CONFERENCE REPORT

THE TRANSLATING RIBOSOME: TOWARDS MATURE PROTEINS

De la traduction ribosomale aux protéines matures

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Résumé (en français)

La conférence internationale « De la traduction ribosomale aux protéines mature » a été organisée entre les 2 et 6 juin 212 à Roscoff dans le cadre des Conférences Jacques Monod, à l'occasion de leur 25ème anniversaire. Le concept original était de rassembler deux communautés travaillant sur des objets similaires tous centrés sur le ribosome come machinerie et interface majeure. La communauté du ribosome et de la traduction est une communauté établie depuis 50 ans qui a été consacrée par les résolutions à l'échelle atomique des ribosomes procaryotes et de bactérie, travail récompensé du prix Nobel de Chimie en 2009. La seconde communauté - qui s'intéresse au devenir (modifications, temps de vie, repliement, localisation, contrôle qualité…) du peptide émergent du tunnel de sortie du ribosome – est de création plus récente et moins structurée. La conférence - conçue comme la première d'une série - était organisée autour des deux thèmes, synthèse des protéines dépendante du ribosome et évènements co-traductionnels. Ces deux thèmes, déclinés chacun en trois sous-thèmes ont été mélangés au cours des sessions orales invitées (26) ou sélectionnées (16) ainsi qu'au cours des quatre plages de sessions de poster (76 affiches). Quatre conférences plus longues – une par jour – données par des chercheurs récompensés du Prix Nobel ou de très grande notoriété et originalité avaient été identifiées pour induire un écho particulier et ont reçu effectivement un franc succès. Les autres conférences toutes de très grande qualité ont conduit à de nombreuses discussions. Globalement, les inscriptions initiales (115 pour 115 places, nécessitant un processus rigoureux de sélection des nombreuses candidatures) et la fréquentation assidue de la conférence conduisent au constat qu'une seconde conférence devrait être organisée dans les deux ou trois ans prochains pour soutenir les efforts d'interface réels entre les deux communautés et permettre de continuer à stimuler le dialogue passionnant qu'elles ont su nourrir au cours de ces 5 jours.

REPORT (In English)

The 2012 Conference on "*the translating ribosome: towards mature proteins*" was thought as the first of a series aiming at bringing together established leaders in the fields of ribosomes and cotranslational events. Two themes each covering 3 sub-themes were addressed and combined during the conference:

Theme 1 Ribosome-directed protein synthesis

"Dynamics in translation": ribosome-mRNA-tRNAs, translation factors

"The ribosome at atomic resolution"

"Single molecules studies"

Theme 2 Cotranslational events (modifications, targeting and folding)

"Cotranslational modifications"

"Dynamics in translation": ribosome-nascent chain-biogenesis factors

"Cotranslational fate of the protein" (targeting and folding)

Program organization

Each day, one sub-theme of each theme was addressed through a dedicated morning or afternoon session. The two poster presentations were split into two sessions with half of the presenters asked to attend one of the two sessions.

In addition to 26 invited speakers, the program gave space to 16 selected presentations to allow for unforeseen discoveries to be presented. A vast majority of the selected presentations was given by recently established PIs, new promising leaders in their field. 5 invited speakers were from outside Europe, 13 from Europe outside France, and 8 from France. 5 selected speakers were from outside Europe, 9 from Europe outside France, and 2 from France. The Female/Male ratio was 8/18 (invited), 4/16 (selected), 29/46 (attendees). The attendees' average age was 36 years old.

Except for two keynote and two Nobel lectures (45 minutes), plenary lectures were allocated 25 plus 5 minutes for discussion. Each short presentation selected from the submitted abstracts was ten plus 5 minutes. One or two dedicated chairpersons were assigned to each session. Each of the two poster sessions lasted twice 2 hours (afternoon and night). They consisted of 40 posters *i.e.* the maximal capacity of the poster room. In addition, the poster room remained accessible until 1 am for additional presentations and discussions. The program also left ample opportunity for

informal discussions amongst the participants both at lunch time and dinner/post-dinner time.

Scientific program

The first evening was dedicated to a Keynote lecture by **Jody Puglisi** (*Stanford, USA*) who gave an impressive and innovative presentation about the dynamics of translation elongation. Using single molecule fluorescence methods to track directly the dynamics of translation elongation in real time on a codon by codon basis, the approach combined FRET measurements to monitor ribosomal inter-subunit conformation with direct tracking of composition using photonic nanostructures (zero mode waveguides). Jody showed how both tRNA and EF-G arrival and departure are correlated to global conformational states of the ribosome, and gave information on how antibiotics interfere with both conformational and compositional dynamics. The newest data of this talk were actually published two months later in a July issue of Nature (Tsai et al. 2012).

Theme 1: Ribosome-directed protein synthesis

Dynamics in translation: ribosome-mRNA-tRNAs-translation factors

Jody Puglisi and Ada Yonath chaired the session.

The session started with the first of the two Nobel lectures given by **Ada Yonath** (*Rehovot, Israel*); Ada showed that peptide bonds are formed and elongated within a universal semi-symmetrical region connecting all of the remote ribosomal features involved in nascent chain creation and elongation. The high conservation of this region implies its existence irrespective of environmental conditions implying that it may represent an ancient/prebiotic RNA apparatus with bonding capabilities, which turned into peptide bond maker, thus capable of creating oligopeptides. **Pascale Romby**'s (*Strasbourg, France*) talk dealt with translation regulation mediated by structured mRNAs in bacteria. She discussed the role of the six domains of r-protein S1 in the formation of active initiation complexes involving structured and regulated mRNAs. **Petra Van Damme** (*Gent, Belgium*) reported that, in higher eukaryotic cells, and depending on the cell or tissue type and origin, about 5-10% of all identified protein N-termini point to alternative translation events to incorrect assignments of the translation start codon or to alternative splicing. By applying

positional proteomics, more than 1,000 unique alternative protein N-termini could be identified in human cell lines and used to classify them according to their origin. **Måns Ehrenberg** (*Uppsala, Sweden*) discussed he rate-accuracy trade-off in genetic code translation by tRNAs. How $tRNA^{Lys}$ selects its cognate codon (AAA) as compared to all neighboring codons, including the other cognate Lys codon AAG was presented and this approach was extended to several other tRNAs. **Reynald Gillet** (*Rennes, France*) focused on a trans-translation, a quality control process performed by a specialized RNA acting as both a tRNA and an mRNA (tmRNA) associated with small protein B. Using cryo-electron microscopy studies, he addressed the movements of tmRNA-SmpB through a stalled ribosome, and more particularly the conformational changes needed to transit from one ribosomal site to one another. **Bruno Sargueil** (*Paris, France*) presented data on translation initiation and the loading of the ribosome on HIV mRNA. An original molecular mechanism was described and compared to other well-known characterized viral Internal Ribosome Entry Sites. **Franck Martin** (*Strasbourg, France*) showed that histone H4 translation is using a novel and unconventional translation mode, a hybrid between canonical and IRES-driven translation initiation process. Using chemical and UV crosslinking approaches, he described the interplay between the two cis-acting elements from the coding region and eIF4F.

The ribosome at atomic resolution

Thomas Steitz chaired the session. Mans Ehrenberg introduced **Thomas Steitz'** (*New Haven, U.S.A.*) Nobel lecture. Thomas made a spectacular presentation – a show - on the various aspects of his research on ribosome structure, making an overview of the major and most recent data. Using two remarkable movies conceived in his laboratory (available on the web site at Yale) both synchronized with most relevant music, Thomas - also dancing - addressed the many insights into the structural basis of ribosome function in protein synthesis from structural studies of the large ribosomal subunit as well as the 70S bacterial ribosome, and their complexes with substrates, protein factors or antibiotics. **Marat Yusupov** (*Illkirch, France*) discussed the structure of the lower eukaryotic (yeast) ribosome at 3.0Å resolution. This analysis captured the ribosome in two different conformations which are believed to reflect intermediate states in course of mRNA and tRNA translocation. **Daniel Wilson** (*Munich, Germany*) reported a cryo-electron microscopy structure of

the RPP TetM in complex with the 70S ribosome at 7.2 Å resolution. The structure reveals the contacts of TetM with the ribosome, including interaction between the conserved and functionally critical C-terminal extension of TetM and the decoding center of the small subunit. **Nenad Ban** (*Zurich, Switzerland*) reported the crystal structures of the higher eukaryotic ribosome and its insights into the regulation of protein synthesis. This provides detailed structural information on the entire eukaryotic ribosome, revealing novel architectural feature. This offers insights into the various eukaryotic-specific aspects of protein synthesis and ribosome evolution. **Olivier Namy** (*Orsay, France*) provided data of ribosome interacting with frameshifter pseudoknots. This gave for the first time a mechanical explanation of the role of a pseudoknot during both -1 and +1 frameshifting and highlight why -1 and +1 frameshifter pseudoknots cannot be inverted. **Matthieu Gagnon** (*New Haven, U.S.A.*) reported a 3.2 Å resolution crystal structure of the rescue factor YaeJ bound to the *Thermus thermophilus* 70S ribosome in complex with the initiator $tRNAi^{fMet}$ and a short mRNA

Single molecules studies

The session was chaired by Wofgang Baumeister and Nenad Ban.

Thomas Becker (*Munich, Germany*) discussed the issue of termination and ribosome recycling in eukaryotes. Using subnanometer cryo-EM structures of stalled ribosomes in complex with mRNA surveillance factors, he showed that the ribosome undergoes a large scale conformational switch of its central domain, which reaches towards the peptidyl-transferase center when bound to ABCE1. He proposed a common mechanism for ABCE1-mediated ribosome recycling after both canonical termination and stalled ribosome rescue. **Bruno Klaholz** (*Illkirch, France*) visualized the translating ribosome as revealed by multi-scale structural analysis including crystal structures; he also analyzed the 3D organization of polyribosomes by using cryo electron microscopy and single- and double-tilt tomography. A regular inter-ribosome arrangement allowed visualizing the higher-order structure of polysomes at molecular level and the overall topology of the polysome chain of translating ribosomes. **Julio Ortiz** (*Martinsried, Germany*) illustrated how cryo-electron tomography allows the visualization of flexible molecular structures both in vitro and in the functional environment of intact cells. Distinctive spatial organizations for translating and hibernating ribosomes were revealed. He

hypothesized that polysomal organizations disfavor interaction between the non-folded nascent chains avoiding protein misfolding. **Satoko Yoshizawa** (*Gif-sur-Yvette, France*) concluded the meeting on nanotechnologies for translational studies and applications. She discussed one of these technologies, where an on-chip method for generating protein arrays with ultra-high density based on microcompartmentalization of protein synthesis was developed.

Theme 2: Cotranslational events (modifications, targeting and folding)

Cotranslational modifications

This session was chaired by Alexander Varshavsky and Kris Gevaert.

Alexander Varshavsky (*Pasadena, U.S.A.*) gave the second Keynote lecture. His provocative and extremely well-thought talk was remarkable and led to many interesting discussions during the session and at dinner. Alexander reported the current knowledge on the N-end rule which relates the *in vivo* half-life of a protein to the identity of its N-terminal residue using the ubiquitin system. The presentation presented the two branches of the pathway and focused on the newest, the Acetylation/N-end rule pathway which recognizes and destroys intracellular proteins with N-terminally acetylated residues. The proposal that this pathway is part of the early quality control of proteins was made. **Wolfgang Baumeister** (*Martinsried, Germany*) discussed the molecular architecture of the 26S proteasome holocomplex as determined by an integrative approach. The modular structure of the proteasome provides insights into the sequence of events prior to the degradation of ubiquitylated substrate. **Toshifumi Inada** (*Sendai, Japan*) examined how stalled ribosomes induce mRNA and protein quality control systems. This included respectively the endonucleolytic cleavage of mRNA, the No-Go-Decay (NGD) and E3 ubiquitin ligases being involved in the degradation of products of translation arrest induced by poly-Lys and rare codons. **Thierry Meinnel** (*Gif-sur-Yvette, France*) made an overview of cotranslational protein N-alpha-Myristoylation at the proteome scale and dynamic; he showed the most recent data of the team, concluding that it is a more frequent modification than initially expected and that its specific recognition motif is extremely complex and how together with S-palmytoylation govern membrane compartmentalization of proteins in eukaryotes. **Thomas Arnesen** (*Bergen, Norway*) could unfortunately not attend the meeting. **Kris Gevaert** (*Gent, Belgium*) provided,

instead of Thomas, an overall presentation on the biology and roles protein N-terminal acetyltransferases and N-terminal acetylation. He next focused on N-terminomics technologies for studying the nature, the extent and the roles of protein alpha-N-acetylation. A SILAC-based pulse-chase method was set to analyze the stability of the different protein variants in their cellular context by analyzing and quantifying their Nt-acetylated peptides and our preliminary data indeed show differences between the stability of database annotated proteins versus their alternative, shorter variants. **Willy Bienvenut** (*Gif-sur-Yvette, France*) was invited a couple of days before the beginning of the meeting to make a talk instead of Thomas Arnesen. He showed that large-scale characterization of plant N-terminal modifications reveals similar N-alpha-acetylation features between animals and plants but that plants have an additional, specific machinery occurring in the plastid. He also revealed that heavy isotope protein N-terminal acetylation was useful methodology to get large scale quantitation of the N-term acetylation yield. **Markus Wirtz** (*Heidelberg, Germany*) concluded the session by presenting his investigation of the plant NatA system, demonstrating that N-terminal protein acetylation is essential for Arabidopsis. He revealed novel functions of acetylation during organization of multi-cellularity in embryogenesis, in vegetative development and in response to environmental stress.

Dynamics in translation: ribosome-nascent chain-biogenesis factors

This session was chaired by Bernd Bukau and Sabine Rospert

Sabine Rospert (*Freiburg, Germany*) described new aspects relative to nascent-polypeptide associated complex (NAC) a eukaryotic factor which interacts with ribosomes and nascent chains. NAC is required for the timely recruitment of SRP to ribosome nascent chain complexes during the early steps of protein targeting to the ER. Sabine showed that both subunits of NAC directly and tightly interact with hydrophobic signal sequences, shielding them from improper interactions in the cytosol. **Elke Deuerling** (*Konstanz, Germany*) reported that that the Ccr4-Not complex with its deadenylation activity involving Caf1 and its E3-ligase activity displayed by Not4 is specifically recruited to ribosome-nascent chain complexes for cotranslational protein quality control functions. **Nora Vazquez-Laslop** *(Chicago, U.S.A.*) described the shortest nascent peptide able to direct ribosome stalling by systematically shortening the ermDL ORF. Antibiotic-promoted stalling can be

supported by the nascent peptide as short as 'MRL' with the peptidyl-tRNA positioned in the P-site. **John Atkins** (*Cork, Ireland*) reported stimulatory effects of nascent peptides encoded 5' of the shift site in certain "non-yeast" fungi which contrasts with inhibitory effects of a nascent peptide encoded 5' of the shift site in *S. cerevisiae* antizyme mRNA. **Bernd Bukau** (*Heidelberg, Germany*) studied co-translational processes promoting folding of newly synthesized proteins. MAP and PDF enzymes associate with ribosomes in close vicinity to the peptide exit tunnel, binding competitively to partially overlapping sites, demonstrating a sequential action with PDF acting first. These data provide a first understanding of the molecular mechanisms integrating the different co-translational processes leading to the maturation of nascent chains. **Carmela Giglione** (*Gif-sur-Yvette, France*) questioned how these two enzymes – PDF ad MAP - bind to ribosome and dialog with the other cotranslational processing proteins such as TF and METAP. In particular, Carmela reported that bindings of METAP and viral PDF have evidenced the same region of ribosomes in the proximity of the exit tunnel. **Marco Chiabudini** (*Freiburg, Germany*) investigated the role of the ribosome-bound chaperone system consisting of the ribosome associated complex, RAC and the Hsp70 homolog Ssb in the process of translational repression and degradation of nonstop-proteins containing C-terminal polylysine. **Axel Innis** (*New Haven, U.S.A.*) presented data indicative of regulatory nascent peptides in the ribosomal exit tunnel. He established that certain nascent peptide sequences interact with rRNA components and ribosomal proteins lining the walls of the tunnel to bring protein synthesis to a complete halt. He discussed the efforts to elucidate the structural basis of nascent chain-mediated translational arrest.

Cotranslational fate of the protein (targeting and folding)

This session was chaired by Ramanujan Hegde and Thierry Meinnel

Silvia Cavagnero (*Madison, U.S.A.*) focused on the early stages of a protein's life. Silvia discussed (i) the role of the highly charged ribosomal surface on nascent protein conformational sampling and (ii) the real-time kinetics of protein departure from the ribosome, to interpret the meaning of the resulting kinetic phases together with their relevance for protein folding in the cell. **Ramanujan Hegde** (C*ambridge, United Kingdom*) made a wonderful survey presentation, asking the question of chaperoning membrane proteins from the ribosome to their destination. Mammalian

cytosol contains factors that preferentially bind to highly hydrophobic domains. Manu showed that by interfacing with downstream components such as receptors or ubiquitin ligases, each chaperone then imparts a distinct fate to its bound client. These findings reveal the logic of hydrophobic protein sorting and traffic through the cytosol. **Suparna Sanyal** (*Uppsala, Sweden*) revealed that two unrelated anti-prion drugs bind to the 23S/25SrRNA and inhibit specifically the protein folding but not the protein synthesis activity of the ribosome. **Lisa Cabrita** (*London, United Kingdom*) presented the use of NMR spectroscopy on ribosomes and ribosome nascent-chain complexes. This approach is providing detailed structural insights of the conformations of protein chains while they are being created on the ribosome. **Martin Pool** (*Manchester, United Kingdom*) focused on proteins which are destined for secretion. These proteins are targeted to the endoplasmic reticulum by N-terminal signal sequences. He described interplay between N-terminal protein modification and Endoplasmic Reticulum targeting factors at the ribosome exit site. **Zoya Ignatova** (*Potsdam, Germany*) focused on pausing translational elongation to fine-tune protein biogenesis. She suggested that the translation process bears multifaceted options to synchronize and fine-tune translation with various cellular processes. **Wolf Holtkamp** (*Göttingen, Germany*) reported the kinetics of the interaction of SRP with ribosomes in different functional states and with FtsY, as followed by stopped-flow monitoring FRET between fluorophores in SRP and the ribosome. **Véronique Albanese** (*Stanford, U.S.A.*) demonstrated that, in addition to their known cytoplasmic roles in *de novo* protein folding, some ribosome-anchored CLIPS chaperones play a critical role in nuclear steps of ribosome biogenesis.

General comments

115 people (*i.e.* the maximum number of people in the lecture room) attended the conference. This included 26 PhD, 20 Postdoctoral fellows, 25 Researchers, 48 PIs and lab heads. 89 attendees registered; 129 applications were received among which 40 had to be rejected after scientific selection including a balance of themes, topics laboratories, ages, genders and positions of the applicant.

The overall quality of the talks was impressive, and the many questions rose after each talk led to numerous discussions. All 7 oral sessions and 4 poster sessions were very well attended from the first evening to the departure. 76 posters were displayed. The choice of Roscoff for organizing this first conference of a new series was particularly appropriate since it fostered informal, yet rigorous scientific exchanges.

We also need to highlight the remarkable commitment and very nice hosting of the Roscoff CNRS staff members. The high quality of the meals, and the rigorous, timely work of Dominique Lidoreau, before, during and after the Conference were another reason why this conference was a big success, meeting the expectations of the attendees.