayton (Heidelberg, Germany)
Anthony Codbreuil (Bordeaux, France)
Nicolas Corre (Strasbourg, France)
Pauline Cossard (Pessac, France)
Fabien Darfeuille (Bordeaux, France)
Grégoire de Bisshop (Paris, France)
Mayara Lucia Del Cistia (Pessac, France)
Arindam De Tarafder (Uppsala, Sweden)
Carmelo Di Primo (Pessac, France)
Wilfrid Dieryck (Bordeaux, France)
Hélène Dumay-Odelot (Bordeaux, France)
Maëlle Duperray (Bordeaux, France)
Denis Dupuy (Pessac, France)
Stéphanie Durrieu (Pessac, France)
Dmitri Ermolenko (Rochester, USA)
Eduardo Eyras (Canberra, Australia)
Juliette Fedry (Utrecht, the Netherland)
Corentin Fostier (Pessac, France)
Sebastien Fribourg (Bordeaux, France)
Valérie Gabelica (Pessac, France)
Philippe Giegé (Strasbourg, France)
Mélanie Gillard (Bordeaux, France)
Dixie Goss (New York, USA)
Lina Hamouche (Toulouse, France)
Yaser Hashem (Pessac, France)
Vojtěch Havlíček (Prague, Czech Republic)
Alba Herrero (Pessac, France)
Zoya Ignatova (Hamburg, Germany)
Axel Innis (Pessac, France)
Isabelle Iost (Bordeaux, France)
Hollie Ireland (Edinburgh, Scotland)
Philip Johnson (Toronto, Canada)
Nora Karnowski (Bonn, Germany)
Alexey Kopylov (Moscow, Russia)
Natalia Korniy (Göttingen, Germany)
Jeanne Leblond Chain (Bordeaux, France)
Antony Lee (Bordeaux, France)
Anaïs Le Rhun (Bordeaux, France)
Elodie Leroy (Pessac, France)
Judy Lieberman (Boston, USA)

Anne-Marie Lobstein (Strasbourg, France)
Piotr Machtel (Moscow, Russia)
Cameron Mackereth (Pessac, France)
Aitor Manteca (Pessac, France)
Sara Masachis Gelo (München, Germany)
Olena Mayboroda (Prague, Czech Republic)
Lionel Minvielle-Sebastia (Bordeaux, France)
Hakim Mireau (Versailles, France)
Jorg Morf (Cambridge, UK)
Catherine Mulholland (Prague, Czech Republic)
Victoriia Murina (Umeå, Sweden)
Saravuth Ngo (Paris, France)
Thomas Oïffer (Paris, France)
Marek Ondrus (Prague, Czech Republic)
Farès Ousalem (Paris, France)
Camila Parrot (Pessac, France)
Tomas Pelletier (Saskatchewan, Canada)
Natacha Perebaskine (Pessac, France)
Thomas Perry (Pessac, France)
Florian Pierre (Pessac, France)
Olga Puchta (Edinburgh, Scotland)
Shreya Pundir (Uppsala, Sweden)
Finaritra Raoelijaona (Bordeaux, France)
Aline Rimoldi (Pessac, France)
Ismaeil Saad (Giza, Egypt)
Suparna Sanyal (Uppsala, Sweden)
Arghya Sett (Bordeaux, France)
Aron Shoara (Toronto, Canada)
Sanjeev Shukla (Bhopal, India)
Marie Sissler (Pessac, France)
Heddy Soufari (Pessac, France)
Murat Sunbul (Heidelberg, Germany)
Katerina Svehlova (Prague, Czech Republic)
Martin Teichmann (Bordeaux, France)
Nguyen Tan-Trung (Versailles, France)
Jean-Jacques Toulmé (Bordeaux, France)
Nicolas Tourasse (Bordeaux, France)
Anne-Xander van der Stel (Pessac, France)
Allessandro Vannini (London, UK)
Marlène Vayssières (Paris, France)
Daniel Wilson (Hamburg, Germany)
Sarah Woodson (Baltimore, USA)

Venue

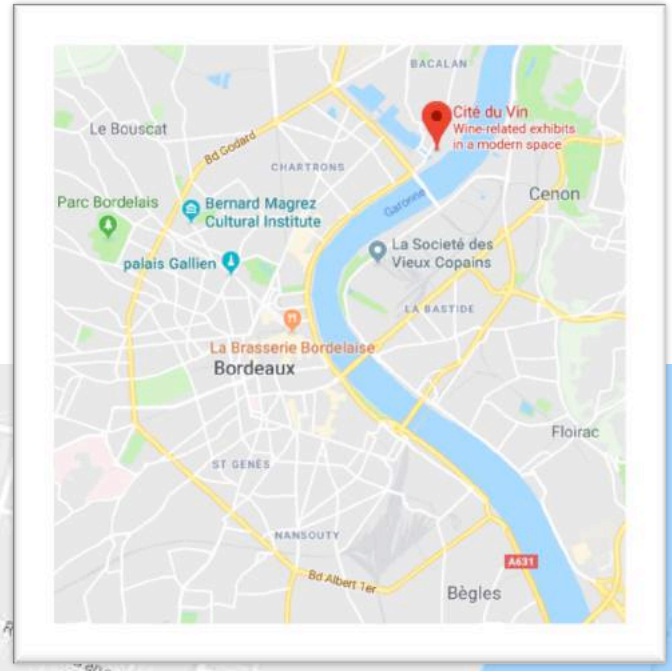
Thursday June 27, 2019

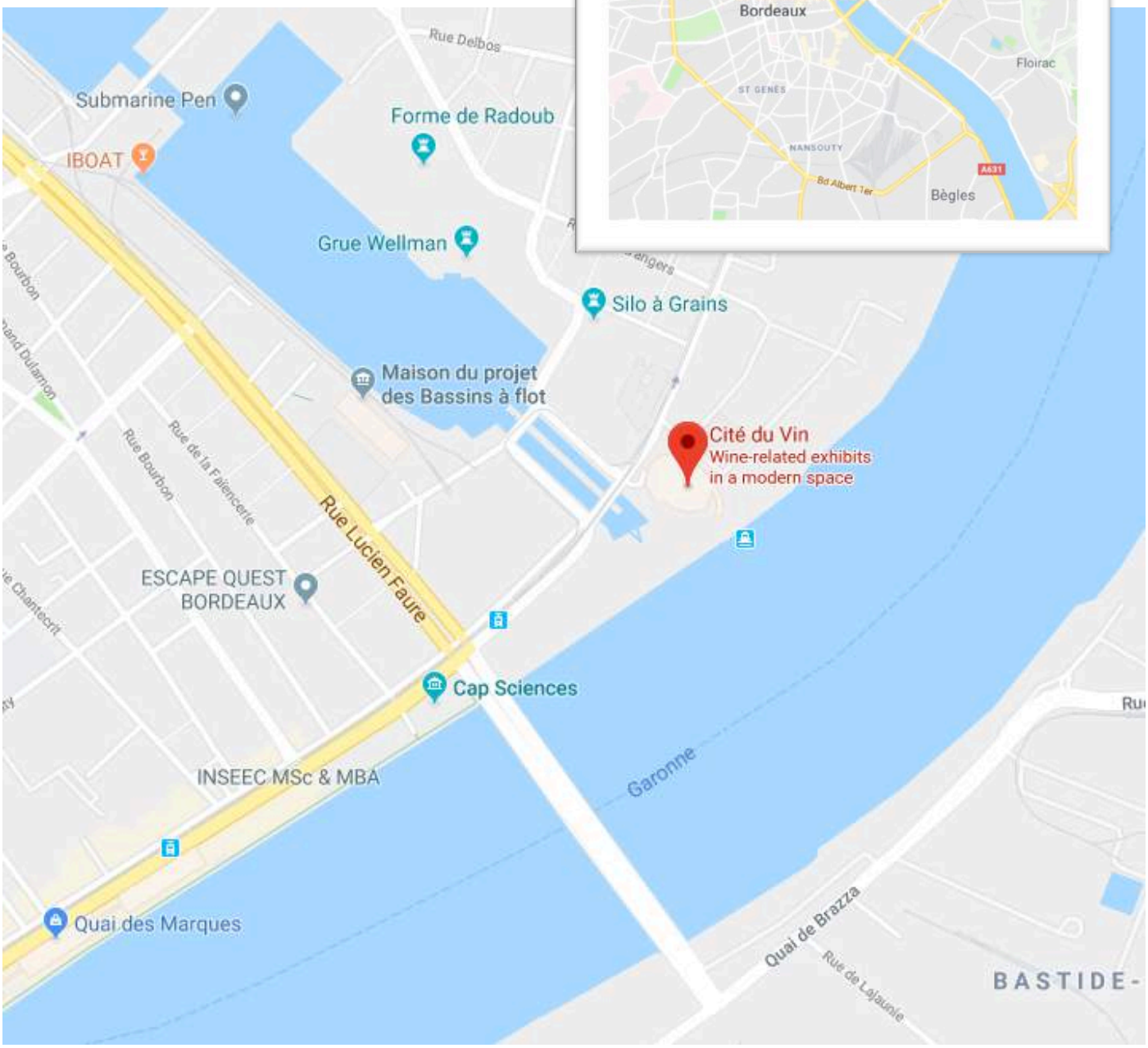
Institut Européen de Chimie et Biologie
2, rue Robert Escarpit
33607 Pessac
France



Friday June 28, 2019

La Cité du Vin
Esplanade de Pontac
134 Quai de Bacalan
33300 Bordeaux
France





Transportation

The instructions below are for reaching the IECB using public transport:

From Bordeaux-Mérignac airport

- Take Bus Liane 1 towards Gare Saint-Jean and get down at Mérignac Soleil (12 min)
- Take Bus Corol 34 towards Rives d'Arcin and get down at Village 2 (32 min)
- The IECB will be in front of you

From the train station

- Take Bus Liane 9 towards Brandenburg and get down at Barrière Saint-Genès (12 min)
- Walk towards Boulevard du Président Franklin Roosevelt for 60 metres, turn right on Place Louis Barthou and walk for 40 metres (2 min)
- Take Tram B from Saint-Genès towards Pessac Centre or France Alouette and get down at Doyen Brus (10 min)
- Walk straight towards the IECB when you exit the tram (5 min)

From the city center

- Take Tram B from Hotel de Ville towards Pessac Centre or France Alouette and get down at Doyen Brus (22 min)
- Walk straight towards the IECB when you exit the tram (5 min)

For more information regarding public transport, please consult the TBM website (https://www.infotbm.com/en).

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

Did you get the message?

HiScribe™ *In Vitro* Transcription Kits *for* RNA RESEARCH -

For rapid synthesis of high yields of high quality RNA

HiScribe T7 High Yield RNA Synthesis Kit

NEB #E2040S

HiScribe T7 Quick High Yield RNA Synthesis Kit

NEB #E2050S

HiScribe T7 ARCA mRNA Kit

with tailing NEB #E2060S

w/o tailing NEB #E2065S

HiScribe SP6 RNA Synthesis Kit

NEB #E2070S

Let NEB help streamline your **RNA-related workflows.**
Get started at **NEBrna.com**

Monarch® Kits for RNA Cleanup

Provide a fast and simple silica column-based solution for cleanup and concentration of RNA after enzymatic reactions (including *in vitro* transcription (IVT), DNase I treatment, capping and labeling), as well as after RNA isolation (e.g., TRIzol® extraction).

Monarch Kit Specifications:

MONARCH RNA CLEANUP KIT	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
Binding Capacity	10 µg	50 µg	500 µg
RNA Size Range	≥ 25 nt (≥ 15 nt with modified protocol)		
Typical Recovery	70–100%		
Elution Volume	6–20 µl	20–50 µl	50–100 µl
Purity	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8		
Protocol Time	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time
Common Downstream Applications	RT-PCR, RNA library prep for NGS, small RNA library prep for NGS, RNA labeling	RT-PCR, RNA library prep for NGS, formation of RNP complexes for genome editing, microinjection, RNA labeling, transfection	RT-PCR, RNA library prep for NGS, RNA labeling, RNAi, microinjection, transfection

Advantages:

- Clean up RNA with simple bind / wash / elute protocol, utilizing a single wash buffer
- Elute in as little as 6µl (NEB#T2030) or 20µl (NEB#T2040)
- Bind up to 500µg of RNA (NEB#T2050)
- Adjust cutoff size down to 15 nt with a slight protocol modification
- Order only what you need, with kit components, including columns and buffers, available separately

Also, try our Monarch Kits for:
- Plasmid miniprep
- DNA cleanup
- DNA gel extraction
- Total RNA extraction

Learn more at NEBMonarch.fr

nqa. ISO 9001 Registered Quality Management

nqa. ISO 14001 Registered Environmental Management

nqa. ISO 13485 Registered Medical Devices

www.neb-online.fr

NEW ENGLAND BioLabs®

New England Biolabs France, 5 rue Henri Desbruères, 91030 Evry cedex, France
Tel.: 0800 100 632, Fax: 0800 100 610, info.fr@neb.com

When proteins matter.

NanoTemper measures protein stability, protein quality and binding affinity in the quickest way possible with just a tiny amount of sample.

MONOLITH

Swiftly measure binding interactions with Monolith and get precise K_d results using very little sample

TYCHO

Quickly identify the quality of any protein using microliters of sample

PROMETHEUS

Precisely characterize thermal unfolding, chemical denaturation and aggregation in a single run with the most flexibility a system has to offer

Cyril Castel, Account Manager
Cyril.castel@nanotempertech.com, phone +33 643961658

When proteins matter.

NanoTemper measures protein stability, protein quality and binding affinity in the quickest way possible with just a tiny amount of sample.

MONOLITH

Swiftly measure binding interactions with Monolith and get precise K_d results using very little sample

TYCHO

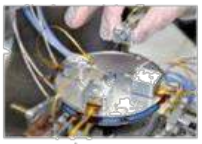
Quickly identify the quality of any protein using microliters of sample

PROMETHEUS

Precisely characterize thermal unfolding, chemical denaturation and aggregation in a single run with the most flexibility a system has to offer

Cyril Castel, Account Manager
Cyril.castel@nanotempertech.com, phone +33 643961658






Solutions de Nano caractérisation

EDEN INSTRUMENTS

MICRO-ANALYSE & MICROSCOPIE ÉLECTRONIQUE

EDEN Instruments est une société high-**tech, spécialisée dans la commercialisation d'équipements pour le secteur de la microscopie électronique. Elle propose une offre dédiée d'accessoires pour l'étude de vos échantillons en micro**-analyse MEB, EDS, EBSD, WDS, pour la microscopie électronique MET in-Situ, ainsi que pour la préparation de vos échantillons ex-Situ.

Notre gamme d'équipements :

➢ *MICRO-ANALYSE :*

EDAX Smart Insight
AMETEK MATERIALS ANALYSIS DIVISION

Solutions de caractérisation des matériaux :
Gamme complète d'outils d'analyse chimique / cristallographique pour MEB avec des technologies intégrées TEAM PEGASUS (EDS-EBSD), TEAM NEPTUNE (EDS-WDS), TEAM TRIDENT (EDS-EBSD-WDS) et d'analyse Micro-XRF. Nouvelle caméra EBSD en technologie CMOS : VELOCITY SUPER à 4500pps.

➢ *IN-SITU :*

Protochips

Solutions pour les expérimentations environnementales In-Situ MET :
Poséidon Select : Porte-objet liquide évolutif liquide/chauffant et électrochimie.
Atmosphère 210 : Porte-objet gaz/température avec détecteur RGA intégré.
Fusion : Porte-objet chauffant 1200 °C avec caractérisation électrique.

irmina technologies

Solutions de Nano-Probing in-Situ MEB :
Manipulation des échantillons en temps réel à l'échelle nanométrique. Mesure de résistivité / conductivité, courant tension (I-V). Robots à 4 degrés de liberté pour une grande flexibilité.
Caractérisation EBIC/EBAC. Probe station avec microscope intégré.

alemnis
Mechanical testing at the micro- and nano-scale

Solutions de Nano-Indentation :
Pour les tests nano-mécaniques In-Situ MEB sur tous types de matériaux pour les applications de déformation, dislocation, compression, courbure en mode statique, dynamique et sous haute température (jusqu'à 800°C).

Kammrath Weiss GmbH

Platines d'essais mécaniques et platines température :
Tests micromécaniques in-situ : Platines de traction/compression jusqu'à 5-10kN.
Tests en température in-situ : Platines chauffantes MEB 300-1500°C, platines froides MEB LN2, He & Peltier. Modules de transfert sous vide.

➢ *IN-SITU :*

Système de Cathodo-Luminescence et microscopie corrélative :
Delmic conçoit des détecteurs de cathodo-luminescence à intégrer sur des MEB : Système SPARC. Nouveau détecteur mini-CL JOLT à installer facilement sur un port EDS.

Solutions complètes de décontaminateurs In-Situ MEB :
Modèle EVACTRON E50, source de plasma déportée jusqu'à 50W pour nettoyage de chambre sous vide et d'échantillons.

➢ *EX-SITU :*

Solutions de métallisation :
Evaporateur Carbone / Métalliseur sous vide secondaire. (2.10^{-6} mbar) Déroulage de tresse automatique, système de mesure d'épaisseur intégré, vaste gamme de cibles métalliques. Fonctions supplémentaires : Glow Discharge & Plasma.

Solutions de préparation d'échantillons par abrasion ionique :
Système Fischione 1061 pour le polissage et le surfaçage automatisés des échantillons MEB (2 canons Argon 10kV, option Cryo).
Système Nanomill pour le nettoyage précis de lames FIB.
Equipement de nettoyage MET par Plasma sans redéposition.
Gamme complète de porte-objets MET pour applications Tomo ou CryoTomo.

Systèmes d'ultramicrotomie, de cryo-ultramicrotomie et de cryo-préparation :
Atumtome et ASH 2 : Systèmes manuels ou automatisés de collecte de coupes sériées pour l'imagerie MEB Tomo 3D.

➢ *UPGRADE MEB :*

Solutions électroniques et logiciels pour MEB :
Rénovation de microscopes, caractérisation.
EBIC/STEBIC/EBAC/RCI, imagerie BSE et topographique 3D calibrée.

Solutions de sources FEG de qualité :
Fabriquant de pointes FEG et électronique associées.
Conversion de MEB tungstène/LaB_6 vers MEB FEG.

EDEN INSTRUMENTS SAS - INEED ROVALTAIN VALENCE TGV - 1 rue Marc Seguin - 26300 ALIXAN
Tél.: +33 (0) 4 81 16 05 10 – Web: www.eden-instruments.com
Contact : Stéphane AGUY – 06 08 06 70 81 – stephane.aguy@eden-instruments.com Alexandru DELAMOREANU – 06 95 70 03 58 - alex.delamoreanu@eden-instruments.com

www.rnaclub.u-bordeaux.fr

facebook.com/RNAbordeaux
@RNAbordeaux

We would like to thank our sponsors and partners

Inserm

cnrs

université de BORDEAUX

EMBO
excellence in life sciences

NANOTEMPER

NEW ENGLAND BioLabs®
be INSPIRED | drive DISCOVERY | stay GENUINE

GATAN

GE Healthcare

EDEN INSTRUMENTS
In Situ NanoCharacterization Solutions

Network (SSID): **iecb-visitors**
Login: **guest**
Password: **13cb2019**