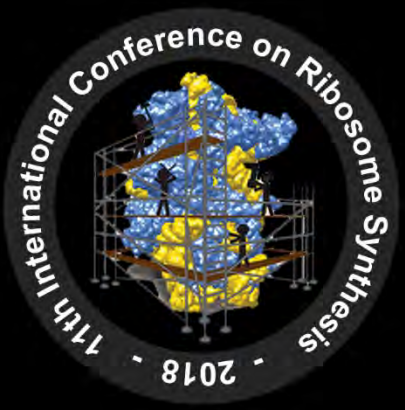

11th International Conference on Ribosome Synthesis

August 1-5, 2018 | Hôtel Manoir des Sables, Orford, Québec, Canada

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11th International Ribosome Synthesis Meeting

Program and Abstracts

August 1st – 5th, 2018

Hôtel Manoir de Sables
Orford, Québec

11th International Ribosome Synthesis Meeting

will be held in Hôtel Manoir de Sables, Orford, Québec from August 1st to August 5th, 2018

Please note that material contained in this booklet should be treated as personal communications and should be cited as such with the author's consent.

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General Information

Visit our website at www.ribosynthesis.riboclub.org You can email us to ribosynthesis@riboclub.org

If you encounter any problems during this event, ask for Leandro Fequino **leandro.fequino@usherbrooke.ca** at the hotel reception.

Oral Presentations

All presenters should see the session chairs at least 30 minutes before the session starts. All presentations should be loaded on the available Mac or PC. A technician will be available on site for file transfer and other logistics.

Please refer to the scientific program for the schedule and the duration of each presentation. All presenters are asked to respect the allocated time. Time will be called if presentations exceed these time limits.

Poster Presentations

You can install your poster from Wednesday August 1st, 2018 at 15:00, and all posters should be removed by Saturday August 4th, 2018 at 13:00. Any necessary equipment for attaching the posters to the board will be available near the poster location. Please use only the provided materials (push pins or Velcro) in order not to damage the panels and to enable a smooth removal afterwards.

Posters MUST be presented in ENGLISH.

No recordings or photographs are allowed to be taken on any talk or poster. This policy will be strictly enforced.

Poster Competition

Posters presented by students or post-doctoral fellows will be evaluated by a team of two judges.

Presentation of the poster will be limited to 10 MINUTES / POSTER (including questions).

The judges will be looking for: Overall presentation; Quality of results; Ability to answer questions; Impact of the work; Length of study versus the amount of results.

All posters should be removed by Saturday August 4th, 2018 at 13:00 h

Poster room: Cherry River

11th Ribosome Synthesis Meeting Committee

Sherif Abou Elela	sherif.abou.elela@usherbrooke.ca
Denis Lafontaine	denis.lafontaine@ulb.ac.be
Tom Meier	tom.meier@einstein.yu.edu
Ute Kothe	ute.kothe@uleth.ca
Joaquin Ortega	joaquin.ortega@mcgill.ca

Keynote Speakers

Ed Hurt	ed.hurt@bzh.uni-heidelberg.de
John Woolford	jw17@andrew.cmu.edu
James Williamson	katrinas@scripps.edu

Session Chairs

Clifford Brangwynne	cbrangwy@princeton.edu
Tom Moss	tom.moss@crhdq.ulaval.ca
Brigitte Pertschy	brigitte.pertschy@uni-graz.at
Claudia Schneider	claudia.schneider@ncl.ac.uk
Rob Britton	robert.britton@bcm.edu
Yi-Tao Yu	yitao_yu@urmc.rochester.edu
Sebastian Klinge	klinge@rockefeller.edu
Roland Beckmann	beckmann@genzentrum.lmu.de
Sharon Savage	savagesh@mail.nih.gov
Jean-Jacques Diaz	jean-jacques.diaz@lyon.unicancer.fr
Lorenzo Montanaro	lorenzo.montanaro@unibo.it
Keqiong Ye	yekeqiong@ibp.ac.cn

Program Schedule

August 1st

15:00 – 17:00 **Registration**

17:00 – 17:10 **Opening Notes** (Salle Champêtre)
Sherif Abou Elela
Université de Sherbrooke, Sherbrooke

Session 1 **Nucleolar organization and dynamics**
Chair: Clifford Brangwynne

17:10 – 17:15 **Introduction**
Clifford Brangwynne
Princeton University, Princeton

17:15 – 17:30 Lighting up the nucleolus
Clifford Brangwynne
Princeton University, Princeton

17:30 – 17:45 Studying ribosome biogenesis and the nucleolar structure of *Chlamydomonas reinhardtii* by in situ cryo-electron tomography
Philipp Sebastian Erdmann (Lab: Baumeister)
Max Planck Institute of Biochemistry, Martinsried

17:45 – 18:00 The path of pre-ribosomes through the nuclear pore complex revealed by electron tomography
Pierre-Emmanuel Gleizes
Centre de Biologie Intégrative, Toulouse

18:00 – 18:15 Engineered rDNA arrays, producing customised ribosomes, reveal the existence of NOR territories within human nucleoli

Hazel Mangan (Lab: McStay)

National University of Ireland, Galway

18:15 – 18:30 A genome-wide RNAi screen for increased nucleolar number reveals new proteins required for ribosome biogenesis

Lisa Ogawa McLean (Lab: Baserga)

Yale University, New Haven

18:30 – 20:00 **Dinner (dining room)**

20:00 – 20:05 **Introduction to keynote speaker**

Denis Lafontaine

Université Libre de Bruxelles, Bruxelles

20:05 – 21:05 Insights into the mechanism of eukaryotic ribosome biogenesis

Ed Hurt - Keynote Lecture I

University of Heidelberg, Heidelberg

21:05 – 01:00 **Get Together (Salle Albatros)**

August 2nd

07:00 – 08:30 **Breakfast (dining room)**

Session 2 **Ribosomal RNA Synthesis**
Chair: Tom Moss

08:30 – 08:35 **Introduction**
Tom Moss
Université Laval, Québec

08:35 – 08:50 Organisation of ribosomal gene chromatin in mouse and human
Tom Moss
Université Laval, Québec

08:50 – 09:05 Dissecting the chromosomal basis for selective ribosomal RNA gene silencing
Craig Pikaard
Indiana University, Bloomington

09:05 – 09:20 The novel roles of histone demethylase KDM4A
Konstantin Panov
Queen's University Belfast, Belfast

09:20 – 09:35 Genetic analysis of RNA polymerase I allowed isolation of alleles leading to over-production of rRNA transcripts
Olivier Gadal
Toulouse University, Toulouse

09:35 – 09:50 Haemanthamine: a powerful drug to inhibit protein synthesis and ribosome assembly processes

Simone Pellegrino (Lab: Yusupov)

IGBMC- CERBM, Strasbourg

09:50 – 10:05 RNA polymerase I therapy: the second generation

Kate Hannan

Australian National University, Acton

10:05 – 10:20 The C-terminal region of *S. cerevisiae* Net1 is a potent activator of RNA polymerase I transcription in vivo and in vitro

Herbert Tschochner

University of Regensburg, Regensburg

10:20 – 10:50 **Coffee break**

Session 3 **Subunit Assembly I**

Chair: Brigitte Pertschy

10:50 – 10:55 **Introduction**

Brigitte Pertschy

University of Graz, Graz

10:55 – 11:10 Assembly, structure and function of the small subunit processome

Sebastian Klinge

The Rockefeller University, New York

11:10 – 11:25 Distinct maturation steps of the human 40S subunit revealed by comprehensive analysis of preribosome composition & compartmentalization

Mercedes Dosil

University of Salamanca, Salamanca

11:25 – 11:40 Coordination of distant 40S ribosomal subunit maturation events

Brigitte Pertschy

University of Graz, Graz

11:40 – 11:55 A novel role of the DUB USP16 in RPS27a/eS31 deubiquitination and 40S subunit maturation

Christian Montellese (Lab: Kutay)

Institute of Biochemistry ETHZ, Zurich

11:55 – 12:10 A dynamic view on ribosome biogenesis

Helmut Bergler

Institute of Molecular Biosciences, Graz

12:10 – 12:25 ATPase activity of the AAA-protein NVL2 regulates its communication with WDR74 during ribosome biogenesis

Robin Evans Stanley

NIEHS/NIH, Durham

12:30 – 14:15 **Networking Lunch (dining room)**

Session 4 **Ribosomal RNA processing**

Chair: Claudia Schneider

14:15 – 14:20 **Introduction**

Claudia Schneider

Newcastle University, Framlington Place

14:20 – 14:35 Adjacent gene co-regulation (AGC) functions in the transcriptional control of ribosome biogenesis genes

Michael McAlear

Wesleyan University, Middletown

14:35 – 14:50 RIOK2 phosphorylation by RSK contributes to the synthesis of the human 40S ribosomal subunit

Yves Romeo

University of Toulouse, Toulouse

14:50 – 15:05 The kinase Rio1 regulates the release of Pno1 and 18S rRNA processing during pre-40S ribosome assembly

Melissa Danielle Parker (Lab: Karbstein)

The Scripps Research Institute, Jupiter

15:05 – 15:20 Characterization of the molecular crosstalk within the essential Grc3/Las1 pre-rRNA processing complex

Monica Pillon (Lab: Stanley)

NIEHS/NIH, Durham

15:20 – 15:35 The nuclear export proteins TbMex67 and TbMtr2 make unique interactions with the 5S RNP component of the 60S ribosomal subunit in *Trypanosoma brucei*

Constance Rink (Lab: Williams)

University at Buffalo, Buffalo

15:35 – 16:05 **Coffee break**

Session 5 **Prokaryotic & Organelle Ribosome Assembly I**

Chair: Rob Britton

16:05 – 16:10 **Introduction**

Rob Britton

Baylor College of Medicine, Houston

16:10 – 16:25 Circular-pre-ribosomal RNAs: archaeal specific pre-rRNA intermediates required for the efficient synthesis of mature rRNAs?
Sébastien Ferreira-Cerca
University of Regensburg, Regensburg

16:25 – 16:40 Single methylation of 23S rRNA regulates late steps of 50S ribosomal subunit assembly by sensing cellular AdoMet concentration
Kensuke Ishiguro (Lab: Suzuki)
University of Tokyo, Tokyo

16:40 – 16:55 Role of the conserved GTPase BipA in assembly of the 50S subunit of the ribosome
Michelle Gibbs (Lab: Fredrick)
The Ohio State University, Columbus

16:55 – 17:10 Assembly factors mediated biogenesis of large subunit of ribosome in prokaryotes
Nikhil Jain (Lab: Britton)
Baylor College of Medicine, Houston

17:10 – 17:25 **Break**

17:25 – 17:30 **Introduction to keynote speaker**
Tom Meier
Albert Einstein College of Medicine, Bronx

17:30 – 18:30 An historical perspective on ribosome biogenesis
John Woolford - Keynote Lecture II
Carnegie Mellon University, Pittsburgh

18:30 – 20:00 **Dinner** (dining room)

20:00 – 22:30 **Poster Session I** (Salle Cherry River)

22:30 – 01:00 **Get Together** (Salle Albatros)

August 3rd

07:00 – 08:30 **Breakfast** (dining room)

Session 6 **snoRNP biogenesis and RNA modification**
Chair: Yi-Tao Yu

08:30 – 08:35 **Introduction**
Yi-Tao Yu
University of Rochester Medical Center, Rochester

08:35 – 08:50 Guide-substrate base-pairing requirement for box H/ACA RNA-guided RNA pseudouridylation
Yi-Tao Yu
University of Rochester Medical Center, Rochester

08:50 – 09:05 Substrate and guide RNA specificity of H/ACA small nucleolar ribonucleoproteins
Ute Kothe
University of Lethbridge, Lethbridge

09:05 – 09:20 Identification of non-annotated snoRNA using a structure insensitive sequencing pipeline
Vincent Boivin (Lab: Scott)
Université de Sherbrooke, Sherbrooke

09:20 – 09:35 Fine-tuning of rRNA 2'-O-methylation by non-snoRNP factors
Nicholas James Watkins
Newcastle University, Newcastle

09:35 – 09:50 Nopp140 knockdown depletes scaRNPs from Cajal bodies impairing spliceosomal snRNA modification and promoting telomere lengthening

Tom Meier

Albert Einstein College of Medicine, Bronx

09:50 – 10:05 Structural studies of the snoRNP assembly machine reveal a family of quaternary chaperones

Edouard Bertrand

Institut de Génétique moléculaire de Montpellier, Montpellier

10:05 – 10:35 **Coffee break**

Session 7 **Subunit Assembly II**

Chair: Sebastian Klinge

10:35 – 10:40 **Introduction**

Sebastian Klinge

The Rockefeller University, New York

10:40 – 10:55 Towards a comprehensive landscape of 60S ribosomal biogenesis

Carolin Sailer (Lab: Stengel)

University of Konstanz, Konstanz

10:55 – 11:10 Puf6 directs correct RNA folding during ribosome assembly

Vikram Govind Panse

University of Zurich, Zurich

11:10 – 11:25 The yeast Npa1 complex is involved in the compaction of the 25S rRNA central core in the earliest pre-60S particles

Anthony Henras (Lab: Henry)

University of Toulouse, Toulouse

11:25 – 11:40 Diverse roles of RNA helicases in driving structural transitions and compositional changes in pre-ribosomal complexes

Markus Bohnsack

University Medical Center Göttingen, Göttingen

11:40 – 11:55 Two distinct domains of Nog1 contribute to the assembly of the peptide exit tunnel and the peptidyl transferase center

Amber Joy LaPeruta (Lab: Woolford)

Carnegie Mellon University, Pittsburgh

11:55 – 12:10 Ribosomal protein L1 is required for efficient nuclear export of nascent large subunit

Sharmishtha Musalgaonkar (Lab: Johnson)

University of Texas at Austin, Austin

12:15 – 14:00 **Lunch** (dining room)

13:30 – 14:00 **Business meeting**

14:00 – 18:00 **Free afternoon** (sport activities, hikes, sight-seeing) (Call the hotel for reservations)

18:00 – 20:00 **BBQ Dinner**

20:00 – 22:30 **Poster Session II** (Salle Cherry River)

22:30 – 01:00 **Get Together** (Salle Albatros)

August 4th

07:00 – 08:30 **Breakfast** (dining room)

Session 8 **CryoEM and other advances**
Chair: Roland Beckmann

08:30 – 08:35 **Introduction**
Roland Beckmann
Munich University, Munich

08:35 – 08:50 Multi-particle cryo-EM of eukaryotic ribosome assembly intermediates
Roland Beckmann
Munich University, Munich

08:50 – 09:05 Subunit joining exposes nascent pre-40S rRNA for processing and quality control
Katrin Karbstein
The Scripps Research Institute, Jupiter

09:05 – 09:20 Moving is maturing: cryo-EM structures of cytoplasmic pre-40S particles unveil chronology of late maturation events
Celia Plisson-Chastang
Centre de Biologie Intégrative, Toulouse

09:20 – 09:35 A regulatory system involving the Hsf1 and Ifh1 transcription factors monitors ribosome biogenesis and promotes ribosomal protein homeostasis
David Shore
University of Geneva, Geneva

09:35 – 09:50 Visualizing late cytoplasmic 60S subunit maturation

Alan Warren

University of Cambridge, Cambridge

09:50 – 10:05 Using cryo-EM to reveal the role of Era in the assembly of the bacterial 30S subunit

Joaquin Ortega

McGill University, Montreal

10:05 – 10:35 **Coffee Break**

Session 9 **Ribosomopathies I**

Chair: Sharon Savage

10:35 – 10:40 **Introduction**

Sharon Savage

National Cancer Institute, Bethesda

10:40 – 10:55 Neuronal ribosomal protein function regulates *Drosophila* growth and development

Lisa Deliu (Lab: Grewal)

University of Calgary, Calgary

10:55 – 11:10 SSU production defects activate p53 via the 5S RNP through stalling LSU maturation

Matthew Eastham (Lab: Schneider)

Newcastle University, Newcastle

11:10 – 11:25 The genetics and clinical manifestations of human ribosome biology disorders

Sharon Savage

National Cancer Institute, Bethesda

11:25 – 11:40 Identification of therapeutic targets for the treatment of Diamond Blackfan Anaemia using a high-throughput screening based approach

Amee George (Lab: Hannan)

Australian National University, Acton

11:40 – 11:55 A novel pathomechanism: are Cockayne syndrome and trichothiodystrophy ribosomopathies?

Sebastian Iben

University of Ulm, Ulm

12:00 – 14:00 **Lunch / Organizer Meeting (dining room)**

Session 10 **Ribosome variants and alternative biogenesis pathways**

Chair: Jean-Jacques Diaz

Session sponsored by The Alberta Epigenetics Network

14:00 – 14:05 Greetings

Raja Singh

The Alberta Epigenetics Network

14:05 – 14:10 **Introduction**

Jean-Jacques Diaz

Centre de Recherche en Cancérologie de Lyon, Lyon

14:10 – 14:25 Determining differential translatomes and composition of 5.8S S and L ribosome variants in *S. cerevisiae*

Christian Trahan (Lab: Oeffinger)

IRCM, Montréal

14:25 – 14:40 The involvement of two intersubunit ribosomal RNA base modifications in the differential translation of stress-response transcripts

Felix Ernst (Lab: Lafontaine)

Université Libre de Bruxelles, Bruxelles

14:40 – 14:55 Specialized ribosomal protein genes regulate ribosome biogenesis and function

Malik Ghulam Mustafa (Lab: Abou Elela)

Université de Sherbrooke, Sherbrooke

14:55 – 15:10 A role for eRpL22 and eRpL22-like paralogue-specific specialized ribosomes in differential translation of distinct classes of testis mRNAs in *Drosophila melanogaster*

Vassie Ware

Lehigh University, Bethlehem

15:10 – 15:25 **Break**

Session 11 **Ribosomopathies II**

Chair: Lorenzo Montanaro

15:25 – 15:30 **Introduction**

Lorenzo Montanaro

Università di Bologna, Bologna

15:30 – 15:45 Ribosomal lesions promote oncogenic mutagenesis

Sergey Sulima (Lab: De Keersmaecker)

University of Leuven, Leuven

15:45 – 16:00 An imbalance in the numbers of the two ribosomal subunits differentially affects subunit stability, but not co-regulation of transcription of r-protein genes from both subunits

Lasse Lindahl

University of Maryland, Baltimore

16:00 – 16:15 Ribosomal protein gene mutations activate a novel regulatory pathway which regulates the translation, the growth and the competitiveness of the cell

Marianthi Kiparaki (Lab: Baker)

Albert Einstein College of Medicine, Bronx

16:15 – 16:30 Combined evaluation of JmjC enzymes KDM2A and KDM2B is associated with nucleolar size and prognosis in primary breast carcinomas

Lorenzo Montanaro

Università di Bologna, Bologna

16:30 – 17:00 **Coffee Break**

17:00 – 17:10 **Poster Awards**

17:10 – 17:15 **Introduction to keynote speaker**

Joaquin Ortega

McGill University, Montreal

17:15 – 18:15 Biophysical and structural studies of bacterial ribosome assembly

James Williamson - Keynote Lecture III

The Scripps Research Institute, La Jolla

18:15 – 20:00 **Cocktail and Music Show** (lobby)

20:00 – 22:30 **Banquet** (Salle Champêtre)

22:30 – 01:00 **Dance** (Salle Albatros)

August 5th

07:00 – 09:30 **Breakfast** (dining room)

Session 12 **Prokaryotic & Organelle Ribosome Assembly II**
Chair: Keqiong Ye

09:30 – 09:35 **Introduction**
Keqiong Ye
Institute of Biophysics - CAS, Beijing

09:35 – 09:50 Early co-transcriptional ribosome assembly in real-time
Olivier Duss (Lab: Williamson)
The Scripps Research Institute, La Jolla

09:50 – 10:05 Altered conformations of *E. coli* ribosomes during starvation
Sarah Woodson
Johns Hopkins University, Baltimore

10:05 – 10:20 The functional core of 5' external transcribed spacer of pre-rRNA
Keqiong Ye
Institute of Biophysics - CAS, Beijing

10:20 – 10:35 Crucial roles of P-site binding of initiator tRNA in fidelity of translation initiation and regulation of ribosome maturation/heterogeneity in *Escherichia coli*
Umesh Varshney
Indian Institute of Science, Bangalore

10:20 – 10:35 **Closing Notes**
Sherif Abou Elela
Université de Sherbrooke, Sherbrooke

11:00 **Departure**

Abstracts for Oral Presentation

Session 1: Nucleolar organization and dynamics

Lighting up the nucleolus

Clifford Brangwynne

Princeton University, Princeton, USA

In this talk I will discuss our work showing that phase transitions play an important role in organizing membrane-less RNA and protein rich condensates, which help control the flow of genetic information within cells. The nucleolus is one such nuclear RNP body that we have focused on, which is important for cell growth and size homeostasis. We've shown that a phase transition model explains many features of nucleolar assembly, and that the internal subcompartments of the nucleolus arise from multi-phase coexistence, which may have important consequences for sequential RNA processing. I will also discuss our new "Optodroplet" approaches, which use light to enable spatiotemporal control of phase transitions within living cells. We are now using these approaches to quantitatively map intracellular phase diagrams. This approach has begun to yield insights into the link between the material state of the nucleolus and its role in rRNA processing.

Studying ribosome biogenesis and the nucleolar structure of *Chlamydomonas reinhardtii* by in situ cryo-electron tomography

Philipp Sebastian Erdmann, Jürgen Plitzko, Wolfgang Baumeister

Max Planck Institute of Biochemistry, Martinsried, Germany

The nucleolus is the most prominent subnuclear structure and the birthplace of ribosomes. In recent years, single particle cryo electron microscopy (cryo EM) in particular has helped grow our understanding of the cellular processes of ribosome biogenesis at a molecular level and has provided structures of both the large (LSU) and small subunit (SSU) precursors with magnificent detail. In addition, the structure of the pre 90S particle (also known as the SSU processome), which is the earliest known ribosomal precursor, has recently been solved independently for two different species (C. thermophilum and S. cerevisiae).

While there is much to be learned from these structures, we are still lacking important information of ribosome biogenesis, including the physiological context of the pre 90S and how its maturation and degradation of recycling intermediates is related to the spatial organization within the nucleolus/nucleus. However, single particle cryo-EM cannot provide this information, as it requires homogenous preparations, and accordingly all information on the native environment of the ribosome precursors is lost.

Trying to address this problem, we combined focused ion beam milling with in situ cryo-electron tomography to image the native molecular environment of the nucleolus in the model organism Chlamydomonas reinhardtii. Using this approach, we were able to observe snapshots of ribosome biogenesis in intact cells and in a close-to life state at high resolution. We here present our findings, including the intricate arrangement of ribosome precursors within the nucleolus and the first in situ averages of the small subunit processome (SSU), as well as nucleolar pre 60S particle of C. reinhardtii.

The path of pre-ribosomes through the nuclear pore complex revealed by electron tomography

Franck Delavoie, Vanessa Soldan, Dana Rinaldi, Jean-Yves Dauxois, Pierre-Emmanuel Gleizes

Center for Integrative Biology (CBI), Institute of Mathematics (IMT), University of Toulouse, CNRS, Toulouse, France

Nuclear export of pre-ribosomes is a key step in their maturation pathway that is intimately coordinated with RNA processing and ribosomal protein assembly. While impressive progress has been recently made on the structure of the nuclear pore complex (NPC), the translocation path of pre-ribosomes, or of any RNP, through the NPC remains poorly defined. Indeed, observing the translocation of single RNP particles through the 100 nm-wide nuclear pore complex remains very challenging due to resolution limitation and RNP labelling constraints. Here, by conjugating the speed of high-pressure freezing with the nanometer-scale resolution of electron tomography, we captured snapshots of native preribosomes translocating through NPCs in yeast. Large globular RNP particles were observed in 5-6% of the NPCs present in the tomograms. These particles were identified as pre-ribosomes based on their morphology and their absence in NPCs upon inhibition of RNA polymerase I. From these 3D-views, we not only quantitatively analyzed the trajectory of pre-ribosomes within the NPC, but also infered dynamic parameters of pre-ribosome nuclear export using a probabilistic model. We found that pre-ribosomes cross the nuclear and inner rings of the NPC following its central axis, but diverge from this axial path at the cytoplasmic exit, consistent with the structure of the NPC and the position of the nucleoporins important for pre-ribosome nuclear export. Application of a probabilistic queueing model to these data led to an estimation of the average translocation time of 90 ms. Interestingly, the distribution of pre-ribosomes showed no bias for NPCs adjacent to the nucleolus, suggesting that all NPCs are equally competent for pre-ribosome nuclear export. These observations by electron tomography show that pre-ribosomal particles are funneled through the center of the NPC without extensive structural remodeling and provide a direct approach to challenge structural and biophysical models describing the translocation of preribosomes through the NPC.

Engineered rDNA arrays, producing customised ribosomes, reveal the existence of NOR territories within human nucleoli

Hazel Mangan and Brian McStay

National University of Ireland, Galway, Ireland

Human ribosomal gene (rDNA) arrays or nucleolar organiser regions (NORs) are situated on the unsequenced p-arms of each acrocentric chromosome (HSA13, 14, 15, 21 and 22). We are only now beginning to understand their genomic architecture. After cell division, nucleoli form around individual active NORs. Subsequently, a poorly understood fusion event leads to the formation of mature nucleoli comprising multiple NORs and nucleolar-associated heterochromatin. As rDNA repeats between individual NORS are indistinguishable, how multiple NORs are organised within large mature nucleoli is unknown.

In order to address this important issue, we have developed a workflow in which rDNA repeats within a single NOR are genetically tagged. This is achieved by performing genome editing of NORs on single human acrocentric chromosomes held within mouse A9 cells. Chromosomes with engineered NORs are then transferred to a human cell line by microcell

mediated chromosome transfer (MMCT). In this context, the engineered NOR is reactivated and its products can be identified by probes that recognise the tags. Tags located within the 5' external transcribed spacer (5'ETS) and 28S rRNA encoding sequences allow us to probe where the early and late events, respectively, in ribosome biogenesis occur within large fused nucleoli. Strikingly, we observe that all the stages in the biogenesis of ribosomes derived from a single NOR occur within a distinct nucleolar territory. Establishment of this territory in underpinned by the NOR and its surrounding genomic architecture. While topologically associated domains (TADs) prevent entanglement in most of the genome, we argue that NOR territories prevent rDNA entanglement and promote rDNA genomic stability.

Finally, the work flow we have developed provides both capabilities in probing ribosome structure and production of customised ribosomes.

A genome-wide RNAi screen for increased nucleolar number reveals new proteins required for ribosome biogenesis

Lisa M. Ogawa McLean, Kathleen L. McCann and Susan J. Baserga

Yale University and the Yale School of Medicine, New Haven, USA

Ribosome biogenesis initiates in the nucleolus from 10 distinct genomic loci, yet, 10 nucleoli are rarely observed. In the MCF10A human breast epithelial cell line we observe on average 2-3 nucleoli per nucleus; however, intriguingly we have shown that siRNA depletion of factors required for ribosome biogenesis cause a decrease in this number from 2-3 to 1 (Freed et al., 2012). This observation led to a genome-wide siRNA screening campaign to identify novel proteins required for making ribosomes in human cells (Farley-Barnes et al., 2018). While the published work focused on proteins revealed by the one nucleolus phenotype, this screen also uncovered hits that cause an increase in nucleolar number to 5 or more (unpublished). Focused on this 5+ phenotype, we screened 18,017 genes and identified 186 significant hits. Hits were then filtered by expression and viability leaving 103 proteins, none of which overlapped with the one nucleolus hits. Of these proteins, 20% localize to the nucleolus and Ingenuity Pathway Analysis revealed functional associations with cancer, cell cycle, development, cellular assembly and organization, and DNA replication and repair. Interestingly, while ribosomal proteins were absent, the screen did identify known ribosomal DNA (rDNA) transcription-associated factors (TAF1D; SUV39H1), a 60S export factor (MDN1), and proteins identified by other screens for human ribosome biogenesis factors (ABCE1; CDCA8; TOPBP1; DYNC1H1). Preliminary data on 8 nucleolar hits reveal that while all 8 hits significantly affect global protein synthesis based on a puromycin incorporation assay ($n=3$, $p<0.05$), only 3 affect transcription of the pre-rRNA (INCENP; CDCA8; RFC1; $n=3$, $p<0.01$), and just 1 may affect pre-rRNA processing (MDN1; $n=1$). Thus, while follow-up on the one nucleolus hits revealed proteins required for pre-rRNA transcription and processing, the hits from the 5+ screen may instead be associated with other steps in ribosome biogenesis, or more broadly associated with nucleolar structure and function through repair of the rDNA, chromatin organization post-mitosis, or the structural integrity of the nucleolus itself, and therefore requires further analysis. The importance of this study thus lies in the putative identification of new proteins required for human ribosome biogenesis, but also in elucidating potential mechanisms required to maintain nucleolar structural integrity.

Session 2: Ribosomal RNA Synthesis

Organisation of ribosomal gene chromatin in mouse and human

Jean-Clement Mars[1,2], Marianne Sabourin-Felix[1,2], Michel G. Tremblay[1] and Tom Moss[1,2]

1 - Laboratory of Growth and Development, St-Patrick Research Group in Basic Oncology, Cancer Division of the Quebec University Hospital Research Centre, Québec, QC, Canada.
2 - Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Laval University, Québec, QC, Canada

To better understand ribosomal RNA (rRNA) gene chromatin and the role it plays in gene activity, we have used ChIP-Seq, Deconvolution and DNA methylation mapping, psoralen X-linking, metabolic labeling, molecular imaging in combination with Mouse Embryonic Fibroblasts (MEFs) and Embryonic Stem Cells (mESCs) conditional for the factors UBF, RRN3 and TAF1B. These have allowed us to reveal factor interdependences and disassembly hierarchies, and transcription related changes in rRNA gene chromatin and its dependence on cell type. The combined data strongly suggest that UBF is the key factor in establishing and maintaining rDNA repeat activity, and the subsequent recruitment of the SL1 TBP-TAF and the RPI-RRN3 complex. Release of RRN3 from the elongating transcription complex occurs stochastically, but varies with cell type and is a target of the inhibitor CX5461. Arrest of RPI complexes at the Enhancer associated “Spacer Promoter” and the formation of a CTCF Enhancer Boundary Complex are conserved between human and mouse, and provide an important insight into the organization and maintenance of rRNA gene activity. The discovery of a UBF ribosomopathy provides us a further window into the potential genome-wide functions of this factor. Together our data provide a framework from which to understand the mechanisms regulating rRNA gene activity.

Dissecting the chromosomal basis for selective ribosomal RNA gene silencing

Dingxia Feng, Chinmayi Chandrasekhara, Gireesha Mohannath, Anastasia Gridasova McKinlay, Dalen Fultz and Craig S. Pikaard

Howard Hughes Medical Institute, Department of Biology, Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN, USA

Eukaryotes can have thousands of RNA Polymerase I-transcribed rRNA genes in their genomes, exceeding the number needed to meet the physiological demands of most somatic cells. As a result, the number of rRNA genes that are active is regulated based on cellular demands for ribosomes. The classic epigenetic phenomenon, nucleolar dominance, which describes the expression of rRNA genes inherited from only one progenitor of a genetic hybrid, is one manifestation of this dosage control system.

In mammals and plants, dosage control is achieved by selectively silencing subsets of rRNA genes via changes in DNA methylation and repressive histone modifications. However, the "choice" mechanisms by which nearly identical rRNA genes are selectively silenced are unclear. Studying Arabidopsis thaliana, we have evidence that rRNA gene silencing is not regulated at the level of individual genes but occurs on a larger scale. In *A. thaliana*, as in all eukaryotes, rRNA genes are tandemly arrayed in hundreds of copies at nucleolus organizer regions (NORs). There are two NORs in *A. thaliana,* each ~4Mbp in size; one onchromosome 2 (*NOR2*) and one on chromosome 4 (*NOR4*). Based on SNPs

and other subtle variation, we defined more than a dozen rRNA gene sequence variants in the accession (strain) Col-0. Half are selectively silenced during development and half are constitutively expressed. Exploiting natural variation for rRNA gene subtypes in the species, we conducted genetic experiments that revealed that the silenced rRNA genes of Col-0 map to *NOR2*whereas active genes map to *NOR4*. Moreover, in plants in which the distal portion of *NOR4* is replaced by *NOR2*sequences, the translocated genes derived from *NOR2*escape silencing. Collectively, our results implicate NORs as the units of regulation, with NOR silencing dependent on undefined chromosomal position effects. Using genetics and chromosome engineering, including targeted deletions and reciprocal translocations moving NORs to other chromosomes, we are defining the chromosome intervals required for *NOR4* dominance over *NOR2*.

The novel roles of histone demethylase KDM4A

Kader Salifou[1], Swagat Ray[2,3], Laure Verrier[1], Marion Aguirrebengoa[1], Didier Trouche[1], Marie Vandromme[1], Tatiana Panova[2], Ross Hannan[4], Konstantin I. Panov[2]

1 - Université de Toulouse and CNRS, Toulouse, France
2 - CCRCB and School of Biological Sciences, Queen's University Belfast, Belfast, UK
3 - Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK
4 - JCMSR, Australian National University, Canberra, Australia

The human genome contains around 350 copies of rDNA repeats encoding rRNA, which exist in three distinct chromatin states: epigenetically silenced heterochromatin and two different forms of euchromatin: non-transcribed, "poised" chromatin and transcribed "active" chromatin. Various stresses (starvation, DNA damage, hypoxia et cetera) lead to accumulation of "poised" form whereas post-stress recovery requires opposite changes. Importantly, rRNA transcription is sensitive to a variety of different stresses that makes it an exceptionally convenient model to study the links between epigenetic mechanisms controlling transcription and signalling pathways activated by specific stimuli (i.e. a stress or a recovery from a stress).

Using this model, we recently discovered a novel role of the histone demethylase KDM4A in converting "poised" rDNA chromatin into its "active" form. We also found that KDM4A association with rDNA is controlled through PI3K/SGK1 signalling pathway and KDM4A stability is regulated in a nutrient dependent manner, via an as yet unidentified signalling pathway.

In subsequent experiments we found that, similar to starvation, various types of DNA damage and heat shock also led to dissociation of KDM4A from rDNA and its reduced stability correlated with down-regulation of rRNA synthesis. Conversely, KDM4A stabilised and relocalised back to the nucleolus during recovery from a stress, correlating with increased levels of Pol-I transcription. Here we will discuss our hypothesis that KDM4A is a master regulator that controls stress and post-stress related changes in rDNA chromatin structure, thus affecting rRNA transcription levels as well as stress related transcription of other genes.

Genetic analysis of RNA polymerase I allowed isolation of alleles leading to over-production of rRNA transcripts

Tommy Darrière[1], Marie-Kerguelen Sarthou[1], Michael Pilsl[2], Adrien Chauvier[1], Titouan Genty[1], Sylvain Audibert[1], Isabelle Leger-Silvestre[1], Christophe Normand[1], Olga Calvo[3], Carlos Fernández-Tornero[4], Herbert Tschochner[2], Christophe Dez[1] and Olivier Gadal[1]

1 - Laboratoire de Biologie Moléculaire Eucaryote, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31000, Toulouse, France
2 - Universität Regensburg, Biochemie-Zentrum Regensburg (BZR), Lehrstuhl Biochemie III, 93053 Regensburg, Germany
3 - Instituto de Biología Funcional y Genómica, IBFG-CSIC, Universidad de Salamanca, Zacarías González 2, 37007 Salamanca, Spain
4 - Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain

Most transcriptional activity of exponentially growing cells is carried out by RNA Polymerase I (Pol I), which produces the large rRNA precursor. The Pol I transcription cycle is achieved through complex structural rearrangements of the enzyme, revealed by recent structural studies. In the yeast *S. cerevisiae* the Pol 1 subunit Rpa49 supports both initiation and elongation. Here, we show that processivity defect of Pol I when lacking Rpa49 results in the accumulation of specific rRNA abortive transcripts. We characterized novel extragenic suppressors of the growth defect caused by the absence of Rpa49. Suppressor mutants restored normal rRNA synthesis, corrected Pol I initiation rate on rDNA genes, but not the processivity defect observed in the absence of Rpa49; Most mutated residues cluster at an interface formed by the jaw in Rpa190, the lobe in Rpa135, and subunit Rpa12 when mapped on the structure of Pol I. Surprisingly, in presence of Rpa49 subunit, Pol I bearing suppressor allleles are exquisitely sensitive to exosome mutations, and massively accumulate pre-rRNA. We propose that our genetic analysis allowed the identification of super-active alleles of Pol I enzyme.

Haemanthamine: a powerful drug to inhibit protein synthesis and ribosome assembly processes

Pellegrino S., Meyer M., Zorbas C., Bouchta S.A., Saraf K., Pelly S.C., Yusupova G., Evidente A., Mathieu V., Kornienko A., Lafontaine D.L.J., Yusupov M.

IGBMC- CERBM, Strasbourg, France

The ribosome is the machinery responsible for protein biosynthesis in all kingdoms of life. The inhibition of its function is one of the most common way used to treat bacterial infections. Similarly, it has been shown that small molecules possessing anti-cancer activities can target and inhibit the eukaryotic ribosome. Our laboratory recently solved the X-ray structure of the eukaryotic ribosome of *Saccharomyces cerevisiae* 80S ribosome (1), providing an outstanding model for the understanding of the mechanism of protein synthesis inhibition in eukaryotes. We present here the crystal structure of the drug haemanthamine (HAE), extracted from the *Narcissus* flowers (commonly known as 'Daffodils'), in complex with the 80S ribosome. The compound binds to the peptidyl transferase center to block the elongation phase of translation. By comparison with other related compounds (so-called *Amaryllidaceae* alkaloids) haemanthamine uses a highly specific and unique mode of interaction with the ribosome. Surprisingly, we tested HAE and

other compounds binding to the same pocket and observed a direct effect of HAE in inhibition of early stages of ribosome biogenesis, more precisely pre-rRNA processing events. Strikingly, this was accompanied by an activation of nucleolar stress and the stabilization of the anti-tumor protein p53. These data show for the first time that an inhibitor of protein synthesis can also specifically affect the process of assembly, thus proving to be a multi-tasking effector (2).

(1) Ben-Shem et al., *Science* (2011); (2) Pellegrino et al., *Structure* (2018)

RNA polymerase I therapy: the second generation

Katherine M. Hannan[1,2,3],Nadine Hein[1], Denis Drygin[4] and Ross D. Hannan[1,2,3,5,6]

1 - ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, ANU, ACT, Australia
2 - Division of Cancer Research, Peter MacCallum Cancer Centre, Victoria, Australia
3 - Department of Biochemistry and Molecular Biology, Uni. of Melbourne, Victoria, Australia
4 - Pimera, Inc, San Diego, CA, USA
5 - Department of Biochemistry and Molecular Biology, Monash Uni., Victoria, Australia
6 - School of Medical Sciences, Uni. of Queensland, Queensland, Australia

Despite the overwhelming evidence of dysregulated RNA Polymerase I (Pol I) transcription in cancer, only 1 selective Pol I transcription inhibitor, CX5461, has entered clinical trials. While pioneering and promising, CX-5461 is associated with additional activities (e.g., Top2a inhibition), which possibly contribute to its efficacy, toxicity profiles and acquired resistance mechanisms.

To address the need for new, improved Pol I inhibitors we have developed a series of orally available 2ndgeneration selective inhibitors with improved toxicology, tissue distribution (penetration of the blood brain barrier), lower plasma protein binding and higher efficacy compared to CX-5461. Preliminary studies on our new lead compound, PMR-116, has demonstrated improved survival administrated at a maximal tolerated dose (MTD:≥300 mg/kg) in murine models of acute myeloid leukemia and B-cell lymphoma. PMR-116 is a selective inhibitor of Pol I transcription**,** with ~200x more selectivity for Pol I vs Pol II. It has a spectrum of activity on viability across a panel of cancer (GI50 ~280nM) and normal (~5μM) cell lines, which was not due to variable rDNA transcription inhibition, thus indicates a clear therapeutic window. Preliminary studies demonstrate that PMR-116 impairs Pol I recruitment to the rDNA repeat in a similar fashion to CX-5461. Most importantly, however, under equivalent IC50 (50% inhibition of rDNA transcription) doses PMR-116 does not activate the DNA damage response pathway observed for CX-5461 (no activation of Chk2). Thus, unlike CX-5461, PMR-116 may not interfere with Top2a function, a significant distinguishing characteristic between these two drugs. We have also completed an investigational new drug-enabling toxicology program which demonstrated that PMR-116 has a high orally bioavailability (82%) and is well tolerated. This approach of mechanistic and preclinical studies is not only critical to develop better clinical compounds, but will likely further expand our knowledge on the mechanism of Pol I dysregulation and drive the clinical potential of this entire drug class.

The C-terminal region of S. cerevisiae Net1 is a potent activator of RNA polymerase I transcription in vivo and in vitro

Katharina Hannig, Ulrike Stöckl, Andreas Maier, Michael Pilsl, Shuang Li, Virginia Babl, Kristin Hergert, Christopher Schächner, Philipp Milkereit, Wolfgang Seufert, Joachim Griesenbeck and Herbert Tschochner

Universität Regensburg, Institut für Biochemie, Genetik und Mikrobiologie, Regensburg, Germany

Eukaryotic RNA polymerase I (Pol I) transcribes ribosomal DNA (rDNA) producing a precursor of the large ribosomal RNAs (rRNAs). This essential process accounts for more than half of total cellular transcription in proliferating cells. To achieve this high output, a specific set of protein factors has evolved to support Pol I transcription initiation. Whereas some of these factors are highly conserved in amino acid sequence from yeast to human, others are conserved rather at the functional and structural level. In *S. cerevisiae* (hereafter called yeast) a minimal set of transcription factors composed of Rrn3, which associates with Pol I to form the initiation competent polymerase, and a three subunit complex called core factor support promoter dependent transcription *in vitro*. Another six subunit complex called upstream activating factor, which forms a complex with TATA binding protein, is required for Pol I transcription *in vivo*. Previously, it has been reported that a large protein, Net1, stimulates cell growth, correlating with its ability to activate Pol I transcription. Net1 is a multifunctional protein, which fulfills additional important roles in regulating the cell cycle and silencing of RNA polymerase II transcription at the rDNA locus. The mechanism by which Net1 stimulates Pol I transcription is unknown.

We show, that the Pol I stimulating function of Net1 can be attributed to an individual domain within the protein. The very C-terminal region (CTR) of Net1, comprised of only 139 amino acids was required for normal cell growth and sufficient to partially rescue the growth defect observed in the absence of full-length Net1. In good correlation, the CTR supported efficient Pol I association with rRNA genes *in vivo* and promoter-dependent transcription in a minimal transcription system *in vitro*. Interestingly, phosphorylation of the CTR appeared to modulate its potential to stimulate Pol I transcription. The data suggests, that the Net1-CTR might be an important regulator of Pol I transcription and cell proliferation. Whereas Net1 is yeast specific, there was evidence that features of the CTR are conserved in higher eukaryotes.

Session 3: Subunit Assembly I

Assembly, structure and function of the small subunit processome

Jonas Barandun, Malik Chaker-Margot, Mirjam Hunziker, Kelly R. Molloy, Brian T. Chait, Sebastian Klinge

The Rockefeller University, New York

Eukaryotic ribosome biogenesis is a complex and multidimensional process involving all three RNA polymerases, many small nucleolar RNAs and more than 200 assembly factors. Co-transcriptional recruitment of biogenesis factors in the nucleolus results in the formation of the earliest stable precursor of the small ribosomal subunit, the small subunit (SSU) processome. Here we present biochemical and structural data of the SSU processome, a large assembly of more than 4.5 MDa. Electron microscopy in combination with protein-RNA, protein-protein crosslinking and mass-spectrometry allowed us to shed light on the early events in ribosome biogenesis. In the SSU processome, U3 snoRNA and 51 assembly factors chaperone a partially processed rRNA bound by 15 ribosomal proteins. The individual domains of the small ribosomal subunit RNA are kept in an open conformation such that enzymes can access RNA regions occluded in the mature ribosome. U3 snoRNA, assisted by several proteins, remodels the central pseudoknot and prevents its premature formation in the particle. An extensive network of peptides and long helices pierce through the particle and contact distant functional sites. Steric hindrance, molecular mimicry, and exosome recruitment modules suggest that the maturing ribosome has to pass an extensive quality control system for productive ribosome assembly to occur.

Distinct maturation steps of the human 40S subunit revealed by comprehensive analysis of preribosome composition and compartmentalization

Blanca Nieto, Sonia G. Gaspar, Xosé R. Bustelo and Mercedes Dosil

Centro de Investigación del Cáncer, University of Salamanca, Salamanca, Spain

We have developed a method that efficiently extracts preribosomal complexes from human cell lysates in a sequential manner. The method renders three separate pools of particles: one containing the cytoplasmic and nucleoplasmic preribosomes, a second one containing the preribosomes present in the outer regions of the nucleolus, and a third pool with the early preribosomes present in internal nucleolar regions. In addition, we have generated CRISPR-edited HeLa-derived cell lines that endogenously express GFP-fused versions of five 40S synthesis factors: NOC4L, BYSTIN, RRP12, LTV1 and NOB1. Preribosomal particles were analyzed in these cell lines using our sequential extraction method, followed by GFP-Trap™ pull-down and mass-spectrometry analyses, in combination with fluorescence microscopy. This approach identified two distinct pools of 18SE-containing complexes that correspond to the first ~40S particles, formed in the inside of the granular component, and the following intermediate-maturation particles, formed in more peripheral regions of the nucleolus. The latter particles are highly vulnerable to degradation and require the factor RRP12 for their stability when they exit the nucleolus. The release of RRP12 only occurs when the intermediate 40S preribosomes mature into more-stable late particles. Our findings unveil several steps of the 40S synthesis pathway in human cells and show that the maturation dynamics, the biochemical properties of nuclear preribosomes and the regulation of some maturation events are different to those in yeast.

Coordination of distant 40S ribosomal subunit maturation events

Valentin Mitterer[1], Ramtin Shayan[2], Guillaume Murat[3], Dieter Kressler[3], Sebastién Ferreira-Cerca[4], Celia Plisson-Chastang[2] and Brigitte Pertschy[1]

1 - University of Graz, Graz, Austria
2 - Université Paul Sabatier, Laboratoire de Biologie Moléculaire Eucaryote, Toulouse, France
3 - University of Fribourg, Department of Biology, Biochemistry, Switzerland
4 - University of Regensburg, Biochemistry, Germany

Freshly exported precursor particles of the small 40S ribosomal subunit still contain ~10 ribosome assembly factors bound at different important functional sites of the 40S. Among them is Rio2, an ATPase positioned on the intersubunit side of the 40S subunit in the so called "head region". Rio2 hydrolyses ATP and is believed to thereby trigger structural re-arrangements. Additionally, ATP hydrolysis results in Rio2 dissociation from pre-40S particles. Another assembly factor, Ltv1 is also positioned in the 40S head, albeit distantly from Rio2 on the solvent exposed side. Its release involves phosphorylation by a protein kinase Hrr25 and allows for the stable incorporation of ribosomal protein Rps3 into 40S subunits. Intriguingly, despite the distant positioning of Rio2 and Ltv1 on opposing sides of the 40S head, their release is mutually linked.

Here, we introduce a communication mechanism controlling the interdependent and coordinated release of these two assembly factors.

A novel role of the DUB USP16 in RPS27a/eS31 deubiquitination and 40S subunit maturation

Christian Montellese, Jasmin van den Heuvel, Ulrike Kutay

Institute of Biochemistry ETHZ, Zurich, Switzerland

The ubiquitin proteasome system (UPS) serves a plethora of purposes, ranging from the regulation of protein degradation to nonproteolytic, regulatory functions in many cellular pathways. The UPS includes a class of enzymes called deubiquitinases (DUBs), which are responsible for the removal of ubiquitin moieties from protein substrates. The DUB USP16 has so far been implicated in the etiology of Down's syndrome, HBV-induced malignancies as well as in stem cell differentiation and developmental processes in mammals, primarily through its established role in the deubiquitination of histone H2A.

Here, we have identified USP16 as a novel component of late pre-40S ribosomes through proteomic and biochemical characterization of different human pre-40S subunits. Our analysis reveals that USP16 is a cytoplasmic protein that possesses a ribosome-associated function, promoting the deubiquitination of the ribosomal protein RPS27a/eS31. Interestingly, although RPS27a is initially synthesized as an in-cis fusion with ubiquitin, mutational analysis shows that USP16 removes a trans-ubiquitin conjugated to an internal lysine of RPS27a. In addition, depletion of USP16 leads to defects in late cytoplasmic steps of 40S biogenesis indicating that the presence of a ubiquitin moiety on RPS27a interferes with the final maturation of 40S subunits. Together, our results reveal a novel function of USP16 in the deubiquitination of RPS27a and a requirement for late 40S subunit maturation.

A dynamic view on ribosome biogenesis

Gertrude Zisser[1], Lisa Kofler[1], Vasileios Kargas[2], Uli Ohmayer[3], Christina Mauerhofer[1], Philipp Milkereit[4], Alan J. Warren[2] and Helmut Bergler[1]

1 - Institute of Molecular Biosciences, Graz
2 - Cambridge Institute for Medical Research (CIMR), University of Cambridge, United Kingdom
3 - Polyquant GmbH, Bad Abbach, Germany
4 - Polyquant GmbH, Bad Abbach, Germany

The formation of ribosomal subunits requires the precisely coordinated activity of more than 200 maturation factors acting in the nucleolus, the nucleoplasm and the cytoplasm. Despite the complex nature of the pathway, the full assembly line leading to the mature subunits in the cytoplasm is finished within few minutes in yeast cells. The investigation of such a highly dynamic pathway is experimentally challenging. We previously identified the drug diazaborine as first specific inhibitor of large ribosomal subunit formation. This compound rapidly diffuses into the cells and blocks the activity of its target protein, the AAA-ATPase Drg1, which is required for the recycling of shuttling proteins back into the nucleus. As a consequence, the large subunit maturation pathway is blocked at an early nucleolar step. The fast onset of inhibition by diazaborine enabled us to study the maturation pathway with high temporal resolution allowing the identification of temporal and functional linkages during ribosome maturation. Here we show how diazaborine and additional, hitherto undescribed inhibitors of ribosome biogenesis can be used to gain deeper insights into the maturation processes of the large ribosomal subunit.

ATPase activity of the AAA-protein NVL2 regulates its communication with WDR74 during ribosome biogenesis

Yu-Hua Lo, Mack Sobhany, Monica Pillon and Robin E. Stanley

Signal Transduction Laboratory National Institute of Environmental Health Sciences, NIH, Durham, USA

Three essential AAA-ATPases are required for pre-60S maturation, including NVL2/Rix7, Midasin and Drg1. The AAA (ATPase associated with various cellular activities) family is a large and functionally diverse group of enzymes that couple ATP hydrolysis with mechanical work. Previous studies revealed that NVL2 is responsible for catalyzing the removal of the assembly factor WDR74/Nsa1 from pre-60S particles, however little is known about the mechanism. Through multiple structural analyses we show that S. cerevisiaeWDR74/Nsa1 is composed of an N-terminal seven bladed-WD40 domain followed by a lysine rich C-terminus that extends away from the WD40 domain and is required for nucleolar localization. Co-immunoprecipitation assays with the mammalian homologues identified a well-conserved interface within WDR74 that is important for its association with NVL2. We further show that WDR74 associates with the D1 AAA domain of NVL2, which represents a novel mode of binding of a substrate with a type-II AAA-ATPase.Moreover, Co-IP analysis showed WDR74 has a greater affinity for binding to an ATP deficient mutant of NVL2, suggesting that ATP hydrolysis drives release of WDR74 from both NVL2 and pre-60S particles. Taken together, our data suggest that ATP hydrolysis of NVL2 drives conformational changes to mediate its cofactor-binding and release during ribosome assembly.

Session 4: Ribosomal RNA processing

Adjacent gene co-regulation (AGC) functions in the transcriptional control of ribosome biogenesis genes

Michael McAlear

Dept. of Molecular Biology and Biochemistry, Wesleyan University, Middletown, USA

Ribosome biogenesis represents one of the most energetically demanding, and tightly controlled biosynthetic process in microorganisms. In budding yeast, ribosome biogenesis is controlled predominately at the transcriptional level, and the ribosomal protein (RP), and the rRNA and ribosome biosynthesis (RRB or ribi) regulons together include some 400 genes. These two regulons are under tight regulatory control, and yeast cells actively up and down regulate ribosome biogenesis in response to a wide variety of changing cellular conditions. Strikingly, an unusually high fraction (> 15%) of the genes of the respective regulons exist on the chromosomes as distinct, immediately adjacent gene pairs. These RP-RP and RRB-RRB gene pairs exist in all three tandem, divergent, and convergent orientations, and they exhibit a higher level of transcriptional co-regulation than the unpaired genes of the same regulons. We used mutational analysis to dissect the convergent RRB MPP10-MRX12 gene pair and observed that promoter motifs from the MPP10 gene were important for regulating the heat shock induced repression of the MRX12 gene, even though the MRX12 promoter is some 4 kbp away, and pointing in the opposite direction. Furthermore, the transcriptional coupling of the two genes – a phenomenon we call adjacent gene co-regulation (AGC) - was reading frame independent, and could be disrupted by splitting with pair with an exogenous, actively transcribed reading frame. A search for relevant trans factors revealed that AGC also depends on the activity of chromatin modifying proteins. While AGC was first described as being associated with the ribosome biogenesis regulons, statistically relevant levels of gene pairing can be seen in many other co-regulated gene sets, and in widely divergent yeast species. Interestingly, while high levels of AGC can be observed across divergent yeast species, the exact pairing arrangements – which genes pair with which – vary considerably. We suggest that AGC represents an operon-like regulatory control system in eukaryotes, one that allows for more efficient transcriptional control of large gene sets (by organizing some members as gene pairs) and for the facile rewiring of mini (i.e. two gene) regulatory circuits.

RIOK2 phosphorylation by RSK contributes to the synthesis of the human 40S ribosomal subunit

E. Cerezo, M.-K. Sarthou, O. Lié, C. Audoynaud, G. Lavoie, M. Halladjian, C. Froment, O. Schiltz, Y. Henry, P. Roux, A. Henras and Y. Romeo

LBME - CBI CNRS - University of Toulouse, Toulouse, France

In eukaryotes, ribosome biogenesis requires scores of assembly factors (AFs) that transiently interact with pre-ribosomal particles and is tightly regulated by signal transduction cascades to accommodate cell growth requirements. In human, the Ras/MAPK-ERK pathway regulates ribosome production at different stages, including transcription by RNA polymerases I and III, and translation of ribosomal proteins. However, little is known regarding its contribution to pre-rRNA processing and maturation of pre-ribosomal particles. In this work, we have addressed the role of RSK, a major downstream effector kinase of ERK, in the maturation of pre-40S pre-ribosomal particles, the precursors to the small ribosomal subunit. We have identified RIOK2, an essential AF involved in late steps of 40S ribosomal subunit biogenesis, as a new substrate of RSK. We showed that

RSK phosphorylates RIOK2 on a specific serine residue. Our results suggest that RSK-dependent phosphorylation of RIOK2 contributes to its dynamic association to pre-40S particles. We are now investigating the impact of this regulation on the maturation of pre-40S pre-ribosomal particles. This work unravels original connections between the Ras/MAPK-ERK signalling pathway and the maturation of pre-ribosomal particles.

The kinase Rio1 regulates the release of Pno1 and 18S rRNA processing during pre-40S ribosome assembly

Melissa D. Parker, Jason C. Collins, Boguslawa Korona, Katrin Karbstein

The Scripps Research Institute, Jupiter, USA

Quality control mechanisms during ribosome synthesis reduce mistakes in translation that would otherwise reduce the fidelity of translation and adversely affect cell growth. This requires the concerted effort of many transiently-binding assembly factors to coordinate rRNA processing with ribosomal protein binding, and to prevent premature ribosomal subunits from initiating translation. Here we present genetic and biochemical data to reveal how the essential kinase Rio1 regulates the final assembly steps in 40S ribosome maturation. Rio1 directly binds the Nob1•Pno1 complex in an ATP-dependent manner. Upon ATP hydrolysis, Rio1 releases Pno1 from the pre-40S ribosome. The data show that release of Pno1 and 18S rRNA processing both promote the release of the Nob1 endonuclease from nascent ribosomes, and suggest strongly that Rio1 promotes 18S rRNA cleavage by rendering 18S rRNA maturation irreversible. In addition, our data demonstrate that Rio1-independent release of Pno1 leads to defects in start and stop codon recognition, reduces decoding fidelity, and affects the incorporation of Rps26, suggesting a role for Rio1 in Rps26 incorporation and in ensuring that 40S ribosomes are mature before initiating translation.

Characterization of the molecular crosstalk within the essential Grc3/Las1 pre-rRNA processing complex

Monica C. Pillon, Mack Sobhany, Mario J. Borgnia, Jason G. Williams, Lalith Perera and Robin E. Stanley

National Institute of Environmental Health Sciences (NIEHS/NIH), Durham, USA

Las1 is a recently discovered endoribonuclease that cooperates with Grc3-Rat1-Rai1 to process precursor ribosomal RNA (pre-rRNA), yet its mechanism of action remains unknown. Disruption of the mammalian Las1 gene has been linked to congenital lethal motor neuron disease and X-linked intellectual disability disorders, thus highlighting the necessity to understand Las1 regulation and function. We report that the essential Las1 endoribonuclease displays weak RNase activity, however in the presence of the polynucleotide kinase Grc3, Las1 is reprogrammed for efficient and specific RNA cleavage both in vitro and in Saccharomyces cerevisiae. Biophysical techniques reveal that Las1 and Grc3 assemble into a tetrameric complex that is required for competent rRNA processing. A comprehensive mutational analysis of conserved residues found at the Grc3 kinase active site uncover an intricate molecular crosstalk that ensures coupling of RNA cleavage and phosphorylation during pre-rRNA processing. The tetrameric Grc3/Las1 crosstalk draws unexpected parallels to endoribonucleases RNase L and Ire1, and establishes Grc3/Las1 as a novel member of the RNase L/Ire1 RNA Splicing Family.

The nuclear export proteins TbMex67 and TbMtr2 make unique interactions with the 5S RNP component of the 60S ribosomal subunit in *Trypanosoma brucei*

Constance Rink and Noreen Williams

University at Buffalo, Buffalo NY, USA

Trypanosoma brucei is the causative agent of Human African Trypanosomasis (HAT), which remains an economic, and health burden in Africa. The current drug treatment for HAT is inadequate resulting in the need for the identification of new drug targets. Our laboratory focuses on the trypanosome specific aspects of ribosome biogenesis. We have previously shown that two trypanosome-specific proteins P34/P37 are necessary for the formation of the pre-60S 5S ribonuclear particle (RNP) component and its nuclear export. In other organisms one of the nuclear export factors responsible for export of the 60S is the heterodimer Mex67-Mtr2. These proteins are not well characterized in T. brucei and have only been functionally linked to mRNA export. We aimed to characterize the function of TbMex67 and TbMtr2 in the nuclear export of the 60S, specifically their interactions with the 5S RNP. We found that TbMex67 associates with P34/P37 and L5 in wild type whole cell extracts, and RNA was not necessary in order to maintain these interactions. In vitro, we have shown that P34 and L5 directly interact with TbMex67. The addition of 5S rRNA abolishes the direct interaction of P34 with TbMex67. In contrast TbMtr2 does not directly interact with P34 or L5. TbMex67 is able to bind 5S rRNA directly in vitro even though it lacks the amino acids previously characterized for 5S rRNA binding. TbMtr2 was unable to bind 5S rRNA with specificity but TbMtr2 enhances the binding of TbMex67 to 5S rRNA. This study demonstrates that Mex67-Mtr2 uniquely interacts with the protein components of the 60S ribosomal subunit.

Session 5: Prokaryotic and Organelle Ribosome Assembly I

Circular-pre-ribosomal RNAs: archaeal specific pre-rRNA intermediates required for the efficient synthesis of mature rRNAs?

Michael Jüttner[1], Matthias Weiß[1], Nina Ostheimer[1], Karina A. Haas[2], Thandi S. Schwarz[2], Michael Kern[1], Robert Knüppel[1], Corina Küttenberger[1], Anita Marchfelder[2] and Sébastien Ferreira-Cerca[1]

1 - Biochemistry III, Institute for Biochemistry, Genetics and Microbiology - University of Regensburg, Regensburg, Germany.
2 - Biology II - University of Ulm, Ulm, Germany.

In the recent years, several studies have described the occurence of circular-RNA (circ-RNA) intermediates in various cell types and tissues. Interestingly, previous studies were also suggesting the presence of circular ribosomal RNA precursors (circ-pre-rRNA) in several archaea. However, the in vivo functional relevance of these circ-pre-rRNA intermediates, for the formation of mature ribosome, has not been investigated so far.

In this work we have analyzed the functional relevance of the circ-pre-rRNA intermediates for the efficient synthesis of mature ribosomal subunits in vivo. To this end we have established an rDNA cis-acting element reporter assay, in the archaeon Haloferax volcanii, allowing the quantitative and qualitative analysis of rRNA mutations. Accordingly, this assay was applied to disturb the synthesis of the circ-pre-rRNA, by generating mutations potentially affecting the formation and processing of these circ-pre-rRNA intermediates. Furthermore, we have identified and functionally analyzed ribosome biogenesis factors involved in the synthesis and maturation of these circ-pre-rRNA intermediates.

Together our results provide first functional evidence suggesting that circ-pre-rRNA formation is a necessary step for the efficient production of stabile mature rRNA in archaea. In addition, we describe a versatile rDNA cis-acting element reporter in archaea that will allow to generate and functionally analyze the fate of specific rRNA variants.

Finally, our results provide new insights into archaeal-specific and evolutionary shared ribosome biogenesis features.

Single methylation of 23S rRNA regulates late steps of 50S ribosomal subunit assembly by sensing cellular AdoMet concentration

Kensuke Ishiguro and Tsutomu Suzuki

University of Tokyo, Tokyo, Japan

Ribosome biogenesis proceeds through rRNA transcription coupled with ribosomal protein assembly. This process is facilitated and regulated by various assembly factors. The rate of ribosome assembly is strongly affected by growth condition of the cell, but its regulatory mechanism is not fully understood.

RlmE is a 23S rRNA methyltransferase responsible for 2'O-methylation of U2552 (Um2552) of Helix 92 in domain V. RlmE uses S-adenosylmethionine (AdoMet) as a methyl donor. Deletion of rlmE results in remarkable growth defect and accumulation of 45S precursor. In our previous study, we demonstrated that late steps of 50S subunit assembly is triggered

by the single Um2552 methylation mediated by RlmE (Arai et al., PNAS, 2015).

We further examined whether RlmE-mediated Um2552 formation and 50S assembly is regulated by sensing AdoMet concentration in the cell. Upon disruption of mtngene which is required for AdoMet recycle, we observed severe growth defect of Δmtn strain with significant accumulation of the 45S precursor bearing 23S rRNA with hypomodified Um2552. Strikingly, overexpression of rlmErescued the growth defect of Δmtn strain, and reduced the accumulation of 45S precursor. AdoMet is a central metabolite not only involved in rRNA methylation but also in various cellular processes. Nevertheless, it is suggested that growth defect of Δmtn is mainly due to down regulation of 50S subunit. These results indicate that late steps of 50S assembly strongly depend on cellular metabolic status by sensing AdoMet concentration.

Role of the conserved GTPase BipA in assembly of the 50S subunit of the ribosome

Michelle R. Gibbs[1], Kyung-Mee Moon[2], Leonard J. Foster[2], Kurt Fredrick[1]

1 - Department of Microbiology and Center for RNA Biology, The Ohio State University, Columbus, Ohio, USA
2 - Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia, Canada

BipA is a conserved translational GTPase that resembles elongation factor EF-G and 30S assembly factor LepA. Recent evidence from the Flower group suggests that BipA functions in 50S subunit assembly, but the precise role of the factor remains unclear. Here, we use stable isotope labeling of amino acids in culture and mass spectrometry (SILAC / MS) to examine the function of BipA in ribosome biogenesis. During growth at suboptimal temperature, loss of BipA leads to accumulation of immature large subunit particles (~40S) that lack several proteins. These include L2, L10, L14, L16, L17, L19, L25, L27, L28 and L32. Parallel analysis of the control (wild-type) strain shows accumulation of virtually identical intermediate particles, although at much lower levels. These data suggest that BipA acts in some way to destabilize or inhibit formation of this 40S intermediate. Loss of BipA causes no apparent defect in 30S subunit assembly. In fact, the proportion of 30S assembly intermediates decreases in the mutant strain, presumably due to an increase in free mature 30S subunits unable to enter the translation pool because they have no functional 50S partner. Notably, LepA and BipA bind similarly to the ribosome, and the GTP hydrolysis activity of each factor depends on the intact 70S ribosome. Based on these observations, we propose that, for each subunit, part of the assembly process occurs in the context of the 70S ribosome.

Assembly factors mediated biogenesis of large subunit of ribosome in prokaryotes

Nikhil Jain[1], Xiaodan Ni[2], Aida Razi[2], Joaquin Ortega[2] and Robert A. Britton[1]

1 - Department of Molecular Virology & Microbiology, Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, USA
2 - Department of Anatomy and Cell Biology, McGill University, Montreal, Canada

Ribosome biogenesis in bacteria requires a large number of assembly factors. The late stages of assembly are regulated by a group of GTPases known as ribosome assembly

GTPases that sense the level of GTP in cell and regulate the final steps of assembly by assisting folding of rRNA and mediating binding of late ribosomal proteins. Our groups have previously established that independent depletion of three ribosome assembly GTPases, RbgA, YphC and YsxC results in the accumulation of subunit intermediates that lack late binding ribosomal proteins. Interestingly, all of these intermediates have similar protein complement and have highly reduced uL16, bL27, bL28, bL33, bL35, and bL36 ribosomal proteins. The cryo-EM structures of the 45SRbgA, 45SYphC and 44.5SYsxC immature particles are similar with each lacking the central protuberance and showing disordered h38, h68 and h69 helices. These results indicate that these intermediates require the action of several assembly factors to progress into a mature 50S subunit. To understand the exact molecular functions of RbgA, YphC and YsxC assembly factors, we are obtaining the cryo-EM structures of these assembly intermediates along with their respective assembly GTPase. Interestingly, RbgA-45SRbgA complex structure revealed that RbgA binds at the P site (it is more on the P site) on the 50S subunit. We are working to get high resolution information to understand precise rearrangements and mechanism of maturation dependent GTP hydrolysis that lead to detachment of RbgA from mature complex. These essential assembly factors would be excellent drug targets as inactivation of these would stall key steps of ribosome maturation that are necessary for bacterial cell survival.

Session 6: snoRNP biogenesis and RNA modification

Guide-substrate base-pairing requirement for box H/ACA RNA-guided RNA pseudouridylation

Meemanage De Zoysa, Guowei Wu, Raviv Katz, Yi-Tao Yu

University of Rochester Medical Center Department of Biochemistry and Biophysics Center for RNA Biology, Rochester, USA

Box H/ACA RNAs are a group of small RNAs found in abundance in eukaryotes (as well as in archaea). Although their sequences differ, eukaryotic box H/ACA RNAs all share the same unique hairpin-hinge-hairpin-tail structure. Almost all of them function as guides that primarily direct pseudouridylation of rRNAs and spliceosomal snRNAs at specific sites. Although box H/ACA RNA-guided pseudouridylation has been extensively studied, the detailed rules governing this reaction, especially those concerning the guide RNA-substrate RNA base-pairing interactions that determine the specificity and efficiency of pseudouridylation, are still not exactly clear. This is particularly relevant given that the lengths of the guide sequences involved in base-pairing vary from one box H/ACA RNA to another. Here, we carry out a detailed investigation into guide-substrate base-pairing interactions, and identify the minimum number of base-pairs (8), required for RNA-guided pseudouridylation. In addition, we find that the pseudouridylation pocket, present in each hairpin of box H/ACA RNA, exhibits flexibility in fitting slightly different substrate sequences. Our results are consistent across three independent pseudouridylation pockets tested, suggesting that our findings are generally applicable to box H/ACA RNA-guided RNA pseudouridylation.

Substrate and guide RNA specificity of H/ACA small nucleolar ribonucleoproteins

Erin Kelly, Ute Kothe

Alberta RNA Research and Training Institute (ARRTI), University of Lethbridge, Lethbridge, Canada,

During ribosome synthesis, many H/ACA small nucleolar Ribonucleoproteins (snoRNPs) bind to ribosomal RNA (rRNA) to introduce pseudouridine modifications and to assist in rRNA processing. The versatility of H/ACA snoRNPs stems from their ability to utilize a variety of H/ACA snoRNAs for the specific interaction with many different regions of rRNA. H/ACA snoRNAs are characterized by the presence of two hairpins each harboring a pseudouridylation pocket and a conserved motif after each hairpin, the H box and ACA box, respectively.

To understand the function of H/ACA snoRNPs during ribosome biogenesis, it is critical to characterize the specific interaction of H/ACA proteins with the many different H/ACA snoRNAs and the base-pairing requirements between rRNA and H/ACA snoRNA. Both interactions pose numerous questions as the known H/ACA snoRNAs share no sequence similarities beyond the H and ACA boxes and their structural similarities are limited to the presence of two hairpins. Similarly, the known base-pairing interactions between H/ACA snoRNAs and rRNA differ widely in the number of base-pairs on either side of the pseudouridylation pocket and in the presence of mismatches.

Using our recently reported reconstituted yeast H/ACA snoRNPs system, we systematically analyzed the features of H/ACA snoRNAs required for binding to the H/ACA proteins and

for directing efficient pseudouridine formation. Interestingly, H/ACA proteins bind H/ACA snoRNAs with a remarkable high affinity that does not rely on a single sequence or structural feature while maintaining their pseudouridylation ability. Thus, our results display an impressive adaptability of H/ACA snoRNAs. Likewise, we dissected the base-pairing necessities between H/ACA snoRNAs and rRNA revealing the importance of a minimal number of base-pairs and the absence of mismatches close to the target uridine for pseudouridylation. Notably, H/ACA snoRNPs bind other RNAs with reasonable affinity, but without modifying these RNAs presumably because the target uridine is not correctly positioned in the active site of the protein Cbf5. This implies that H/ACA snoRNPs interact with many areas of the rRNA while searching for the correct modification sites. In conclusion, our results provide a better understanding of the versatility of H/ACA snoRNAs and their interaction with rRNA during ribosome synthesis which does not always result in pseudouridine formation.

Identification of non-annotated snoRNA using a structure insensitive sequencing pipeline

Vincent Boivin, Olivier Boisvert, Sonia Couture, Sherif Abou Elela and Michelle Scott

Université de Sherbrooke, Département de biochimie, Sherbrooke, Canada

Modification and processing of rRNA requires a large number of guide snoRNA. The majority of snoRNAs were identified based on sequence complementarity, structure prediction and association with snoRNA binding proteins. However, the expression pattern and tissue specific distribution of the majority of snoRNAs remain unclear due to difficulty in the detection and quantification of snoRNA using standard sequencing method. Recently we have developed a new sequencing pipeline capable of detecting and accurately quantifying structured non-coding RNA in unfragmented total RNA samples (abbreviated URT). Using this new method, which substitutes the retroviral RT with a Thermostable Group II Intron Reverse Transcriptase (TGIRT) we were able to accurately map snoRNA termini and establish their relative abundance in different cell lines. Here we show that URT could also be used as a tool for de novo detection of stable non-coding RNA including snoRNAs. Due to the read consistency and transcripts profile homogeneity of URT we were able to identify hundreds of new non-annotated non-coding RNAs, including 26 snoRNA genes. The majority (17/23) newly identified snoRNA are associated with Alu repeats, which may explain the difficulty in predicting them using traditionally methods. About half of the newly detected snoRNA have known rRNA modifications targets while the other half have no apparent rRNA target suggesting possible non-canonical functions. The majority of snoRNA detected using this sequencing method adopts the predicted snoRNA structure and their expression validated using qRT-PCR. The expression of these snoRNA was not restricted to a specific cell type suggesting possible housekeeping functions. Together, our work presents a new tool for de novo annotation independent prediction of snoRNA and identifies a new group of snoRNA that are difficult to predict using traditional methods.

Fine-tuning of rRNA 2'-O-methylation by non-snoRNP factors

Rebecca Cunningham[1], Daniela Zeitler[1], Nicolai Krogh[2], Robert van Nues[1], Katherine Sloan[3], Mark Ashe[4], Markus Bohnsack[3], Henrik Nielsen[2], Claudia Schneider[1], Nicholas James Watkins[1]

1 - Newcastle University, Newcastle, United Kingdom
2 - University of Copenhagen, Copenhagen, Denmark
3 - Georg-August-University, Germany
5 - University of Manchester, United Kingdom

Modification of ribosomal (r)RNA is essential for rRNA folding and ribosome function. In yeast, 54 rRNA 2'-O-methylations are catalysed by Nop1 (fibrillarin) in the box C/D small nucleolar (sno)RNPs. Little is known about how this mostly co-transcriptional event is mediated.

To identify regulators of rRNA 2'-O-methylation, we performed a genome-wide yeast genetic screen in which we expressed a toxic snoRNA targeting a naturally unmodified site in the 18S rRNA. Importantly, the core snoRNP proteins Nop1 and Nop56 were identified as top suppressors. Intriguingly, our screen revealed multiple enhancers of rRNA 2'-O-methylation. DAmP strains for the RNA helicase Has1, the Pol I regulator Rrn9 and the large subunit biogenesis factor Rrs1 all showed enhanced 2'-O-methylation at naturally undermodified sites. Excitingly, the gene encoding Srp40 was a prominent suppressor in our screen. RiboMeth-seq analysis demonstrated significantly reduced (often < 50%) 2'-O-methylation at 43 natural sites in the absence of Srp40. We are now exploring whether another protein functions as a snoRNP co-factor at the remaining 11 Srp40-independent sites, which are present at the core of the ribosome. Lack of Srp40 affects translation, with a major subunit joining defect observed on polysome gradients, and cells lacking Srp40 showed altered sensitivity to ribosome-binding antibiotics. Srp40 interacts with both RNA pol I and the box C/D snoRNPs and switching rRNA transcription to RNA pol II indeed impacted rRNA modification. In summary, our data provide exciting new insights into the complex regulation of snoRNP-mediated rRNA modification.

Nopp140 knockdown depletes scaRNPs from Cajal bodies impairing spliceosomal snRNA modification and promoting telomere lengthening

Jonathan Bizarro[1], Svetlana Deryusheva[2], Joseph G Gall[2], U.Thomas Meier[1]

1 - Albert Einstein College of Medicine, Bronx, USA
2 - Carnegie Institution, Baltimore, USA

Cajal bodies (CBs) are small nuclear organelles identified by the marker protein coilin. They concentrate small CB-specific (sca)RNPs and their modification targets, spliceosomal small nuclear (sn)RNAs. Nopp140 is a highly phosphorylated nucleolar and CB protein that associates with both major classes of small nucleolar (sno)RNPs, box H/ACA and C/D, including scaRNPs. Although CBs have been implicated in snRNA modification and Nopp140 in ribosome biogenesis, their function remains to be established. We show that CRISPR/Cas9-mediated knockdown of Nopp140 causes loosening of the dense fibrillar component of the nucleolus (the home of Nopp140 and snoRNPs) without affecting nucleolar snoRNP localization. In contrast, in Nopp140 knockdown cells, scaRNPs (but not snRNPs) are lost from CBs leading to a dramatic reduction of snRNA 2'-O-methylation. Unlike knockdown or mutation of the CB localizing and scaRNP protein Wdr79/TCAB1,

which cause telomere shortening, the loss from CBs of telomerase (a specialized scaRNP) leads to telomere lengthening in Nopp140 knockdown cells. Our findings document a role for the intrinsically disordered Nopp140 in packaging of nucleoli and CBs, and in the function of CBs in snRNA modification and telomere length maintenance.

Structural studies of the snoRNP assembly machine reveal a family of quaternary chaperones

Chloé Maurizy[1], Marc Quinternet[2], Yoann Abel[1], Céline Verheggen[1], Paulo E. Santo[3], Maxime Bourguet[4], Claire Abeza[1], Philippe Fort[6], Franck Vandermoere[7], Pedro M.F. Sousa[3], Jean-Christophe Rain[8], Bruno Charpentier[5], Sarah Cianférani[4], Tiago M. Bandeiras[3], Bérengère Pradet-Balade[6], Xavier Manival[5], Edouard Bertrand[1]

1 - IGMM, CNRS, Université de Montpellier, Montpellier, France
2 - IBSLOR, Université de Lorraine, CNRS, INSERM, Nancy, France
3 - Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal and iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
4 - LSMBO, Université de Strasbourg, Strasbourg, France
5 - IMoPA, Université de Lorraine, CNRS, Nancy, France
6 - CRBM, CNRS, Université de Montpellier, Montpellier, France
7 - IGF, CNRS, Université de Montpellier, Montpellier, France
8 - Hybrigenics Services, Paris, France

R2TP is an HSP90 co-chaperone composed of an RPAP3-PIH1D1 heterodimer associated with the two essential AAA+ ATPases RUVBL1/RUVBL2 (also known as pontin and reptin). The R2TP chaperone is unique in that it appears specialized in the assembly of macro-molecular complexes. Accordingly, it has been shown to play a role in the assembly of a number of cellular machineries, including: (i) box C/D and box H/ACA snoRNPs; (ii) telomerase RNP; (iii) U4 and U5 snRNPs; (iv) the nuclear RNA polymerases; (v) PIKK-containing complexes such as mTORC1 and mTORC2. Here, we resolved the structure of the RPAP3-PIH1D1 interface, as well as of the RPAP3-Cterminal domain. We further show that this latter domain binds the RUVBL1/2 ATPases and helps recruiting clients to the chaperone. Together with other structural and interaction data, this suggests a possible model for how the R2TP chaperone may work.

The human genome encodes two other proteins bearing RPAP3-C-terminal-like domains and three containing PIH-like domains. Systematic interaction analyses show that one RPAP3-like protein, SPAG1, binds PIH1D2 and RUVBL1/2 to form an R2TP-like complex, termed R2SP. Structural studies further reveal the simililarities between R2TP and R2SP. A yeast two-hybrid screen identified potential R2SP clients, and, remarkably, we found that R2SP is required for their expression and assembly into larger complexes. R2TP and R2SP thus form a family of chaperones functioning in quaternary protein folding.

Session 7: Subunit Assembly II

Towards a comprehensive landscape of 60S ribosomal biogenesis

Carolin Sailer[1], Jasmin Jansen[1], Axel Reiser[2], Jan Erzberger[3] and Florian Stengel[1]

1 - Department of Biology, University of Konstanz, Germany
2 - Institute for Parallel and Distributed Systems, University of Stuttgart, Germany
3 - Department of Biophysics, UT Southwestern Medical Center, USA

Crosslinking coupled to mass spectrometry (XL-MS) has evolved to an indispensable tool for the structural analysis of macromolecular protein complexes [reviewed in 1] and we could show before, that the ribosome and ribosome associated particles can be investigated by XL-MS and crosslinking guided integrative modeling [2].

Especially during the last years, Cryo-EM structures of select 60S pre-ribosomal particles helped to uncover the site of action of many assembly factors and to describe principles of ribosome assembly [3]. However, some assembly factors still evade structural analysis and the molecular dynamics of rRNP interaction networks remain not fully understood.

Here, we used XL-MS and large scale biochemical enrichment of pre-ribosomal particles in order to obtain a comprehensive landscape of 60S ribosomal biogenesis. We captured 163 known assembly factors and potentially novel ones. Furthermore, the dataset allowed us to reconstruct a detailed timeline for 60S ribosome biogenesis.

[1] Leitner, A. et al., Trends in Biochemical Sciences, 2016. 41(1): p.20-32
[2] Erzberger, J.P. et al., Cell, 2014. 158(5): p.1123-35
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Puf6 directs correct RNA folding during ribosome assembly

Stefan Gerhardy[1,2], Michaela Oplova[1,2], Ludovic Gillet[1], Rob Van Neus[3], Richard Börner[4], Olga T. Schubert[1], Sander Granneman[3], Ruedi Aebersold[1,4], Roland K. O. Sigel[4] and Vikram Govind Panse[2]

1 - ETH Zurich, CH-8093 Zurich, Switzerland
2 - Institute of Medical Microbiology, University of Zurich, CH-8006 Zurich, Switzerland
3 - Centre for Synthetic and Systems Biology, University of Edinburgh, Edinburgh, EH9 3BF, UK.
4 - Faculty of Science, University of Zurich, CH-8057 Zurich, Switzerland

Long-range interactions between RNA-tetraloops (RNA-TL) and their receptors drive higher order RNA folding during ribosome assembly. How RNA-TL:receptor interactions are regulated in vivo remains unclear. By applying high-resolution data independent acquisition mass spectrometry to budding yeast, we uncovered several RNA-binding proteins that are induced at low temperature (LT), a condition where non-native RNA folding intermediates tend to accumulate. Functional analyses of one identified factor, Puf6, unveiled its critical requirement at LT to regulate interactions between a specific RNA-TL and its RNA-receptor, a kissing loop (KL), during early nucleolar 60S subunit assembly. Bulk fluorescence resonance energy transfer studies revealed that Puf6 employs its binding energy to prevent this RNA-TL from engaging with non-cognate receptors, therefore directing productive RNA compaction. We propose that Puf6 directs eukaryotic rRNA folding at low temperature by preventing incorrect tertiary contacts during 60S assembly.

The yeast Npa1 complex is involved in the compaction of the 25S rRNA central core in the earliest pre-60S particles

Anthony Henras, Clément Joret, Mariam Jaafar, Régine Capeyrou, Kamila Belhabich-Baumas,Odile Humbert, Célia Plisson-Chastang, Nicolas Leulliot, Simon Lebaron and Yves Henry

LBME / CBI CNRS - University of Toulouse, Toulouse, France

The early steps of the production of the large ribosomal subunit are probably the least understood stages of eukaryotic ribosome biogenesis. We demonstrate that five assembly factors required for the production of the first pre-60S pre-ribosomal particles, Npa1p, Npa2p, Nop8p, Rsa3p and the helicase Dbp6p form a complex. Npa1p is a crucial component of this complex since it is required for its formation and stable integration within pre-ribosomes. Mapping of the Npa1p-rRNA contacts by CRAC indicates that Npa1p binds adjacent to ribosomal protein Rpl3, structural protein Rrp5p and helicase Prp43p, all key players in early pre-60S particle assembly. Npa1p also crosslinks efficiently to several snoRNAs involved in decoding center and peptidyl transferase center modifications and in the immediate vicinity of the base-pairing sites of these snoRNAs on 25S rRNA. One of these snoRNAs emerges as a novel RNA chaperone required for normal yeast growth, whose depletion affects the early stages of pre-60S particle assembly and maturation. Our data strongly suggest that the Npa1p complex plays a key role in the compaction of the central core of 25S rRNA and the control of snoRNA-pre-rRNA interactions.

Diverse roles of RNA helicases in driving structural transitions and compositional changes in pre-ribosomal complexes

Lukas Brüning, Philipp Hackert, Roman Martin, Katherine E. Sloan, Markus T. Bohnsack

University Medical Center Göttingen, Göttingen, Germany

RNA-protein complexes (RNPs) play key roles at all stages of gene expression and in various other pathways of RNA metabolism. Production of eukaryotic ribosomal subunits is a highly dynamic process involving numerous structural rearrangements of the ribosomal RNAs (rRNAs) and the hierarchical recruitment of approximately 80 ribosomal proteins. Such remodelling events not only establish the architecture present in mature complexes, but also serve as key checkpoints, ensuring the fidelity of ribosome assembly. RNA helicases are important regulators of such structural transitions and multiple helicases have been implicated in ribosome synthesis. However, the molecular functions and sites of action of many of these enzymes during subunit assembly have remained elusive. Using in vivo crosslinking and analysis of cDNA (CRAC), we have identified the pre-ribosomal binding sites of the three RNA helicases Has1, Mak5 and Spb4. By elucidating the precise targets of these enzymes, we uncover direct roles for Has1 in triggering dissociation of a cluster of early pre-60S biogenesis factors from domain I of the 25S rRNA and mediating release of the U14 snoRNP, a critical event during early 40S maturation. Furthermore, remodelling of helix 39 of the 25S rRNA sequences by Mak5 enables recruitment of the ribosomal protein Rpl10, which is necessary for subunit joining and ribosome function. Finally, our data show that Spb4 binds to a molecular hinge at the base of ES27, which forms a flexible arm that anchors the export factor Arx1 to pre-60S complexes. We show that Spb4 facilitates the pre-ribosomal recruitment of Arx1, implying that helicase-mediated remodelling of this region contributes to establishing the export competence of pre-60S

particles. Taken together, our data provide important new insights into the driving forces behind key remodelling events during ribosomal subunit assembly.

Two distinct domains of Nog1 contribute to the assembly of the peptide exit tunnel and the peptidyl transferase center

Amber J. LaPeruta, Daniel M. Wilson, John L. Woolford

Carnegie Mellon University, Pittsburgh, USA

The polypeptide exit tunnel (PET) and the peptidyl transferase center (PTC) are essential and conserved functional centers of the large ribosomal subunit. However, the mechanisms involved in their assembly remain elusive. Recent cryo-EM models of assembling pre-ribosomes have revealed that Nog1, a GTPase assembly factor, interacts with both of these functional centers. Specifically, Nog1 splits H89 of the PTC with its N-terminal helix bundle domain and reaches around two-thirds of the pre-ribosome to probe the PET with its C-terminus. Adjacent to the helix bundle, Nog1 has an Obg-like GTPase domain. How the GTPase and C-terminal domain function in ribosome assembly and in the context of these functional centers is unknown. We used biochemical and molecular genetic methods to determine how Nog1 contributes to maturation of the PTC and PET. To study the role of the GTPase domain of Nog1 in PTC assembly, we assayed effects of nog1G223A, a mutation predicted to prevent GTP hydrolysis. In this mutant, cleavage at the C2 site in the ITS2 spacer is blocked, Erb1 and Brx1 fail to be released from pre-ribosomes, and Nog2 fails to assemble. Based on cryo-EM structures of early nucleolar assembly intermediates, Spb1 occupies a portion of the Nog2 binding site proximal to the helix bundle and GTPase domain of Nog1. Additionally, Erb1 and Brx1 are bound on top of Spb1. We hypothesize that the GTPase activity of Nog1 is essential for initiating the removal of Spb1, which destabilizes or removes Erb1 and Brx1 and allows Nog2 to bind to the PTC. This mechanism outlines a novel remodeling event leading to C2 cleavage and identifies a new function for both Nog1 and Spb1 in the assembly of the PTC. To investigate the role of the C-terminus of Nog1 that extends throughout the PET, we truncated the last 52 amino acids of C-terminal amino acids which is the full extent of Nog1 in the PET. This resulted in a cold-sensitive phenotype and accumulation of 27SB pre-rRNA. However, there was no observable effects on the assembly of proteins required for 27SB pre-rRNA processing. We hypothesize that these defects we observe are caused by a failure to fold rRNA helices in domains II and V that make up part of the mature PET. Cryo-EM shows that these helices adopt immature conformations just prior to insertion of the Nog1 C-terminus. Altogether, our data suggest that Nog1 acts as a coordinator of maturation events in two functional centers in the large subunit, the PTC and the PET.

Ribosomal protein L1 is required for efficient nuclear export of nascent large subunit

Sharmishtha Musalgaonkar, Arlen Johnson

University of Texas at Austin, Austin, USA

Eukaryotic ribosomal Protein L1 (Rpl1/uL1) is an essential large (60S) subunit protein that binds to the L1 stalk and interacts with E site ligands during translation. It has been reported that 60S subunits lacking Rpl1 are exported to the cytoplasm and participate in translation1. We recently showed that a domain of the nuclear export factor Nmd3 is a mimic of the elongation factor eIF5A and makes extensive contacts with L1. In light of this

interaction we re-evaluated the impact of the lack of L1 on nuclear export of 60S subunits. We found that depletion of Rpl1 lead to mislocalization of the assembly factors Nmd3, Tif6 and Mrt4 as well as 60S reporter Rpl25 to the nucleoplasm, indicating that loss of Rpl1 leads to inefficient nuclear export of nascent 60S subunits. Surprisingly, recruitment of Nmd3 seems unaffected, suggesting that inefficient export may be due to a defect in recruiting another export factor. Truncation of the RNA of the L1 stalk rRNA also slowed nuclear export of 60S subunits. However, these subunits are apparently exported to the cytoplasm and incorporated into polyribosomes. Analysis of the protein composition of nascent subunits from Rpl1-repressed cells revealed altered levels of several proteins including the heterodimer Mex67 and Mtr2 that are essential for 60S export. Overexpression of this complex suppressed the 60S export defect in Rpl1 repressed cells. Although Mex67 and Mtr2 do not appear to bind directly to the L1 stalk, our results suggest that the L1 stalk is important for efficient recruitment of Mex67 and Mtr2 for efficient export of 60S subunits.

Session 8: CryoEM and other advances

Multi-particle cryo-EM of eukaryotic ribosome assembly intermediates

Roland Beckmann

Gene Center Munich University, Munich, Germany

Ribosome assembly in eukaryotic cells extends from rRNA transcription in the nucleolus to the final maturation in the cytoplasm and is facilitated by hundreds of assembly factors (AFs). During this process, the modification and processing of the rRNA is coordinated with the developing structural maturation and localization in the different compartments of the cell. Here, the application of cryo-electron microscopy (cryo-EM) in combination with single particle analysis has provided a wealth of information on the compositional and structural transitions that take place along the assembly pathway of the subunits. Mostly native assembly intermediates purified via affinity tags have been studied so far, which has been complemented by the analysis of in vitro reconstituted particles using isolated mature ribosomal subunits and recombinant late acting assembly factors. A particular advantage of cryo-EM in this context turned out to be the possibility to classify heterogeneous particle populations into more homogeneous ones with increasingly efficient algorithms. Thereby, single preparations now often reveal numerous intermediate states, which have already provided evidence for complex folding and assembly pathways, which follow mostly sequential but also parallel routes. With respect to rRNA modification and processing, substantial differences exist between lower (yeast) and higher eukaryotes. Novel high-resolution cryo-EM studies on native and reconstituted yeast and human particles provide information on the degree of structural conservation between the well characterized fungal model and the human system.

Subunit joining exposes nascent pre-40S rRNA for processing and quality control

Jay Rai, Melissa Parker, Homa Ghalei, M. Elizabeth Stroupe, and Katrin Karbstein

The Scripps Research Institute, Jupiter, USA

Correct ribosome assembly is required to ensure the faithful translation of the genetic code and the accurate interpretation of sequence elements in the mRNA to maintain protein homeostasis. During their maturation, nascent 40S subunit enter a translation-like quality control cycle, where they are joined by mature 60S subunits. Maturation continues within these 80S-like ribosomes, and bypass of individual steps leads to loss of translational fidelity. How assembly factors enable joining of 60S subunits, and how the presence of 60S subunits promotes maturation and quality control via structural and functional mimicry of translation events, remain unknown. To address these questions, we have solved the structure of an 80S-like ribosome assembly intermediate to a resolution of 4.35 Å. Unexpectedly, the structure shows that to accommodate the 60S subunit, the universally conserved assembly factor Dim1 has rotated to bridge the P and E-sites. This opens up the platform to construct a new Dim1-stabilized intersubunit bridge, which leaves Dim1 poised for release via Fap7 ATPase-induced subunit rotation. In addition, the opened platform also repositions the pre-rRNA in the nuclease active site. Thus, this structure, together with functional data from mutants, demonstrates how quality-control and 40S maturation are

linked during ribosome assembly.

Moving is maturing: cryo-EM structures of cytoplasmic pre-40S particles unveil chronology of late maturation events

Ramtin Shayan, Dana Rinaldi, Julien Marcoux, Simon Lebaron, Stéphanie Balor, Natacha Larburu, Pierre-Emmanuel Gleizes, Celia Plisson-Chastang

CNRS-LBME Centre de Biologie Integrative, Université Toulouse III - Paul Sabatier, Toulouse, France

Assembly of functional small ribosomal subunits (40S) is performed in several successive and/or parallel maturation steps taking place from the nucleolus to the cytoplasm in eukaryotic cells. It requires the intervention of scores of ribosome biogenesis factors (RBFs), that will be progressively shed by the forming particles. In the cytoplasm, only a handful of RBFs are stably bound to the pre-40S particles, but their precise role in the last maturation steps, as well as their timing of disassembly from the forming particle remains to be elucidated for most of them. Using cryo-EM and single particle analysis, we have solved the 3D structures of several cytoplasmic pre-40S particles, purified from yeast S. cerevisiae at intermediate maturation steps, to resolutions ranging from 3.1 to 3.7 Å. Here we will present the atomic models deriving from these cryo-EM maps, and propose possible chronology(ies) of remodelling events leading to mature, functional small ribosomal subunits. In particular, we will focus on long-range interactions which connect maturation events that occur on the beak to these on the platform of the forming small ribosomal subunits.

A regulatory system involving the Hsf1 and Ifh1 transcription factors monitors ribosome biogenesis and promotes ribosomal protein homeostasis

Benjamin Albert[1], Martin Kos[2], Isabelle Kos[2], Anthony Henras[3], Christophe Dez[3], Olivier Gadal[3], Maria Paula Rueda[1], Xu Zhang[1] and David Shore[1]

1 - Department of Molecular Biology and Insitute for Genetics and Genomics in Geneva, Switzerland
2 - Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany;
3 - Laboratoire de Biologie Moléculaire Eucaryote, Université Paul Sabatier, Toulouse III, France

We found that induced degradation of Topoisomerase 1 (Top1) in budding yeast leads to a rapid, highly specific, but transient re-organization RNA polymerase II transcription in which nearly all ribosomal protein (RP) genes are down-regulated and a small group of heat shock factor 1 (Hsf1) target genes are up-regulated. RP gene down-regulation is correlated with the loss of an essential activator protein, Ifh1, from the promoters of these genes and is independent of Hsf1 activation or the CURI complex.

We present evidence that this transcriptional program is a response to a defect in ribosome biogenesis, most likely provoked by a rapid decline in rRNA transcription when Top1 is depleted. Importantly, an identical transcriptional response is observed when ribosome biogenesis is blocked by rapid degradation of assembly factors. Experiments will be described that support a model in which unassembled RPs lead to both Hsf1 activation and Ifh1 sequestration, which we interpret as a homeostatic mechanism to avoid the toxic accumulation of free RPs in the cell.

Visualizing late cytoplasmic 60S subunit maturation

Vasileios Kargas, Pablo Castro-Hartmann, Norberto Escudero Urquijo, Gertrude Zisser, Helmut Bergler, Alan J. Warren

University of Cambridge, Cambridge, United Kingdom

The final steps in maturation of the eukaryotic large ribosomal subunit remain poorly understood. Using single-particle cryo-EM, we have determined the structures of multiple native cytoplasmic pre-60S intermediates, ordering them into a sequential pathway. On the intersubunit face, the GTPase Lsg1 forms a ternary complex with the N-terminus of the export adaptor Nmd3 and the anti-association factor Tif6. Integration of the last remaining ribosomal proteins destabilizes Nmd3 binding by promoting extensive rRNA rearrangement. Partial retraction of the L1 stalk is coupled to a conformational switch in Nmd3 (while retaining the Tif6 interaction) that allows full accommodation of uL16 into the peptidyl transferase center, where it competes with Nmd3 for an overlapping binding site. Importantly, we resolve a key late maturation intermediate that provides compelling evidence for the release of Tif6 after Nmd3. Our data provide new insights into the mechanism of late 60S subunit maturation, defining a coherent pathway corrupted in multiple leukemia-associated ribosomopathies.

Using cryo-EM to reveal the role of Era in the assembly of the bacterial 30S subunit

Aida Razi[1], Joseph H. Davis[2], Brett Thurlow[1], Jean Philippe Cotte[3], Eric Brown[3], James R. Williamson[2] and Joaquin Ortega[1]

1 - Department of Anatomy and Cell Biology, McGill University, Montreal. Canada
2 - Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, USA
3 - Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada.

In Escherichia coli, the ribosome is made from more than 50 different components organized into the small (30S) and large (50S) ribosomal subunits. In spite of its complexity, each bacterial cell assembles more than 20,000 ribosomes in less than 30 minutes. Cells are able to maintain this assembly rate because a number of auxiliary factors make the process extremely efficient. Our recent work focuses on the Era GTPase assembly factor. To gain new key insights regarding the function of Era, we created an E. coli strain in which the era gene is under the control of an arabinose inducible promoter. Under Era depletion conditions this strain accumulates a 30S subunit assembly intermediate. We hypothesized that this assembly intermediate constitutes the substrate for the Era protein and that the biochemical and structural analysis of this assembly intermediate would be informative on the function of Era. We have obtained the 3D structure of these immature subunits at 3.8Å resolution using cryo-electron microscopy. The map shows a 30S subunit at the late stages of maturation with helices 44 and 45 in the decoding center still in an immature state. Similarly, helices 23 and 24 in the platform region are still not adopting the mature conformation. Density for late entry ribosomal proteins uS2, uS3 and bS21 is also missing and that is consistent with our analysis of these particles using quantitative mass spectrometry. These results indicate that Era is an important factor acting at the late stages of assembly of the 30S subunit playing a role in the maturation of the platform region in the 30S ribosomal subunit. In addition, we will be presenting cryo-EM structures at 3.5Å resolution that provide new insights on how Era coordinate its function with other assembly factors such as YjeQ. The theme that emerges from this work is that these assembly

factors work as “local chaperones” dedicated to fold regions of the rRNA that have a native folding which is not thermodynamically favored. We believe the analysis of atomic resolution structures of immature subunits by themselves and in complex with assembly factors has the potential to ultimately describe the sequence of events leading to a mature 30S subunit and elucidate the role of these assembly factors.

Session 9: Ribosomopathies I

Neuronal ribosomal protein function regulates Drosophila growth and development

Lisa P. Deliu, Abhishek Ghosh, Savraj S. Grewal

University of Calgary, Calgary

Ribosome biogenesis is essential for growth. Most studies have investigated links between ribosome function and growth at the cellular level. We use Drosophila as a model to study how ribosome biogenesis controls tissue and body growth. In Drosophila growth is restricted to the larval stage of the life cycle, in which animals increase in mass 200-fold over a five-day period of development. Minutes are a class of dominant ribosomal protein (rp/+) mutants that exhibit a characteristic delay in larval development, classically thought to be caused by reduced overall protein synthesis. However, when we examined three Minutes (rpS13/+, rpS26/+ and rpL38/+) we saw little or no change in either global ribosome numbers or in protein synthesis rates, when compared to wild-type controls. Instead, we found evidence of a cell type-specific function for one RP (S13) in the control of development. The termination of the larval period is controlled by a neuroendocrine circuit that leads to a pulse of secretion of the steroid hormone ecdysone from the prothoracic gland (PG). We found that rpS13/+ animals had a delayed ecdysone pulse. We also found that re-expression of RpS13, either in the entire brain or specifically in serotonergic neurons – some of which directly innervate the PG to control ecdysone - could partially rescue the delay in development in rpS13/+ animals. These findings suggest that RpS13 may mediate a neuron-specific ribosome function to govern overall body growth. We are currently investigating the molecular and cellular basis for this neuronal specific function of RpS13.

SSU production defects activate p53 via the 5S RNP through stalling LSU maturation

Matthew Eastham, Andria Pelava, Graeme Wells, Maria Deichner, Regina Hertle, Justine Lee, Nicholas Watkins, Claudia Schneider

ICaMB, Newcastle University, Newcastle, United Kingdom

Defects in ribosome biogenesis are linked to more than 20 genetic diseases (ribosomopathies) and multiple forms of cancer. Large ribosomal subunit (LSU) defects cause accumulation of the 5S RNP (ribosomal proteins RPL5 and RPL11 and the 5S ribosomal RNA), an LSU assembly intermediate. The 5S RNP binds to, and suppresses the p53 inhibitor MDM2, leading to the activation of the tumour suppressor p53. Unexpectedly, defects in the production of the small ribosomal subunit (SSU), seen in multiple ribosomopathies, also activate p53 through the 5S RNP.

Here, we show that knockdown of factors involved in the early, middle, or late/cytoplasmic phases of either SSU or LSU production activate p53 in a 5S RNP-dependent manner. In time-course experiments, we found that p53 is activated as rapidly as 3-6 hrs after treatment with siRNAs or ASOs that block the production of either subunit. At these early time points there was no change in mature rRNA levels. However, pre-rRNA processing defects were observed indicating that p53 activation is coupled to ribosome biogenesis, but not to a change in cellular ribosome levels.

Interestingly, inhibition of SSU production resulted in reduced levels of the late, cytoplasmic

pre-5.8S rRNA and impaired nuclear export of newly synthesised LSU. A similar 5.8S rRNA processing defect, together with p53 activation, was also observed upon knockdown of the late LSU biogenesis factor LSG1. Our results therefore indicate that SSU production defects cause 5S RNP-mediated p53 activation via stalling late stages of LSU maturation. We are currently screening for factors that link the SSU and LSU production pathways.

The genetics and clinical manifestations of human ribosome biology disorders

Sharon A. Savage, Neelam Giri, Blanche P. Alter

Clinical Genetics Branch Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, USA

Germline mutations in genes essential for ribosome biosynthesis and function are associated with an array of human phenotypes including congenital anomalies, bone marrow failure (BMF), and elevated cancer risk. Diamond Blackfan anemia (DBA) is caused predominantly by heterozygous mutations in genes encoding the small and large ribosomal subunit components. It typically presents with pure red blood cell aplasia in infancy. Patients with DBA may also have short stature, abnormal thumbs, high arched or cleft palate, genitourinary defects, and cardiac anomalies. They are at increased risk of BMF, myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), colon adenocarcinoma and osteosarcoma. Shwachman Diamond syndrome (SDS) is an autosomal recessive disorder most commonly caused by germline mutations in SBDS, a protein important in joining the 40S and 60S ribosomal subunits. Patients with SDS are usually diagnosed in childhood with neutropenia and failure to thrive due to exocrine pancreatic insufficiency. Physical anomalies, such as short stature, metaphyseal dysostosis, and thoracic dystrophy, may also be present in SDS. BMF may develop and patients with SDS are at risk of progression to MDS and AML. Germline mutations in DKC1are responsible for the X-linked recessive form of dyskeratosis congenita (DC), a telomere biology disorder. DC often presents with the triad of nail dysplasia, abnormal skin pigmentation, and oral leukoplakia. Patients with DC are at high risk of BMF, head and neck squamous cell cancer, MDS, AML, pulmonary fibrosis, and other problems. 13 genes, with all modes of inheritance, have been reported in association with DC. DKC1 is a key component of the telomerase holoenzyme complex and some studies suggest it is also important in H/ACA small RNA function.

The NCI's longitudinal cohort study of inherited BMF syndromes, including DBA, SDS, and DC, opened to accrual in 2001, and has prospectively evaluated hundreds of patients with these disorders and their family members. This study has led to improved characterization of the wide-array of clinical phenotypes, identified numerous genes with causative mutations, and improved estimates of cancer risk in these patients. Clinical, genetic and epidemiologic data from patients with ribosome biology disorders will be presented; these aid in understanding of the human phenotypes associated with aberrations in these key ribosome biology genes.

Identification of therapeutic targets for the treatment of Diamond Blackfan Anaemia using a high-throughput screening based approach

Amee J. George[1], Lorena Nuñez Villacis[1], Mei S. Wong[1,2], Priscilla Soo[1], Maurits Evers[1], Nadine Hein[1], Sheren J. Al-Obaidi[1], Jeannine Diesch[2], Megan Pavy[1], Perlita Poh[1], Cathryn M. Gould[3], Piyush Madhamshettiwar[4], Lorey K. Smith[2], Jun Chen[5],Melissa Ilsley[5], Hamish S. Scott[6], Katherine M. Hannan[1], Thomas J. Gonda[7], Kaylene J. Simpson[1], Johan Flygare[5], Richard B. Pearson[1], and Ross D. Hannan[1,2]

1 - ACRF Department of Cancer Biology and Therapeutics, The John Curtin School of Medical Research, The Australian National University, Acton, ACT, 2601, Australia
2 - The Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, VIC, 3010, Australia
3 - Garvan Institute of Medical Research, Darlinghurst, NSW, 2010, Australia
4 - Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre, East Melbourne, VIC 3002, Australia
5 - Lund Stem Cell Centre, Biomedical Centre, Lund University, Lund, S-22184, Sweden
6 - Centre for Cancer Biology, Adelaide, SA, 5000, Australia
7 - School of Pharmacy, University of Queensland, Wooloongabba, QLD, 4102, Australia

One molecular mechanism to account for the impaired proliferation and cell death associated with bone marrow failure in Diamond Blackfan Anaemia (DBA) is the aberrant activation of the nucleolar surveillance pathway. In this model, mutations or insults that disrupt ribosome biogenesis result in the sequestration of the E3 ubiquitin ligase murine double minute 2 (MDM2) by free RPs (predominantly the 60S RPs, L5 and L11) in a complex with 5S rRNA, leading to the accumulation of p53 and subsequent induction of cell cycle arrest or apoptosis. In the case of DBA, the nucleolar surveillance response is aberrantly activated, and elevated p53 protein results in preferential apoptosis or cell cycle arrest of the erythroid progenitors required for red blood cell development and normal development. Moreover, it has also been proposed that the reduced levels of functional ribosomes in surviving erythroid cells exhibit altered translation of mRNAs that encode proteins critical for erythropoiesis. Our studies are built on the central hypothesis that understanding the molecular mechanism(s) by which DBA-causing ribosomal protein mutations activate p53, leading to the death of erythroid progenitors, will allow us to identify new therapeutic targets and drugs for the treatment of patients with Diamond-Blackfan Anaemia, as well as other ribosomopathies.

To address this, we have used a high content screening based approach to perform genome-wide loss-of-function (RNAi) and gain of function (ORF overexpression) screens, and have also screened compound libraries of all current clinically approved therapeutics, to identify the critical genes and pathways implicated in the p53-mediated nucleolar surveillance response due to ribosomal protein S19 insufficiency (as observed in DBA). We will present our current data, which has uncovered a suite of novel genes/biological processes involved in this process, as well as a number of clinically approved therapeutics that can ameliorate nucleolar sureveillance activation/p53 stabilisation. We are currently validating these targets/drugs using in vivo models. We predict that these studies will enable the development of novel treatments for patients with DBA.

A novel pathomechanism: are Cockayne syndrome and trichothiodystrophy ribosomopathies?

Fatima Khalid, Tamara Phan, Marius Alupei, Karin Scharffetter-Kochanek and Sebastian Iben

Department of Dermatology, University of Ulm, James-Franck Ring N27, 89081 Ulm, Germany

Premature aging, loss of proteostasis, ribosome, translational fidelity

The childhood premature aging diseases Cockayne syndrome(CS) and trichothiodystrophy (TTD) are attributed DNA-repair diseases as the affected genes code for DNA-repair proteins. However, no accumulating DNA-damage nor an elevated cancer incidence as a consequence of unrepaired DNA-damage has been reported in patients. The DNA-repair proteins are also involved in the key step of ribosomal biogenesis, transcription by RNA polymerase I. Here we investigated the cellular consequences of a disturbed ribosomal biogenesis and discovered that translational fidelity of the ribosomes of all patient cell lines tested is reduced. This reduced accuracy of the translation process produces misfolded proteins that provoke endoplasmic reticulum stress and an unfolded protein response that in turn represses transcription of RNA polymerase I and ribosomal biogenesis. This vicious circle can be disrupted by pharmaceutical chaperones offering a treatment opportunity for the affected children. Interestingly this pathomechanism, identified in CS-cells (Alupei et al, 2018), is also active in cells of TTD patients that also suffer from retarded growth, microcephaly and premature aging. Loss of proteostasis due to an inaccurate translation has not been described in human pathology before and offers one plausible explanation for the developmental problems and premature aging characterising these children.

Session 10: Ribosome variants & alternative biogenesis pathways

Determining differential translatomes and composition of 5.8S S and L ribosome variants in *S. cerevisiae*

Trahan Christian, Pollet Leah, van Neus Rob W., Brown Jeremy, Pechmann Sebastian and Oeffinger Marlene

IRCM, Montréal. Canada

The notion of heterogeneous ribosomes first emerged in the 1970s but regardless, over the last three decades, ribosomes have been considered monolithic in nature, identical in structure and composition within different cells and tissues of an organism. In recent years, however, an increasing number of studies have identified so called 'specialized', or differential, ribosomes differing in either ribosomal protein (r-protein) content or ribosomal RNA (rRNA) modifications in various tissues but even within the same cell type.

Another, so far unexplored, source of ribosome heterogeneity at the RNA level is the constitutive differential processing of 5.8S rRNA, which results in the production of distinct ratios of a minor long (L) and a major short (S) form of mature 5.8S rRNA; while the ratio is divergent across all eukaryotes, the presence of both 5.8S forms is highly conserved. Interestingly, it was previously observed that deletion of the RNase MRP component NME1 resulted in a shift of 5.8S S:L ratio in S.cerevisiae, and, using an in vitro translation assay, that the translation profile was altered in cells predominantly producing 5.8SL rRNA. Moreover, a point mutation in NME1 that was linked to cartilage-hair hypoplasia caused a change in the 5.8S S:L production suggesting that an imbalance in S:L ratio, and thus the number of ribosomes carrying one or the other, has severe consequences.

Here, we are using modified S. cerevisiae strains, in which the production of 5.8S rRNA is altered to create either predominantly S- or L- ribosomes, in order to investigate their differential translatomes, i.e. the mRNAs translated by either S- or L-containing ribosomes. Translatomes are identified by ribosome profiling in either strain as well as wild-type, and cross-linking coupled to mass spectrometry is used to elucidate r-protein structures, rearrangements, accessory factors and overall composition within differential mature L vs S ribosomes.

The involvement of two intersubunit ribosomal RNA base modifications in the differential translation of stress-response transcripts

Felix GM Ernst[1], Lionel Tafforeau[1], Alice Griselin[1], Virginie Marchand[2], Yuri Mmotorin[2], and Denis L.J. Lafontaine[1]

1 RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S./FNRS), Université Libre de Bruxelles (ULB), Belgium
2 Next-Generation Sequencing Core Facility, CNRS-Lorraine University, Vandoeuvre-les-Nancy, France

Ribosomal RNA modifications are largely assumed to optimize ribosomal function yet in most cases a precise role for individual modified nucleotide has not been established. Here we studied the role in translation of two large ribosomal subunit RNA modifications located on the intersubunit surface, namely: m1A2142 and m5C2278 deposited on the yeast 25S

rRNA by BMT2 and RCM1, respectively. Using ribosomal profiling, we show that mRNAs encoding proteins involved in stress-response are differentially translated in cells producing ribosomes lacking either modification. On the basis of the differential translation observed, we predicted and validated by using phenotypic assays that specific cellular pathways are impacted. Unmethylated ribosomes were found to have distinct 5' and 3' UTR presence on stress-related transcripts and distinct global codon occupancy, providing initial mechanistic insights onto differential translation. Ribosomes differing by a single methyl group are thus endowed with specific translational capacity. A conclusion that bears implications for the role of specialized ribosomes in normal and pathophysiological processes.

Specialized ribosomal protein genes regulate ribosome biogenesis and function

Mustafa Malik Ghulam, Mathieu Catala, Sherif Abou Elela

Laboratory for Functional Genomics, RNA Group, Département de microbiologie et d'infectiologie, Faculté de médecine, Université de Sherbrooke, Cancer Research Pavillon, Sherbrooke, Canada

In yeast, the majority of ribosomal proteins are produced from duplicated genes and 62% of these differ by one or more amino acid. In this study, we show that these duplicated genes produce specialized protein isoform with distinct functions. Homogenization of ribosomal protein genes resulted in defects in ribosome biogenesis, even when the overall level of the ribosomal protein is maintained, and resistance to drug. As would be expected, the absence of the major isoform affected growth under normal condition and was more likely to cause defect in ribosome biogenesis even when the amount of total protein produced is not limiting. Surprisingly, the absence of minor isoforms that represent 10 % of the total amount of proteins produced resulted in severe sensitivity to drug with little or no effect on growth under normal condition. Profiling the translation for wild type and strain expressing two copy of the one protein isoform or another in the presence or absence of drugs indicated that exposure to drugs influences translation in a ribosomal protein gene dependent manner. Indeed, exposure to cell wall related drugs like the protein kinase inhibitor Staurosporine inhibited the translation of several cell wall associated mRNAs and these inhibitions was dependent on the nature of ribosomal protein isoform. Indeed, the presence of the minor copy of ribosomal protein genes induced the expression of a specific subset of mRNA sharing common function. Together the data provide a new model of drug resistance were changing the ribosome composition promote cell tolerance to stress by remodeling the mRNA translation.

A role for eRpL22 and eRpL22-like paralogue-specific specialized ribosomes in differential translation of distinct classes of testis mRNAs in *Drosophila melanogaster*

Vassie Ware, Catherine Mageeney

Department of Biological Sciences, Lehigh University, Bethlehem, USA

Recent studies (e.g., Slavov et al., 2015; Shi et al., 2017) evaluating differences in core ribosomal protein (RP) stoichiometry and translational profiling have consolidated support for the existence of heterogeneous populations of ribosomes that regulate translation of distinct mRNAs. Our approach to understanding functional consequences of ribosome heterogeneity in development focuses on ribosomes within germ cells that differ in RP content due to assembly of a tissue-specific or constitutively-expressed paralogous RP. The eRpL22 family in Drosophila melanogaster includes paralogous genes eRpL22 and eRpL22-like. eRpL22 is expressed ubiquitously and eRpL22-like expression is tissue-restricted with highest levels in the male germline. In developing, early mitotic stage germ cells, mutually exclusive assembly of eRpL22 or eRpL22-like into ribosomes establishes heterogeneous populations of paralogue-specific ribosomes. In primary meiotic spermatocytes, eRpL22 undergoes testis-specific post-translational modifications and is sequestered in the nucleoplasm, excluding significant amounts of eRpL22 from the cytoplasmic ribosomal pool (Kearse et al., 2013). We tested the hypothesis that specialized functions are ascribed to paralogue-specific ribosomes assembled in germ cells during spermatogenesis. We performed RNA-seq on paralogue-specific polysomes (captured from testis ribosome profiles by immunoprecipitation using paralogue-specific antibodies) and revealed both unique and overlapping mRNAs and noncoding RNAs. mRNAs from constitutively expressed genes are enriched on eRpL22 ribosomes. Early mitotic stage transcripts associate with both ribosome types, with a slight bias toward eRpL22 ribosomes. Most meiotic and late stage testis-specific transcripts associate with eRpL22-like ribosomes. We conclude that eRpL22 paralogue-specific ribosomes translate distinct testis mRNAs. To investigate if testis-specific factors are required for specific mRNA association with eRpL22-like ribosomes, we expressed Shal and TIMP mRNAs (that show high fold-change differences in association with testis eRpL22-like ribosomes), in transfected S2 cell lines expressing eRpL22-like and control S2 lines. Transcripts were loaded onto both ribosome types, but were preferentially loaded onto eRpL22-like ribosomes. Results suggest that testis-specific factors are not required for preferential loading onto eRpL22-like ribosomes but may be influenced by abundance of ribosome type.

Session 11: Ribosomopathies II

Ribosomal lesions promote oncogenic mutagenesis

Sergey O. Sulima[1], Kim R. Kampen[1], Stijn Vereecke[1], Jelle Verbeeck[1], Jonathan D. Dinman[2], Kim De Keersmaecker[1]

1 - Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Leuven, Belgium
2 - Department of Cell Biology and Molecular Genetics, University of Maryland, College Park Maryland, USA

Ribosome dysfunction is known to cause disease - mutations in ribosomal proteins (RPs) predominantly affect hematopoietic tissues and are found in congenital ribosomopathies and somatic cancers. These diverse RP-mutant disorders share an intriguing paradox, also known as “Dameshek’s Riddle”: ribosomopathy patients initially suffer from cellular insufficiency (e.g. anemia), but have a high risk to develop cancer (e.g. leukemia) later in life. Moreover, the somatic, recurrent R98S missense mutation in ribosomal protein L10 (RPL10-R98S), which we have identified in pediatric leukemia, induces elevated reactive oxygen species levels and a growth defect in cell and animal models. Collectively this raises the questions of how ribosome-mutant cells can survive under high oxidative stress, and how initial cellular hypo-proliferation can ultimately transition to hyper-proliferation.

We observed a specific increase of IRES-mediated translation of the anti-apoptotic factor BCL-2 in RPL10-R98S human leukemia cells, facilitating survival but not proliferation. Instead, this proliferation defect was rescued with time by acquisition of additional mutations. Specifically, RPL10-R98S induced 5-fold more secondary mutations than RPL10-WT in cell models. The presence of RPL10-R98S and of other RP mutations also correlated with a higher mutational load in leukemia patients, with an enrichment in activating lesions in the transcription factor NOTCH1. The RPL10-R98S-induced cellular oxidative stress promoted DNA damage and impaired cell proliferation. NOTCH1 expression eliminated these phenotypes in RPL10-R98S cells, with no effect on WT cells. RP-mutant patients with other hematologic cancers also demonstrated higher mutational burdens, enriched for mutations that may diminish oxidative stress.

Our results indicate that oxidative stress due to ribosome dysfunction causes DNA damage and hypo-proliferation in ribosomopathies and RP-mutant cancer. This drives surviving cells, potentiated by genomic instability, to acquire rescuing mutations which can compensate for these initial defects. We propose that RP mutations are intrinsic cellular stressors that provide a fertile ground for mutagenesis, thereby enabling a larger mutagenic pool and in turn making oncogenesis more accessible.

An imbalance in the numbers of the two ribosomal subunits differentially affects subunit stability, but not co-regulation of transcription of r-protein genes from both subunits

Lasse Lindahl, Ananth Bommakanti, Brian Gregory, Nusrat Rahman, Md Shamsuzzaman, Mamata Thapa, Janice Zengel

University of Maryland, Baltimore County, USA

Co-transcription of rRNA for the two ribosomal subunits normally ensures biogenesis of equal numbers of the two subunits, but mutations in ribosomal genes can distort this balance. Little is known about mechanisms that can rectify an imbalance between ribosomal subunits. Here we show that repressing 60S assembly inhibits accumulation of not only 60S subunits, but also of 40S subunits, because excess 40S subunits are turned over after assembly. In contrast, cessation of the 40S pathways does not affect 60S accumulation, but does, however, lead to fragmentation of the 25S rRNA in 60S subunits. Thus the stability of each of the subunits depends on matching numbers of the two subunits.

In contrast to the differential effect on subunit stability, transcription of the ribosomal genes specific to either subunit remains co-regulated during cessation of subunit assembly. Our RNA-seq experiments show that mRNAs encoding both 40S and 60S ribosomal proteins are increased during inhibition of 60S ribosome assembly. However, ribosomal protein mRNA decreases when ribosome function is inhibited due to repression of the gene for Translation Elongation Factor 3. Thus, the ribosome regulatory systems respond differently to changes in ribosome concentration and efficiency of ribosome function.

We conclude that cells rectify an imbalance in the numbers of subunits by turning over excess subunits, not by differentially adjusting transcription for r-protein genes specific to each subunit. Since the degradation of 40S subunits involves “waste” of resources, it seems like it must be important to eliminate excess of 40S.

Ribosomal protein gene mutations activate a novel regulatory pathway which regulates the translation, the growth and the competitiveness of the cell

Marianthi Kiparaki*, Chang-Hyun Lee*, Jorge Blanco, Amit Kumar, Virginia Folgado, Zhejun Ji, Gerard Rimesso, Nicholas. E. Baker (* equal)

Albert Einstein College of Medicine, Bronx, USA

Heterozygous inactivating mutations in ribosomal protein (RP) genes have been identified in several diseases, known as ribosomopathies, which have some shared defects including bone marrow failure and growth abnormalities. In Drosophila, heterozygous mutations in many of the RP genes lead to the Minute syndrome, which includes developmental delay, slow translation and growth and reduced cell competitiveness. During cell competition, RP heterozygous mutant cells (Rp/+) are actively eliminated from mosaic tissues containing wild type cells. In our laboratory, previous genetics screensidentified two genes which block the elimination of Rp/+ cells by wt cells. One of them is RpS12 and the other one is Xrp1, which is a putative transcription factor. By employing genetic experiments, Click-chemistry, Northern and RNAseq analysis we showed that both proteins are components of a cellular

stress pathway that monitors ribosomal proteins, regulates more than 80% of the transcriptome changes that occur in Rp/+ cells and mediates the developmental delay of the Rp/+ flies. Interestingly, even the major defects in global translation are regulated by this pathway and are indirectly related to the initial RP gene mutation. This pathway may be advantageous, to eliminate the Rp/+ cells by cell competition before translation is affected non-specifically.

Combined evaluation of JmjC enzymes KDM2A and KDM2B is associated with nucleolar size and prognosis in primary breast carcinomas

Igor De Nicola, Ania Naila Guerrieri, Alice Galbiati, Marianna Penzo, Davide Treré, Lorenzo Montanaro

Università di Bologna, Bologna, Italy

Ribosome biogenesis is a fine-tuned cellular process and its deregulation is linked to neoplastic progrssion. Indeed, tumors characterized by an intense ribosome biogenesis often display a more aggressive biological and clinical behavior. rRNA synthesis is controlled at several levels, the higher one being the epigenetic regulation of the condensation of chromatin portions containing rRNA genes. KDM2A and KDM2B (Lysine (K)-specific demethylase 2A / B) are JmjC histone demethylases modulating the accessibility of rDNA genes, thereby repressing their transcription. Both enzymes are able to demethylate lysins at relevant and specific sites on histone H3: KDM2A has a target on lysine 36 monomethylate/demethylated sites (H3K36m1 / H2K36me2) whereas KDM2B is involved in the demethylation of lysine 4 trimethylated site (H3K4me3) and lysine 36 dimethylated site (H3K36me2). We previously demonstrated that KDM2B is one of the factors regulating ribosome biogenesis in human breast cancer. In the present study we aimed to define the combined contribution of KDM2A and KDM2B in a series of primary breast carcinomas evaluating KDM2A and KDM2B mRNA levels by RT-qPCR. We observed that tumors characterized by reduced levels of both KDM2A and KDM2B displayed a particularly aggressive clinical behavior and increased nucleolar size, which is considered a morphological parameter related to the activity ribosome biogenesis. We also investigated rDNA methylation status in cases where DNA was available using the MassARRAY EpiTYPER assay, a sensitive and quantitative method with single base resolution. Altogether our results suggest that KDM2A and KDM2B may cooperate in regulating ribosome biogenesis thus influencing the biological behavior of human breast cancers.

Session 12: Prokaryotic and Organelle Ribosome Assembly II

Early co-transcriptional ribosome assembly in real-time

Olivier Duss[1,2], Gala Stepanyuk[1], Seán O'Leary[2], Jody Puglisi[2], Jamie Williamson[1]

1 - The Scripps Research Institute, La Jolla, CA, USA
2 - Stanford University, Palo Alto, CA, USA

The ribosome is a large macromolecular machine that synthesizes the proteins present in the cell. In bacteria, the ribosome consists of three large RNA molecules and more than 50 proteins, which are co-transcriptionally assembled in a living cell within ~2 minutes. However, it is poorly understood how transcription and RNA folding are coupled to protein binding at the molecular level.

Here, we present a novel single-molecule approach that allows simultaneously monitoring transcription elongation and the binding of fluorescently labeled proteins to the nascent rRNA in real-time. We have investigated and compared the early assembly of the central and 3'-domains of the bacterial 30S subunit, in which either primary ribosomal proteins S15 or S7 initiate assembly, respectively.

We find that at physiological temperatures, the majority of the central domain RNA molecules bind primary r-protein S15 kinetically stably and immediately after transcription of the binding site as expected for a primary binding protein that is initiating the assembly process.

In contrast, the majority of nascent 3'-domain RNA molecules are incompetent to bind S7 or can do so only several minutes after transcription. RNA structures remote from the S7 binding site are responsible for RNA misfolding and higher temperature increases RNA folding efficiency. Analyzing the subset of nascent RNA molecules that can bind S7, we find that the S7-bound lifetime decreases from ~ 30 seconds to only ~ 4 seconds when increasing the temperature from 20° C to 35° C. The presence of all three secondary binding r-proteins S13, S9 and S19 is required and sufficient to increase the S7-bound lifetime to more than several minutes. Notably, the presence of all 3'domain r-proteins, significantly increases the nascent 3'-domain RNA folding efficiency, demonstrating their ability as RNA folding chaperones.

Overall, our results establish that during the course of assembly of the difficult-to-fold 3'-domain RNA, highly dynamic protein-RNA interactions gradually become stabilized by the binding of subsequent r-proteins, in order to eventually obtain a stable ribosome. Dynamic binding of S7 early in assembly could prevent stable trapping of misfolded RNA conformations in which the binding sites for subsequent r-proteins are not yet properly formed.

Altered conformations of E. coli ribosomes during starvation

Ryan Hulscher[1], Yumeng Hao[1], Jen Bohon[2], Peter Lobue[3], Patrick Limbach[3], and Sarah A. Woodson[1]

1 - Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218 USA
2 - Center for Proteomics and Bioinformatics and Case Center for Synchrotron Biosciences, Case Western Reserve University, Upton, NY11973 USA
3 - Department of Chemistry, University of Cincinnati, Cincinnati, OH, USA

Ribosome synthesis in all organisms responds to cell growth rates, nutrient availability, and various forms of metabolic stress. During starvation, bacterial ribosomes are either degraded and their components reused, or protected from turnover by hibernation or stress response factors. We used uridine pulse chase experiments to compare the lifetimes of ribosomal complexes in E. colicells during stationary phase or after transfer to minimal media lacking a specific nutrient. Under all conditions, 30S subunits outlasted 50S subunits, with the slowest depletion of 50S ribosomes in low glucose or low nitrogen conditions. We probed the structures of ribosomes in starved cells were by DMS modification (DMS-MaP-Seq) or X-ray hydroxyl radical footprinting (HRF-Seq). The footprinting results revealed widespread perturbations to the rRNA conformation, with strong protection of active sites under certain conditions. These results indicate that specific stress responses in E. colimodify the structures of ribosomal subunits, in some cases protecting them for future use and in other cases exposing them to turnover enzymes. We are currently analyzing the protein composition of ribosomes in starved cells. Our results suggest that specific structural perturbations to bacterial ribosomes not only optimize survival but also influence recovery of protein synthesis capacity upon nutrient upshift.

The functional core of 5’ external transcribed spacer of pre-rRNA

Jing Chen, Liman Zhang, Kegiong Ye

Institute of Biophysics, CAS Center for Excellence in Biomacromolecules, Chinese Academy of Sciences, Beijing, China

Eukaryotic ribosomes are initially assembled on a long precursor ribosomal RNA (pre-rRNA) that contains 18S, 5.8S and 25S rRNAs and four spacers. The spacers are important for ribosome assembly and need to be processed during ribosome maturation. The 90S pre-ribosome is the earliest assembly intermediate of small ribosomal subunit and is formed co-transcriptionally on the 5' part of pre-rRNA. After the pre-rRNA is cleaved at A0, A1 and A2 sites, the 90S is transformed into the pre-40S ribosome that matures into the small subunit in the cytoplasm.

The 5’ external transcribed spacer (5' ETS) is the most prominent spacer of pre-rRNA. It recruits U3 snoRNA and many assembly factors and scaffolds the assembly of 90S. The recently determined cryo-EM structures of yeast 90S have shown that the 5' ETS forms a highly branched structure that interacts extensively with the 90S structure. To study the functional role of 5' ETS, we systematically mutated the 5' ETS on a plasmid-expressed pre-18S RNA and examined how the rRNA processing and 90S assembly are affected. The data reveal the functional core of 5' ETS and provide new insights into the assembly hierarchy of 90S.

Crucial roles of P-site binding of initiator tRNA in fidelity of translation initiation and regulation of ribosome maturation/ heterogeneity in *Escherichia coli*

Umesh Varshney

Indian Institute of Science, Bangalore, India

Initiator tRNAs (i-tRNAs) are special in their direct binding to the ribosomal P-site, to decode the start codon and to determine the reading frame in an mRNA during the regulatory step of translation initiation. In all domains of life, i-tRNAs are characterized by a highly conserved feature of the presence of three consecutive G-C base pairs (3GC pairs) in the anticodon stem. In addition, eubacterial i-tRNAs (tRNAfMet) possess a mismatch at the top of the acceptor stem, which together with the second and the third base pairs, forms a major determinant for recognition of tRNAfMet by the formylating enzyme (Fmt). We have shown that the two highly conserved features (i. e. the 3GC pairs and the property of formylation) license bacterial tRNAfMet to pass through the distinct checkpoints in translation initiation. A deficiency of tRNAfMet in E. coli results in loss of fidelity of i-tRNA selection in the P-site, and initiation with even an elongator tRNA suggesting a role for regulation of i-tRNA expression in proteome diversity especially under the conditions of stress. Furthermore, we show that participation of tRNAfMet in the first round of initiation complex formation licenses (via the 3GC pairs) the final steps of ribosome maturation by signaling RNases to trim the terminal extensions of immature 16S rRNA. A deficiency of tRNAfMet results in accumulation of ribosomes with immature 16S rRNA (heterogenous ribosomes) and facilitates translation of mRNAs with variant SD sequences, and use of tRNAfMet that lacks the 3GC pairs. Implication of the role of heterogenous ribosomes in generating proteome diversity will be discussed.

Abstracts for Poster Presentation

Ordered by presenter’s last name

Starts with poster number #n

#1. Structural basis for 5'-ETS recognition by Utp4 at the early stages of ribosome biogenesi

Yasar Luqman Ahmed, Markus Kornprobst, Fabiola Rodriguez-Calvino, Klemens Wild, Ed Hurt, Irmi Sinning

Biochemistry Center BZH - University of Heidelberg, Germany

Eukaryotic ribosome biogenesis begins with the co-transcriptional assembly of the 90S pre-ribosome. The 'U three protein' (UTP) complexes and snoRNP particles arrange around the nascent pre-ribosomal RNA chaperoning its folding and further maturation. The earliest event in this hierarchical process is the binding of the UTP-A complex to the 5'-end of the pre-ribosomal RNA (5'-ETS). This oligomeric complex predominantly consists of beta-propeller and beta-solenoidal proteins. Here we present the structure of the Utp4 subunit from the thermophilic fungus Chaetomium thermophilum at 2.15 Å resolution and analyze its function by UV RNA-crosslinking (CRAC) and in context of recent cryo-EM structures of the 90S pre-ribosome. Utp4 consists of two orthogonal and highly beta-propellers that perfectly fit the EM-data. The Utp4 structure highlights an unusual Velcro-closure of its C-terminal beta-propeller as relevant for protein integrity and potentially Utp8 recognition in the context of the pre-ribosome.

#2. Structures of human 40S assembly intermediates

Michael Ameismeier, Jingdong Cheng, Otto Berninghausen, Roland Beckmann

Gene Center Munich and Center of Integrated Protein Science-Munich (CiPS-M), Department of Biochemistry, University of Munich, Munich, Germany

Eukaryotic ribosome biogenesis spans from the nucleolus to the cytoplasm and entails hundreds of assembly factors that ensure correct processing, modification and folding of rRNA and concomitant incorporation of ribosomal proteins. Significant differences in rRNA processing illustrate deviating pathways in ribosome formation between yeast and humans. At the same time, however, there is a remarkable structural conservation of many assembly factors and of the ribosome itself. Yet, high-resolution structural information of assembly intermediates has so far been limited to fungi. Using cryo-electron microscopy and single particle analysis, we solved structures of late human 40S assembly intermediates, ranging from nuclear to late cytoplasmic states. Differences in particle conformation and factor composition allowed their chronological arrangement. In our earliest particles, distinct immature rRNA conformations, supported by the ribosome biogenesis factor RRP12, define the formation of the 40S subunit head. Together with insights from molecular models of late-acting assembly factors TSR1, RIOK1, RIOK2, ENP1, LTV1, PNO1 and NOB1 from our later particles these results highlight the sequential and step-wise nature of human 40S subunit assembly. Furthermore, the molecular model of NOB1 and the unprocessed rRNA 3' end reveal an inactive nuclease conformation that requires structural rearrangements of the PNO1-bound 3'-rRNA, thereby coordinating the final rRNA folding steps with site 3 cleavage.

#3. Regulation of rRNA transcription by SUMO pathway

Yicheng Luo[1], Elena Fefelova[2] and Alexei A. Aravin[1,2]

1 Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA USA
2 Institute of Molecular Genetics, Russian Academy of Sciences, Moscow 123182, Russia

Similar to other organisms, the genome of Drosophila melanogaster contains many copies of identical rDNA units organized in extended arrays. It is believed that only fraction of available rDNA unites are expressed in each cell, however, molecular mechanisms responsible for selection of active rDNA units and silencing of inactive rDNA remain poorly understood. We studied regulation of expression of normal rDNA and units damaged by integration of retrotransposons and found that their expression is strongly regulated by SUMO pathway.

#4. Cell competition exploits ribosomal protein genes as sensors of genome damage and aneuploidy

Zhejun Ji and Nicholas E. Baker

Genetics Dept, Albert Einstein College of Medicine, Bronx, NY 10461, USA

All organisms devote considerable resources to the DNA damage response, but it is not known if it would be possible to recognize those genomes that have been irreversibly altered in the process of replacing damaged nucleotides. One theory is that ribosomal protein genes could serve as sentinels for damaged genomes. Most of the ribosomal protein family are encoded by single copy genes dispersed around the genome, and changes in their relative expression level could be detected during ribosome assembly in cells with mutations, genome copy number changes, or aneuploidy. In Drosophila, cells with ribosomal protein mutations can be eliminated by cell competition. We made use of a mis-sense mutation of RpS12 that prevents cell competition to test whether cell competition is required to eliminate genetically damaged cells. Here we report that after irradiation in vivo, cell competition helps eliminate a subset of damaged cells in a p53-independent manner, and many of these cells show signs of radiation-induced ribosomal protein defects. We then used FLP-FRT recombination to generate heterozygous, somatic deletions of hundreds of genes from cells in growing tissues, and found that these cells survive when no ribosomal protein loci are affected but are eliminated by cell competition if they reduce ribosomal protein gene copy number. Therefore, mutation or heterozygous loss of ribosomal protein gene loci is indeed a signal for elimination of damaged cells in Drosophila. We also showed that Drosophila Minute mutants, constitutively lacking one copy of any of many ribosomal protein genes, are defective for cell competition and unable to remove cells with segmental aneuploidy. The Minute mutations model Diamond-Blackfan Anemia patients who harbor constitutive mutations in ribosomal protein genes. The cancer predisposition of Diamond-Blackfan Anemia patients is consistent with a significant role for ribosomal proteins and cell competition in sensing genome damage and aneuploidy during tumor surveillance in normal individuals.

#5. Characterizing the structure and interaction of an mRNA regulator responding to ribosomal protein S15 from Mycobacterium smegmatis

Daniel M. Beringer, Josephine Tang, Michelle M. Meyer

Boston College, Chestnut Hill, MA, USA

Precise stoichiometric ratios of ribosomal proteins and ribosomal RNAs (rRNAs) are required for optimal ribosome assembly and function. When present in excess, some ribosomal proteins bind to a structured portion of the 5' untranslated region of their own mRNA (in addition to their primary binding partner, the rRNA) and repress translation to form a negative feedback loop. While autogenous cis-regulation of ribosomal proteins is widespread across diverse bacterial species, in some cases the mRNA regulatory structures known in E. coli are narrowly distributed and alternative structures are found in other bacterial clades. Ribosomal protein S15 has a highly conserved binding interaction with the 16S rRNA. However, four unique mRNA regulators for S15 have been identified previously across four different classes of bacteria. Here we validate the regulatory activity of a fifth putative S15 regulator that originates from Mycobacterium smegmatis. Extensive mutational analysis reveals that only very severe mutations to the RNA sequence abolish regulation in vivo, suggesting a robust binding interaction dependent on a G•U/GC motif that mimics the 16S rRNA. In vitro binding assays using the S15 homolog from the thermostable Mycobacterium thermoresistible confirm the importance of the G•U/GC motif for binding.

#6. Auxiliary Factors of the RNA helicase Prp43 in ribosome biogenesis

Priya Bhutada, Stefan Unterweger, Lisa Kofler, Valentin Mitterer and Brigitte Pertschy

University of Graz, Austria

Ribosome biogenesis is a complex, tightly regulated and highly energy demanding process. To our current knowledge ribosome biogenesis needs participation of RNA-polymerases, 82 ribosomal proteins, 66 (small nucleolar RNA) snoRNAs and more than 200 non ribosomal proteins. Vast development and progress in the field keep updating new players in the crucial process. These factors carry out molecular rearrangements, modifications and rRNA processing steps within ribosomal precursors for maturation and functional assembly of ribosome. These include assembly factors, endo –and exonucleases, ATPases, transport factors, RNA helicases.

Among the RNA helicases, Prp43 DEAH/RHA helicase plays multiple roles at different stages in both 40S and 60S ribosomal subunit biogenesis and also participates in mRNA splicing. As Prp43 is responsible for essential processes, it necessitates the engagement of many coordinating auxiliary factors for the accomplishment of its functions. These co-factors of Prp43 include a group of proteins sharing a glycine-rich domain, termed G-patch domain. G-patch proteins Ntr1, Pxr1, Pfa1 and Cmg1 are known to stimulate ATPase and RNA helicase activity of Prp43. In mRNA splicing G-patch protein Ntr1 is responsible for Prp43 helicase activation, while the non G-patch auxiliary factor Ntr2 functions as adaptor for the recruitment of Prp43 to the spliceosome. Pxr1 and Pfa1 are involved in ribosome

biogenesis.

Our research on Prp43 and its associated G-patch and non G-patch factors reveals interactions between them and also focuses on their functional roles in ribosome biogenesis. This will introduce new members of ribosome biogenesis and also helps to understand molecular interactions important to carry out functional roles in the complicated process of ribosome biogenesis.

#7. The DEAD-box helicase Drs1 remodels nucleolar pre-60S subunits prior to C2 cleavage

Stephanie Biedka and John L Woolford

Carnegie Mellon University, USA

During assembly of 60S ribosomal subunits in yeast, cleavage of 27SB pre-rRNA at the C2 site requires the hierarchical recruitment of 11 ribosomal proteins and 14 assembly factors. Recent cryo-EM structures combined with biochemical studies revealed that these ribosomal proteins and assembly factors are necessary for remodeling events several steps upstream of C2 cleavage, rather than the cleavage event itself. Now that we better understand the changes in pre-60S subunit composition and structure that take place prior to C2 cleavage, our new goal is to understand how these remodeling events are regulated and powered. The DEAD-box helicase assembly factor Drs1 has previously been shown to be necessary for C2 cleavage, but it remains unclear exactly what roles Drs1 might play. We are investigating the effects of inhibiting the ATP binding activity of Drs1, as well as the significance of interactions between Drs1 and assembly factors bound near the nascent polypeptide exit tunnel. Preliminary results suggest that Drs1 may be responsible for powering or triggering removal of one or more groups of assembly factors prior to C2 cleavage.

#8. Utp14 interaction with the Small Subunit Processome.

Joshua J. Black[1], Zhaohui Wang[1], Lisa M. Goering[2], Arlen W. Johnson[1]

1 - The University of Texas at Austin, Austin, Texas, USA
2 - St. Edward's University, Austin, Texas, USA

The SSU Processome (sometimes referred to as 90S) is an early stable intermediate in the small ribosomal subunit biogenesis pathway of eukaryotes. Progression of the SSU Processome to a pre-40S particle requires a large-scale compaction of the RNA and release of many biogenesis factors. The U3 snoRNA is a primary component of the SSU Processome and hybridizes to the rRNA at multiple locations to organize the structure of the SSU Processome. Thus, release of U3 is prerequisite for the transition to pre-40S. Our lab proposed that the RNA helicase Dhr1 plays a crucial role in the transition by unwinding U3 and that this activity is controlled by the SSU Processome protein Utp14. How Utp14 times the activation of Dhr1 is an open question. Despite being highly conserved, Utp14 contains no recognizable domains, and how Utp14 interacts with the SSU Processome is not well characterized. Here, we used UV crosslinking and analysis of cDNA and yeast two-hybrid interaction to characterize how Utp14 interacts with the pre-ribosome. Moreover, proteomic analysis of SSU particles lacking Utp14 revealed that Utp14 is needed for efficient recruitment of the RNA exosome. Our analysis positions Utp14 to be uniquely poised to communicate the status of assembly of the SSU Processome to Dhr1 and

possibly to the exosome as well.

#9. Role of low temperature viability protein (LTV1) in Arabidopsis ribosome biogenesis

Peta Bonham-Smith and Kumarakurubaran Selvaraj

University of Saskatchewan, Canada

Ribosome biogenesis is a process that is well described in bacteria, yeast and mammals, however, little is known in plants. In yeast, HRR25 is an essential gene encoding a casein kinase1-like (CK1L) protein that interacts with the RNA helicase LOW TEMPERATURE VIABLE1. This HRR25-LTV1 complex regulates the incorporation of RPS3 and RPS10 onto the 40S small subunit. In Arabidopsis thaliana, 14 CK1L isoforms (1-13, 9α-β) have been reported as plasmodesmata-associated kinases. Five of the 14 (including one pseudogene) closely cluster with yeast HRR25p. Each of the four AtHRR25A-D isoforms differentially associate with AtLTV1. Differential, tissue-specific expression of the four isoforms suggests that there is tissue-specific, developmental-specific and/or stress-specific AtHRR25A-D:LTV1 interactions in Arabidopsis. Unlike yeast, knockout mutations of *AtLTV1*are not lethal to Arabidopsis.

#10. Annotation and characterization of human snoRNAs with snoDB: an updated relational database

Philia Bouchard-Bourelle, Darren Mathurin St-Pierre, Gabrielle Deschamps-Francoeur, Vincent Boivin, Jean-Michel Garant, Sherif Abou Elela, Michelle Scott

RNA group - Département de Biochimie - Faculté de médecine - Université de Sherbrooke, Canada

Small nucleolar RNAs (snoRNAs) are an abundant and highly structured class of RNA essential for modifications in ribosomal RNA (rRNA) and were recently implicated in the regulation of gene expression. snoRNAs are classified into H/ACA and C/D box snoRNAs based on their structure and type of modification they guide. The majority of snoRNAs are encoded in introns and identify their target RNA through base-pairing with the guide sequence. While the basic function of snoRNAs in the modification of rRNA is well studied the mechanism that regulate their expression, their link to their host gene and potential non-ribosomal targets remain unclear. Here we present snoDB: an updated snoRNA database that not only allows for the identification and characterization of snoRNAs but also includes the expression profile of their host genes and potential targets. By using data generated from total RNA sequencing methods capable of detecting both snoRNA and mRNA in a single sample, we have created a comprehensive catalog of the host gene, snoRNA and mRNA target expression profile. To better understand the data and its archival roots we have provided links to more established sources of genomic and transcriptomic data like snoRNAbase, Ensembl, Rfam as well as to relevant literature. This unique database features expression data in a growing number of different tissues and cell lines along with continuously updated snoRNA target information and more. The information can easily be sorted and viewed thanks to multiple integrated search engines and the dynamic table system that enables the selective listing and moving of data. All of which is complimented by individual pages profiling each snoRNA entry along with available graphical data. All of

the data is readily available for download on the snoDB website which can be found at the following address: http://scottgroup.med.usherbrooke.ca/snoDB/.

#11. Ribosomal RNA Biogenesis in rice

Runlai Hang, Zhen Wang, Xian Deng, Chunyan Liu, Bin Yan, Chao Yang, Xianwei Song, Beixin Mo, Xiaofeng Cao

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

Ribosome biogenesis is highly complicated and energy consuming, as one of the most fundamental cellular progress, It begins with transcription of the rDNA by RNA polymerase I (Pol I). The resulting pre-rRNA transcript undergoes systematic processing, where multiple endonucleolytic and exonucleolytic cleavages remove the external and internal transcribed spacers (ETS and ITS). rRNA biogenesis has been deciphered in Saccharomyces cerevisiae and, to some extent, in Xenopus, mammalian cells, and Arabidopsis thaliana. However, the processing sites and pathways remain largely unknown in crops, particularly in monocots such as rice, one of the most important food resources in the world.

We identified the rRNA precursors produced during rRNA biogenesis and the critical endonucleolytic cleavage sites in the transcribed spacer regions of pre-rRNAs in rice. We further found that two alternative pre-rRNA processing pathways coexist in rice.

Rice originated from tropical and subtropical regions; therefore, rice cultivated in temperate zones can exhibit more sensitivity to chilling stress than other crops such as barley (Hordeum vulgare L.) and wheat (Triticum aestivum L.). Accordingly, rice has evolved to adapt to chilling stress. We identified that exposing rice to chilling stress resulted in the inhibition of rRNA biogenesis mainly at the pre-rRNA processing level, suggesting that these energy-intensive processes may be reduced to increase acclimation and survival at lower temperatures.

Overall, our study identified the pre-rRNA processing pathway in rice and showed that ribosome biogenesis is quickly inhibited by low temperatures, which may shed light on the link between ribosome biogenesis and environmental acclimation in crop plants.

#12. Determining the Molecular Pathogenesis of the Emg1 D86G Mutation in the Bowen-Conradi Hutterite Syndrome Ribosomopathy

Trent Nelson, Courtney L. Harris, and J. Michael Charette

Brandon University, Manitoba, Canada

Bowen-Conradi Syndrome (BCS) is a developmental disorder prevalent in the Hutterite population of the Canadian Prairies. It is due to a D86G mutation in the ribosome assembly/SSU processome protein Emg1 making it a ribosomopathy, a new group of genetic disorders associated with ribosome assembly defects. Ribosomopathies are associated with cancer and display an unexpected tissue proclivity. Emg1 is a pseudouridine methyltransferase that targets the 18S rRNA in the decoding center of the ribosomal SSU, a region involved in decoding and translational fidelity, and is also believed to chaperone pre-rRNA folding in SSU assembly. The BCS mutation is suggested to structurally perturb Emg1, significantly altering its structure and protein-protein interactions. One such interaction is between Emg1 and another SSU processome component, Utp2

(NOP14 in humans). It has been reported that this interaction is required for Emg1 to localize to the nucleolus, the region of the nucleus in which ribosome assembly occurs. This loss of interaction would severely affect ribosome assembly and could potentially be the molecular cause of BCS. Using Saccharomyces cerevisiae, we have created a yeast model system of BCS. This was done by replacing the endogenous Emg1 promoter with a conditional promoter allowing for genetic depletion of the wild-type protein. The BCS and other mutant and truncated Emg1 constructs were FLAG-tagged and constitutively expressed from a plasmid. This yeast BCS model system allows us to monitor SSU processome assembly and ribosome biogenesis. Growth curve analysis, a proxy for ribosome assembly, shows a distinct growth defect, and thus likely ribosome mis-assembly, between wildtype and BCS mutant Emg1 and is currently being characterized by pre-rRNA processing northern analysis. Antibiotic sensitivity assays suggest that BCS mutant ribosomes may be structurally and thus translationally altered, as is also found in other cancer-associated ribosomopathies. We have also analyzed the interaction between Emg1 and Utp2, another SSU processome component using the yeast-two hybrid (Y2H) system. We performed Y2H plate and liquid assays using two different reporter genes (HIS3 and ADE) and are currently validating these interactions by co-immunoprecipitation (co-IP). Through our comprehensive assays, we show that both yeast and human Emg1 and Utp2 exhibit a strong interaction, and that in the presence of the BCS mutation, this interaction is abolished. Thus, we have constructed and partially validated a yeast model system of BCS, showing that the mutation causes a growth defect and is likely to perturb the structure and thus the translational fidelity of the ribosome. We have additionally shown that the BCS mutation alters the protein-protein interaction between Emg1 and Utp2, an interaction reported to be essential for Emg1 to localize to the nucleus/nucleolus for ribosome assembly.

#13. 3.2-Å-resolution structure of the 90S preribosome before A1 pre-rRNA cleavage

Jingdong Cheng[1], Nikola Kellner[2], Otto Berninghausen[1], Ed Hurt[2] and Roland Beckmann[1]

1 - Gene Center Munich and Center of integrated Protein Science-Munich (CiPS-M), Department of Biochemistry, University of Munich, Munich, Germany
2 - Biochemie-Zentrum der Universität Heidelberg, Heidelberg, Germany

The 40S small ribosomal subunit is co-transcriptionally assembled in the nucleolus as part of a large chaperoning complex called 90S pre-ribosome or small subunit processome. We present the structure of the 90S pre-ribosome from Chaetomium thermophilumat near-atomic resolution of 3.2Å, allowing to build atomic structures for 34 assembly factors including Mpp10 complex, Bms1, Utp14 and Utp18, and the complete U3 snoRNP. Moreover, we visualize the U3 RNA hetero-duplexes with5' external transcribed spacer (5' ETS) and pre-18S RNA, and their stabilization by 90S factors. Overall, thestructure explains how a highly-intertwined network of assembly factors and pre-rRNA can guide the sequential and independent folding of the individual pre-40S domains, while the RNA regions forming the later 40S active sites are kept immature. By identifying the unprocessed A1 cleavage site and its nearby Utp24 endonuclease, we finally suggest a proofreading model for regulated 5' ETS separation and 90S>pre-40S transition.

#14. Casein Kinase 2 Catalytic Subunits a1/a2 of the SSU Processome's UTP-C Sub-Complex Regulate Growth Likely Through Ribosome Biosynthesis

James J.L. Cluff, Elise A. Poole and J. Michael Charette

Brandon University, Manitoba, Canada

The nucleolus, the site of ribosome assembly, has long been used as a diagnostic and prognostic marker of cancer. However, a direct role for the ribosome in cancer has only recently been uncovered. It is now known that cellular growth rate is directly dependent on the rate of ribosome biosynthesis and that ribosome assembly defects, collectively called ribosomopathies, are associated with various cancers, such as in Diamond-Blackfan anemia and myelodysplastic syndrome. In support of this link, examples abound where the expression of ribosome assembly factors and of ribosome assembly itself is upregulated in many cancers. In addition, select chemotherapeutic agents are known to inhibit ribosome assembly. The small subunit processome (SSU Processome) is a large ribonucleoprotein complex responsible for the assembly of the SSU of the ribosome. It consists of five known sub-complexes, one of which (the UTP-C subunit) is believed to contain the protein kinase/casein kinase CK2 complex. This heterotetramer consists of the catalytic CKα/a1 and CKα'/a2 and the regulatory CKβ/b1 and CKβ'/b2 subunits. CK2 is a ubiquitous and constitutively active serine/threonine kinase implicated in many cellular processes including growth, differentiation, and neoplasia and is the target of chemotherapeutic agents currently under development. In addition to its membership in the SSU processome, CK2 is also known to regulate the activity of RNA Polymerase I and to stoichiometrically coordinate ribosomal protein production with ribosome assembly. Using the yeast Saccharomyces cerevisiae as a model system, we are determining the role of CK2 in the regulation of ribosome assembly by genetically depleting cells of individual and pairs of CK2 subunits. This was done by replacing the endogenous promoter of each of the CK2 components with a galactose inducible/glucose repressible promoter, thus allowing for genetic depletion of individual or pairs of CK2 proteins. As growth is directly correlated to and dependent on ribosome assembly, growth curves were used as a surrogate for ribosome assembly. The growth of wildtype cells was compared to that of cells genetically depleted of individual or pairs of CK2 components. We find that genetic depletion of individual catalytic subunits CKα/a1 or CKα'/a2 results in a reduction in growth while simultaneous depletion of both catalytic subunits is lethal, as seen by growth arrest. Overexpression of catalytically dead CKα/a1 or CKα'/a2 unexpectedly yields a dominant negative phenotype. Single and double depletion of the regulatory CKβ/b1 and CKβ'/b2 proteins shows no change in growth. Northern analysis of pre-rRNA processing will be used to identify putative changes in pre-rRNA processing upon depletion of the catalytic CKα/a1 and CKα'/a2 subunits. Membership of CK2 in the SSU processome will be confirmed by co-IP of each of the individual CK2 proteins with the known SSU processome components Mpp10 and U3 snoRNA.

#15. Thermodynamic characterization of a Mpp10 fragment binding to Imp3 and Imp4

Xin Liu[1], Binal Shah[1], Xiaomeng Shah[1], Sanna Kenney[2], Shrasta Tamrakar[2], Haozhen Xu[2], Lihua Jin[2] and Carl Correll[1]

1 - Department of Biochemistry and Molecular Biology, Rosalind Franklin University of Medicine and Science, North Chicago, IL
2 - Chemistry Department, DePaul University, Chicago IL

The terminal knob structures in 'Miller chromatin spreads' of actively transcribing pre-rRNA contain the UTP-A and UTP-B complexes, the Mpp10-Imp3-Imp4 heterotrimer and the U3 snoRNP. The UTP-A and UTP-B complexes assemble on the 5' external transcribed spacer (5' ETS) with the heterotrimer and U3 snoRNP bridging the interface between this 5' ETS base and the pre-18S body. Here, we investigate in vitro assembly of the heterotrimer, specifically Imp3, Imp4 and Mpp10*, which is a fragment of Mpp10 containing the residues (265-505) necessary and sufficient for binding to Imp3 and Imp4. Proteins from Saccharomyces cerevisiae were recombinantly expressed in E. coli. Imp3 and Imp4 were observed binding to Mpp10* by gel shift assays and by fluorescence polarization. Using isothermal titration calorimetry, we determined the thermodynamic properties of the Mpp10*-Imp3 and Mpp10*-Imp4 complexes. Imp3 and Imp4 each bind to Mpp10* in a 1:1 molar ratio with nM affinity that decreases with increasing temperature in accord with the exothermic nature of the interaction. Imp4 binds more tightly to Mpp10* than Imp3 does. Both Imp3 and Imp4 binding to Mpp10* is enthalpically driven as the exothermic binding is accompanied by a negative entropy change. We will discuss these thermodynamic properties in light of the recent 90S preribosome cryoEM structures and the sequence conservation of the Imp3-Mpp10 and the Imp4-Mpp10 binding surfaces.

#16. Using targeted box C/D snoRNP-mediated RNA methylation to investigate pre-ribosomal RNA cleavage events in budding yeast

Rebecca Jade Cunningham, Nicholas Watkins, Claudia Schneider

Newcastle University, Newcastle, United Kingdom

Endonucleases cleave the pre-ribosomal RNA at multiple sites to initiate release of the mature rRNAs and to enable removal of external and internal transcribed spacer regions. All pre-rRNA cleavage events take place within large pre-ribosomal particles. It is therefore challenging to identify individual enzymes and to assess how their activity is regulated within these complexes. As a consequence, the enzyme cleaving at one of the three early sites important for 18S rRNA release (A0) is still unknown and proposed candidates for another site remain controversial.

Here, we present a novel "modification-interference" method in S. cerevisiae to investigate processing events at individual sites (A0, A1, D, A2, A3, B1L), without depleting or mutating or even knowing the identity of the respective endonuclease(s). In this system, artificial box C/D snoRNPs are designed to block individual pre-rRNA cleavage events from occurring, by directing methylation of the pre-rRNA, either directly at or in the vicinity of the cleavage site. Conditional expression of snoRNPs targeting nucleolar (site A3, RNase MRP) and cytoplasmic (site D, Nob1) cleavage sites strongly affected growth and pre-rRNA processing, while snoRNPs targeting sites A0 and A2 had a milder effect. Importantly, cleavage at sites A3 and D was not affected when the snoRNP was designed to methylate

3 nt downstream of the cleavage site, suggesting a direct impact of the pre-rRNA modification on the catalytic activity of the enzymes.

Taken together, our approach can be used to further characterise pre-rRNA cleavage events and also provides us with a new tool to stall pre-ribosomal complexes at defined processing steps.

#17. The Atypical Dual Specificity Phosphatase hYVH1 Associates with Multiple Ribonucleoprotein Particles

Qiudi Geng, Ashley DaDalt, and Panayiotis O. Vacratsis

University of Windsor, Windsor, ON, Canada

Human YVH1 (hYVH1, also known as DUSP12) is a poorly characterized atypical dual specificity phosphatase widely expressed in human tissues and is evolutionarily conserved. It contains an N-terminal phosphatase domain and a novel C-terminal zinc-binding domain (ZBD). Recent findings have demonstrated that hYVH1 expression affects cellular DNA content and that it is a novel cell survival phosphatase, preventing both thermal and oxidative stress induced cell death. Moreover, the hyvh1 gene has been found significantly amplified in various cancers that have progressed to advanced stages. In yeast models, YVH1 has been identified as having a significant role in ribosome biogenesis; more specifically, it is a “releasing factor” required for proper assembly of the ribosome stalk.

Here, we found that hYVH1 associates with numerous ribonucleoprotein particles using affinity chromatography coupled with mass spectrometry. hYVH1 co-sediments with the 60S ribosomal subunit and the ZBD is indispensable for 60S co-fractionation, which is consistent with what was found in yeast. Also, hYVH1 associates with stress granules under arsenic stress and modulates their size and architecture. siRNA-mediated silencing of hYVH1 expression does not affect stress granule formation; however, recovery experiments demonstrated hYVH1 is involved in the disassembly of stress granules. Moreover, deletion analysis revealed that the ZBD is likely the primary mediator, as this region is sufficient for the hYVH1-mediated effects. Finally, the tyrosine kinase Src, an oncogene, was found to regulate the interaction between hYVH1 and a binding partner, Hsp70. Interestingly, Hsp70 also functions in stress granule disassembly, suggesting that Src-mediated phosphorylation of hYVH1-Hsp70 may represent a novel temporal/spatial mechanism regulating mRNP dynamics and cell survival.

#18. Ribosomal proteins L14 and L16 contribute to the early assembly of 60S ribosomal subunits in Saccharomyces cerevisiae

Olga Rodríguez-Galán[1], Francisco Espinar-Marchena[1], José Fernández-Fernández[1], Jan Linnemann[2], and Jesús de la Cruz[1]

1 - Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, and Departamento de Genética, Universidad de Sevilla, Seville, Spain. Avda. Manuel Siurot, E-41013 Seville, Spain.
2 - Institut für Biochemie III, Universität Regensburg, 93053, Regensburg, Germany.

The contribution of most ribosomal proteins to ribosome synthesis has been quite well analysed in yeast. However, few ribosomal proteins still await characterisation. Herein, we

show that L14 and L16, which are two essential neighbouring 60S proteins, assemble in the nucleolus at an early stage into pre-60S particles. Depletion of either protein result in a deficit in 60S subunits and defective processing of 27SA2 and 27SA3 to 27SB pre-rRNAs. As a result, 27S pre-rRNAs are subjected to turnover and export of pre-60S particles is blocked. These phenotypes likely appear as the direct consequence of the reduced pre-60S particle association not only of L14 or L16 upon their depletion but also of a set of other neighbouring ribosomal proteins located at the solvent interface of 60S subunits and the adjacent region surrounding the polypeptide exit tunnel. These pre-60S intermediates also lack some essential trans-acting factors required for 27SB pre-rRNA processing but accumulate practically all factors required for processing of 27SA3 pre-rRNA. We have also analysed the functional interaction between the eukaryote-specific C-terminal extensions of L14 and L16. Our results indicate that removal of the most distal parts of these extensions cause slight translation alterations in mature 60S subunits.

#19. p53 induces a survival transcriptional response following inhibition of ribosome biogenesis

Claire Mauvais and Catherine Denicourt

The University of Texas Health Science Center at Houston, USA

Inhibition of ribosome biogenesis generally elicits a nucleolar stress response leading to p53 activation and the establishment of a G1 cell cycle arrest. Besides the transcriptional activation of the cell cycle inhibitor p21, the extent of the p53-specific transcriptional program activated after nucleolar stress is currently unknown. Given the growing interest in targeting ribosome synthesis as a novel therapeutic approach for cancer, we performed a transcriptomic analysis to better understand the p53 specific transcriptional response triggered by nucleolar stress. Our analysis revealed that instead of upregulating apoptotic genes after inhibition of rRNA synthesis, p53 elicited the activation of a pro-survival transcriptional program, which included genes involved in autophagy, basal metabolism, ROS control and cell migration. We also found that blocking p53-dependent transcription of metabolic genes or the induction of autophagy dramatically reduced the viability of cancer cells treated with selective RNA pol I inhibitors. These results demonstrate that targeting p53-regulated pathways could be used to enhance the efficacy of ribosome biogenesis drugs used for the treatment of cancers with active p53.

#20. Upregulation of H/ACA snoRNAs promotes cell proliferation and tumour aggressivity

Laurence Faucher-Giguère*, Audrey Roy*, Sonia Couture*, Michelle Scott, Sherif Abou-Elela

*co-author
Université de Sherbrooke, Sherbrooke, Canada

Small nucleolar RNAs (snoRNAs) are a ubiquitous class of non-coding RNA involved in the modification and processing of rRNA. In addition, snoRNAs were also associated with non-ribosomal functions including mRNA surveillance and their expression was linked to different types of cancers. However, the nature of snoRNA association with cell function and cancer biology remains unclear. Here we show that H/ACA snoRNA expression

increases tumour aggressivity by promoting cell survival and resistance to apoptosis. Comparison between borderline and high-grade ovarian cancer tumours indicated that while the majority of C/D box snoRNAs are downregulated or unchanged, many H/ACA snoRNA are upregulated in high-grade ovarian cancer. Strikingly, the abundance levels of specific H/ACA snoRNA were capable of accurately discriminating between borderline and high-grade cancer and their knockdown severely impaired cell proliferation and induced apoptosis. Remarkably, the effect of H/ACA snoRNA on the growth of tumour cell lines was more pronounced than snoRNA involved in rRNA processing like U3 snoRNA suggesting a major impact on ribosome biogenesis. Indeed, analysis of rRNA expression indicated that snoRNA depletion inhibits the production of the 28S rRNA consistent with a defect in ribosome production. Together the data reveal a new model where cancer-induced changes in the expression of H/ACA snoRNA may contribute to the malignant potential of ovarian tunours through the modification of ribosome production.

#21. Repression of ribosomal genes in Drosophila

Elena Fefelova[1], Mikhail Klenov[1], Vladimir Gvozdev[1], Alexei Aravin[1,2]

1 - Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russian Federation
2 - Division of Biology, California Institute of Technology, Pasadena, CA, USA

In many organisms clusters of ribosomal genes are composed of several hundred units, however, not all rDNA units are expressed. How the units that have to be repressed are selected and molecular mechanism of their silencing are poorly understood. We are interested in identifying mechanisms of rDNA silencing in Drosophila melanogaster. The characteristic of Drosophila melanogaster ribosomal genes is the presence of specific retrotransposons, R1 and R2, which are inserted in 28S rDNA, lacking their own promoters and are expressed as part of the rRNA precursor. In most cases, rDNA units with R1 and R2 insertions are strongly repressed, and any change in their expression might serve as an indicator of silencing failure. We identified mutations that lead to strong derepression of ribosomal retrotransposons. Chromatin immunoprecipitation revealed a decrease in the typical marker of heterochromatin, the trimethylation of histone H3 on lysine 9 (H3K9me3) located on retrotransposons and rDNA regions in mutants compared with control. However, lysine-to-arginine mutations at H3K9 using the histone replacement system leads to insignificant up-regulation of R2. This suggests that the repressive histone marks are not sufficient for repression and distribution of transcriptional activity in rDNA clusters.

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#22. Characterization of Diamond-Blackfan Anemia Silent Carriers via Induced Pluripotent Stem Cell Lines

Germain DR, Mirea M, Doblinger M, Stemberger R, Weitzer S, Volz J, Kager L, Penninger JM, Agu CA, Martinez J.

Max F. Perutz Laboratories/ Medical University of Vienna, Austria

Diamond-Blackfan anemia (DBA) is a ribosomopathy characterized by erythroid aplasia in early childhood. Mutations in numerous large and small ribosomal protein genes can result in disease onset in a dominant fashion thought to occur due to disrupted rRNA processing.

While other hematopoeitic cell lineages are unaffected, red blood cell (RBC) counts are reduced to extremely low levels, with some patients indefinitely reliant on RBC transfusions. Investigation of hereditary cases have shown that some related individuals are “silent carriers”, who carry the disease causing allele without the disease state. We have generated induced pluripotent stem cell (iPSC) lines from each individual from a family carrying the most common DBA allele (C>T 184, RPS19). To date, we have generated duplicate cell lines from two healthy control, two silent carriers and two DBA patients. Northern blot analysis of ribosomal precursors has revealed that the silent carrier iPSC lines have disrupted rRNA processing, similar to DBA cell lines. Utilizing iPSC lines provides us with unique opportunities to reveal differences between DBA and silent carrier biology that may lead to novel treatment options.

#23. Mutations in RPL17 change ribosome composition and inform the biochemical signatures of Diamond-Blackfan anemia

Florence Fellmann*, David W. Reid*, Carol Saunders*, Marie-Françoise O’Donohue*, Kelsey A. McFadden, Nathalie Montel-Lehry, Jinlong Liang, Pietro Farinelli, Narjesse Karboul, Jason R. Willer, Lorraine Fievet, Zahurul Alam Bhuiyan, Alissa L. Wall, Jacques S. Beckmann, Joy Fulbright, Carlo Rivolta, Nicholas Katsanis, Lydie Da Costa, Christopher V. Nicchitta, Pierre-Emmanuel Gleizes^, Erica E. Davis^

University Hospital Lausanne, University of Lausanne / Children's Mercy Hospital Kansas City / Paris 7 University, INSERM, AP-HP / University of Toulouse, CNRS / Duke University Medical Center

Diamond-Blackfan anemia (DBA) is a congenital erythroblastopenia associated with haploinsufficiency of more than fifteen ribosomal protein genes essential for ribosome biogenesis. Despite this large number of genes, genotype-phenotype correlations are limited, and the tissue specificity of the disease remains a central question given the ubiquitous nature of ribosome synthesis. Here, we report one large pedigree and two sporadic individuals with novel mutations in ribosomal protein-encoding gene RPL17. As observed with other DBA-associated genes, rpl17 loss-of-function in developing zebrafish embryos resulted in micrognathia and anemia. Consistent with the phenotype observed in cells depleted of RPL17, lymphoblastoid cell lines (LCLs) from RPL17 mutated DBA patients displayed a strong defect in pre-rRNA cleavage at site 2. Strikingly, patient LCLs contain a 5' truncated form of 5.8S rRNA (5.8SC) in addition to the known 5.8SL and 5.8SS. This unusual form of 5.8S rRNA is present in up to 15-20% of the 60S subunits engaged in translation. Using ribosome profiling, we characterized a repertoire of mRNAs differentially translated in RPL17 mutated patient cells. Gene ontology analysis showed significantly reduced translation of mRNAs related to DBA features. In contrast, genes encoding components of the translation machinery were upregulated. Importantly, a similar pattern of translation was found in LCLs harboring mutations in RPS19. These results link a new ribosomal protein gene to DBA and show an unambiguous change in ribosome composition in a ribosomopathy. Despite this specific impact of RPL17 mutation on the 5.8S rRNA, our findings suggest a common translational pattern in DBA cells that may result from deficient ribosome synthesis and contribute directly to the pathophysiological mechanism underlying DBA irrespective of the mutated ribosomal protein gene.

#24. Distinct maturation steps of the human 40S subunit revealed by comprehensive analysis of preribosome composition and compartmentalization

Blanca Nieto, Sonia G. Gaspar, Xosé R. Bustelo and Mercedes Dosil

Centro de Investigación del Cáncer. University of Salamanca. Campus Unamuno, 37007 Salamanca, Spain.

We have developed a method that efficiently extracts preribosomal complexes from human cell lysates in a sequential manner. The method renders three separate pools of particles: one containing the cytoplasmic and nucleoplasmic preribosomes, a second one containing the preribosomes present in the outer regions of the nucleolus, and a third pool with the early preribosomes present in internal nucleolar regions. In addition, we have generated CRISPR-edited HeLa-derived cell lines that endogenously express GFP-fused versions of five 40S synthesis factors: NOC4L, BYSTIN, RRP12, LTV1 and NOB1. Preribosomal particles were analyzed in these cell lines using our sequential extraction method, followed by GFP-Trap™ pull-down and mass-spectrometry analyses, in combination with fluorescence microscopy. This approach identified two distinct pools of 18SE-containing complexes that correspond to the first ~40S particles, formed in the inside of the granular component, and the following intermediate-maturation particles, formed in more peripheral regions of the nucleolus. The latter particles are highly vulnerable to degradation and require the factor RRP12 for their stability when they exit the nucleolus. The release of RRP12 only occurs when the intermediate 40S preribosomes mature into more-stable late particles. Our findings unveil several steps of the 40S synthesis pathway in human cells and show that the maturation dynamics, the biochemical properties of nuclear preribosomes and the regulation of some maturation events are different to those found in yeast.

#25. Single methylation of 23S rRNA regulates late steps of 50S ribosomal subunit assembly by sensing cellular AdoMet concentration

Kensuke Ishiguro, Tsutomu Suzuki

University of Tokyo, Tokyo, Japan

Ribosome biogenesis proceeds through rRNA transcription coupled with ribosomal protein assembly. This process is facilitated and regulated by various assembly factors. The rate of ribosome assembly is strongly affected by growth condition of the cell, but its regulatory mechanism is not fully understood.

RlmE is a 23S rRNA methyltransferase responsible for 2'O-methylation of U2552 (Um2552) of Helix 92 in domain V. RlmE uses S-adenosylmethionine (AdoMet) as a methyl donor. Deletion of rlmE results in remarkable growth defect and accumulation of 45S precursor. In our previous study, we demonstrated that late steps of 50S subunit assembly is triggered by the single Um2552 methylation mediated by RlmE (Arai et al., PNAS, 2015).

We further examined whether RlmE-mediated Um2552 formation and 50S assembly is regulated by sensing AdoMet concentration in the cell. Upon disruption of mtn gene which is required for AdoMet recycle, we observed severe growth defect of Δmtn strain with

significant accumulation of the 45S precursor bearing 23S rRNA with hypomodified Um2552. Strikingly, overexpression of rlmE rescued the growth defect of Δmtn strain, and reduced the accumulation of 45S precursor. AdoMet is a central metabolite not only involved in rRNA methylation but also in various cellular processes. Nevertheless, it is suggested that growth defect of Δmtn is mainly due to down regulation of 50S subunit. These results indicate that late steps of 50S assembly strongly depend on cellular metabolic status by sensing AdoMet concentration.

#26. YjeQ (RsgA) - A quality control manager in the bacterial ribosome assembly factory?

Dushyant Jahagirdar and Joaquin Ortega

Department of Anatomy and Cell Biology, McGill University, Quebec, Canada

Ribosomes are essential cellular complexes for synthesis of functional proteins and do so by maintaining translational fidelity. Recent structural studies from our lab suggest the existence of bacterial assembly factors that are involved in quality control once the ribosome has completed its maturation. Escherichia coli YjeQ is one such assembly factor suggested to be involved in quality control after maturation of the 30S ribosome subunit unit by probing the fidelity mechanisms required during mRNA translation. Structural analysis of YjeQ complexed with mature 30S subunit indicated that residues Phe48 and Arg68 on YjeQ flip out the base A1492 on helix 44 and stabilize it. Interestingly, A1492 along with A1493 have been known to play a role during the translational proof reading mechanism by monitoring the geometry of the codon-anticodon base pairing. The purpose of this project is to study if testing the fidelity mechanisms of the 30S subunit is a function of YjeQ. To investigate the proposed role of YjeQ, we are using several approaches encompassing in vivo and in vitro mutational, biochemical, biophysical and structural methods. Rapid emergence of antibiotic resistance is pushing us on the edge of the dark pre-antibiotic era. This research ultimately aims to uncover the biology of new antimicrobial targets within the assembly process of the ribosome.

#27. Role of ribosome assembly in E. coli ribosomal RNA degradation

Chaitanya Jain

University of Miami Miller School of Medicine, USA

DEAD-Box proteins (DBPs) constitute a prominent class of RNA remodeling factors that play a role in virtually all aspects of RNA metabolism. To better define their cellular functions, deletions in the genes encoding each of the E. coli DBPs were combined with mutations in genes encoding different Ribonucleases (RNases). Significantly, double-deletion strains lacking Ribonuclease R (RNase R) and either the DeaD or SrmB DBP were found to display growth defects and an enhanced accumulation of ribosomal RNA (rRNA) fragments. As RNase R is known to play a key role in removing rRNA degradation products, these observations initially suggested that these two DBPs could be important for this process. However, additional investigations indicated that DeaD and SrmB-dependent rRNA breakdown is caused by delays in ribosome assembly that increase the exposure of nascent RNAs to endonucleolytic cleavage. Consistent with this notion, mutations in factors

known to be important for ribosome assembly also resulted in enhanced rRNA breakdown. Additionally, significant levels of rRNA breakdown products could be visualized in growing cells even in the absence of assembly defects. These findings reveal a hitherto unappreciated mechanism for rRNA degradation under conditions of both normal and abnormal ribosome assembly.

#28. Identifying Critical Interactions in the Unique Trypanosoma brucei 5S Ribonucleoprotein (RNP) Complex and their Role in Ribosome Biogenesis

Daniel S. Jaremko and Noreen Williams

University at Buffalo, Microbiology and Immunology, USA

Trypanosoma brucei is a eukaryotic parasite causing Human African Trypanosomiasis (HAT), which is nearly always fatal without treatment. As current medications are expensive and rife with adverse side effects, the development of new drugs is imperative. One approach is to target processes that are essential and pathogen-specific. Formation and incorporation of the 5S RNP, which contains the 5S rRNA and a number of proteins, is a key checkpoint in 60S formation. The presence of parasite-specific components in this vital process makes it promising for drug development.

While extensively studied in Saccharomyces cerevisiae, ribosome biogenesis in T. brucei is poorly understood. Work in our laboratory identified the trypanosome-specific proteins P34/P37 as an essential component of the T. brucei 5S RNP. We have shown direct interactions between P34/P37 and the proteins L5 and Rpf2, and 5S rRNA, well-studied components of the 5S RNP. Recently, we identified T. brucei homologues of the proteins L11 and Rrs1, which are members of the 5S RNP in yeast. Initial work with L11 has shown that it interacts with P34/P37 in vivo, has a direct interaction with 5S rRNA in vitro, and that RNAi knock-down is lethal in T. brucei. Furthermore, we have previously shown that Rpf2 has unique properties in T. brucei, including being both functional and stable in the absence of Rrs1. Recently, we purified Rpf2 in tight association with Rrs1 using an in vitro co-expression system, and showed that Rpf2/Rrs1 together bind 5S rRNA similarly to Rpf2 alone. Our future work will continue to analyze L11, Rrs1 and the Rpf2/Rrs1 complex to expand on our previous work with the T. brucei 5S RNP.

#29. Snapshots of 60S cytoplasmic maturation

Yi Zhou, Sharmishtha Musalgaonkar, Arlen Johnson, David Taylor

The University of Texas at Austin, Austin, TX, USA

Export of pre-60S subunits depends on the nuclear export adapter Nmd3, which assembles into the pre-60S particle during the later stages of maturation in the nucleus. The pre-60S particle undergoes remodeling in the cytoplasm, including insertion of Rpl10 to complete the peptidyl transferase center, after which Nmd3 is released by the GTPase Lsg1. We have used single particle cryo-EM to determine the structures of intermediates of late nuclear and cytoplasmic maturation. We used a C-terminal truncation mutant of Rlp24, which fails to recruit Drg1, the AAA-ATPase that initiates cytoplasmic remodeling. We captured two classes of particles; one contains Nog2 representing a late nuclear particle

and the second an early cytoplasmic particle with Nmd3 but before Rpl10 binding. In this class, helix38 is highly distorted, bent down toward the L1 stalk and captured by Nmd3. This conformation holds open the Rpl10 binding cleft which we suggest promotes the loading of Rpl10. We also affinity purified particles via Nmd3 after treatment with diazaborine, a drug that blocks Drg1 function. However, the particles we identify have progressed through several steps of maturation, presumably because of wash out of drug. This allowed us to capture two additional classes of particles that contain Nmd3, Rpl10 and with or without Lsg1. In these particles, helix 38 has been released from Nmd3 and adopts its mature conformation, sandwiching Rpl10 into its binding cleft. The presence of Rpl10 on the Nmd3-containing particles suggests that although the loading of Rpl10 is necessary for release of Nmd3, it is not sufficient. Remarkably, the presence of Lsg1 correlates with a dramatic conformational change in Nmd3. In the absence of Lsg1, the N-terminal domain of Nmd3, stretching from the A site to Tif6, interacts with helix89, which forms another side of the Rpl10 binding cleft. In the presence of Lsg1, this domain of Nmd3 rotates about 120°, away from helix89, to interact with Lsg1. This dramatic reorganization of the N-terminus of Nmd3 likely accounts for previous difficulty in resolving this domain. This rotation of Nmd3 may communicate the loading of Rpl10 to the GTPase Lsg1.

#30. Visualizing Nucleolar Steps of pre-60S Ribosome Assembly

Lukas Kater, AG Beckmann

Gene Center Munich and Center of Integrated Protein Science-Munich (CiPS-M), Department of Biochemistry, University of Munich, Munich, Germany

The eukaryotic 60S ribosomal subunit is composed of three RNAs (5S, 5.8S and 25S/28S rRNA) and ~50 ribosomal proteins. The initial steps of 60S subunit formation take place in the nucleolus, but this process is only poorly understood due to lack of structural insight on how dozens of non-ribosomal factors help to fold and organize early pre-60S particles. Here, we solve by cryo-EM several of these intermediates at 3.3 Å to 4.5 Å resolution, giving unprecedented insight into the gradual folding pathway of the maturing pre-60S subunit in the nucleolus. Besides distinct immature rRNA conformations, we map 25 assembly factors in 6 different assembly states on these pre-60S particles. Notably, the Nsa1-Rrp1-Rpf1-Mak16 module stabilizes the solvent side of the 60S subunit. The Erb1-Ytm1-Nop7 complex organizes and connects eight assembly factors, three ribosomal proteins, and three 25S rRNA domains through Erb1's meandering N-terminal extension. Our structural snapshots reveal the order, integration and compactation of the six major 60S domains within the nucleolar 60S particles developing stepwise from the solvent side around the exit tunnel to the central protuberance.

#31. Universal KsgA/Dim1-dependent modification of 16S rRNA: from paradigm to "paradigm-shift"

Robert Knüppel[1], Alexander Wagner[2], Sonja-Verena Albers[2] and Sébastien Ferreira-Cerca[1]

1 - University of Regensburg, Germany Biochemistry III AG Ferreira-Cerca, Germany
2 - University of Freiburg | Albert-Ludwigs-Universität Freiburg · Institute of Biology II, Germany

Ribosomes are intricate molecular machines ensuring proper protein synthesis in every cell. The biosynthesis of functional ribosomes is a complex process that has been

intensively analyzed in bacterial and eukaryotic model organisms. In contrast, our understanding of the in vivo archaeal ribosome biogenesis pathway remains so far largely unexplored. Here we have characterized the in vivo role of the almost universally conserved ribosomal RNA dimethyl transferase KsgA/ Dim1 in archaea.

Using both genetic and biochemical approaches we have been characterizing the function of archaeal Haloferax volcanii and Sulfolobus acidocaldarius KsgA/ Dim1 homologues in vivo and in vitro.

Interestingly and in contrast to previous studies, our work suggests that the KsgA-dependent methylation can be rate-limited in phylogenetically related sub-groups of archaea, and is more dynamic than previously envisioned. Moreover, our functional and phylogenetic-based analysis indicates that the efficiency rate of h45 modifications is intrinsically-determined by organisms-specific h45 folding properties. Together our study provides a structural/ functional account of how rRNA sequence/structure variability can control the extent of the KsgA/ Dim1-dependent modifications and its subsequent release in the different domains of life.

Altogether our current work provides new insights into the molecular mechanisms of this well characterized enzyme, and sheds light on a possible molecular and functional specialization of these rRNA modifications. Finally, we will discuss functional implication(s) of this particular modification-status on the function and dynamics of the translation machinery in these organisms.

#32. Identification of novel inhibitors of eukaryotic ribosome biogenesis

L. Kofler, I. Rössler, M. Pennauer, D. Awad, M. Loibl, B. Pertschy and H. Bergler

Institute of Molecular Biosciences, University of Graz, Austria

Ribosome biogenesis is one of the most intricate and energy consuming processes within the cell. To guarantee the formation of one ribosome more than 200 trans-acting factors have to be spatially and timely coordinated. Due to the assembly and disassembly of these factors, pre-ribosomal particles undergo dramatic conformational changes along their way from the nucleolus to the cytoplasm. A major challenge the investigation of ribosome biogenesis faces, is its immense speed since the whole process is finished within minutes. Common genetic and biochemical approaches provide rather static information about single maturation states and are thus not valuable to map the dynamics of this rapid pathway.

A promising strategy to investigate highly dynamic processes is the use of chemical compounds that rapidly block cellular pathways. With the compound Diazaborine our lab has identified the first known inhibitor of ribosome biogenesis. By using Diazaborine we were not only able to determine the AAA-ATPase Drg1 as a key-player of eukaryotic ribosome biogenesis (e.g. Loibl et al. 2014) but also provided a focused view on the compositional changes pre-60S particles undergo during their maturation (Zisser et al. 2017). However, new inhibitors are needed to block different maturation steps in order to enable the investigation of ribosome biogenesis with high temporal resolution.

In this study we screened more than 1000 chemical compounds and identified several novel inhibitors of ribosome biogenesis targeting different steps all along the pathway.

Among these inhibitors some lead to severe rRNA processing defects and dramatic compositional changes of different pre-ribosomal particles within minutes.

#33. Towards the identification of novel dedicated chaperones of ribosomal proteins

Sébastien Favre, Benjamin Pillet, Michael Stumpe, Jörn Dengjel, and Dieter Kressler

Department of Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland

The majority of ribosomal proteins (r-proteins) need to travel from their site of cytoplasmic synthesis to the nucleus where they get incorporated into the nascent pre-ribosomal subunits. Owing to their special properties, i.e. high content in basic residues, long extensions, and large internal loops, r-proteins are especially prone to aggregation and degradation in their unassembled state. Recent evidence has highlighted that some r-proteins, besides solely relying on the general chaperone and transport systems, associate with specific binding partners, referred to as dedicated chaperones of r-proteins, that enable their fail-safe delivery to the pre-ribosomal assembly site [1]. To ascertain an immediate protection, most of these dedicated chaperones already capture their r-protein clients while they are synthesised by the ribosome [2, 3]. So far, eight of the 79 r-proteins have been shown to associate with seven different dedicated chaperones [1]; thus, suggesting that additional r-proteins may also require such specific binding partners. Here, we present our strategy to identify novel dedicated chaperones of r-proteins, which is based on the biochemical purification of individual r-proteins coupled to the detection of co-enriched proteins by mass spectrometry. In support of the feasibility of this approach, the one-step GFP-Trap purification of five GFP-tagged 60S r-proteins (Rpl3, Rpl4, Rpl5, Rpl10, and Rpl23) revealed the selective enrichment of the respective dedicated chaperone (Rrb1, Acl4, Syo1, Sqt1, and Bcp1). We are now in the process of testing all r-proteins of the 60S subunit for specifically associated candidate proteins. Upon validation of the binary interactions, we will then address the functional relevance of each novel dedicated chaperone for the life cycle of its r-protein client.

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#34. System-wide proteomic profiling integrates the SUMO isopeptidase SENP3 in the mammalian ribosome biogenesis network

Kathrin Kunz*, Tanja Piller*, Kristina Wagner, Jan Keiten-Schmitz, Soraya Hölper§ and Stefan Müller

§ current address: Sanofi AG, Frankfurt / * equal contribution
Institute of Biochemistry II, Goethe University, Medical School, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

Post translational protein modification by members of the ubiquitin-related SUMO family is a rapid and reversible way to control cellular signalling processes. SUMO-specific isopeptidases of the SENP family catalyse the deconjugation of SUMO from target proteins and thus are key determinants of the cellular SUMOylation status. We previously uncovered a critical role of the nucleolar SUMO isopeptidase SENP3 in mammalian ribosome biogenesis (Haindl et al., EMBO Rep. 2008; Finkbeiner et al., EMBO J, 2011; Raman et al., Mol Cell, 2016). In particular, we found that SENP3 is involved in pre-60S ribosome maturation by controlling the SUMOylation state of the mammalian Rix1 orthologue PELP1. We further demonstrated that SUMOylation/deSUMOylation of PELP1 regulates the ordered recruitment and release of the AAA ATPase MDN1/Rea1. To uncover additional functions of SENP3 we initiated a system-wide proteomic analysis of SENP3-associated protein complexes and profiled SENP3 target specificity by SUMO proteomics in cells lacking SENP3. With these complementary approaches we confirm the critical involvement of SENP3 in pre-60S maturation through the mammalian PELP1 complex. Importantly, our data also point to a physical and functional link of SENP3 to the 40S assembly pathway. Details of these findings will be presented.

#35. The translation release factor methyltransferase Mtq2 is required for ribosome biogenesis

Caroline Lacoux[1], Kritika Saraf[2], Ludivine Wacheul[2], Leslie Muller[3], Emmeline Huvelle[1], Sabine Figaro[1], Marc Graille[4], Sarah Cianférani[3], Denis L.J. Lafontaine[2], Valérie Heurgué-Hamard[1]

1 - Université Paris Diderot, Sorbonne Paris Cité, France
2 - Université Libre de Bruxelles, Belgium
3 - Université de Strasbourg, Strasbourg, France
4 - Ecole Polytechnique, Palaiseau, France

Mtq2 is a Rossmann-like fold domain-containing methyltransferase which methylates the glutamine of the catalytic GGQ motif of the eukaryotic translation release factor eRF1. MTQ2 is not essential for growth but loss of Mtq2 function leads to severe growth impairment, in particular at low temperatures. Here we report an unexpected function of Mtq2 in ribosome biogenesis. We reveal that Mtq2 interacts with nuclear pre-ribosomes, comigrates with them on velocity gradients and copurifies with numerous ribosome assembly factors and nucleoporins. We demonstrate that the catalytic activity of Mtq2 is required for efficient processing of large nucleolar pre-rRNAs, maturation of the 3' end of 5.8S rRNA, and pre-60S export. Collectively, this results in a substantial reduction in production of mature 60S. We conclude that, in addition to its role as a translation release factor modification enzyme, Mtq2 is a ribosome assembly factor important for large

ribosomal subunit formation. We speculate that within pre-60S ribosomes, Mtq2 may be involved in a quality control mechanism aimed at proof reading the binding site of eRF1.

#36. Using the iNo score to discriminate normal from altered nucleolar morphology, with applications in basic cell biology and potential in human disease diagnostics

Vassiliki Stamatopoulou[1], Pascaline Parisot[2], Christophe De Vleeschouwer[2] and Denis L.J. Lafontaine[3]

1 - Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece.
2 - ICTEAM-ELEN, Université catholique de Louvain, Louvain-la-Neuve, Belgium
3 - RNA Molecular Biology, F.R.S./FNRS, Université Libre de Bruxelles, Charleroi-Gosselies, Belgium

Ribosome biogenesis is initiated in the nucleolus, a cell condensate essential to gene expression, whose morphology informs cancer pathologists on the health status of a cell. Here we describe a protocol for assessing both qualitatively and quantitatively the involvement of trans-acting factors in nucleolar structure. The protocol involves depleting cells of factors of interest using small interfering RNAs, fluorescence imaging of nucleoli in an automated high-throughput platform, and use of a dedicated software to determine an index of nucleolar disruption, the iNo score. This scoring system is unique in that it integrates in a parametric equation the five most discriminant shape and textural features of the nucleolus. Determining the iNo score enables both qualitative and quantitative factor classification with prediction of function (functional clustering), which is not achieved by competing approaches, and stratification of their effect (severity of defects) on nucleolar structure. The iNo score has the potential to be useful in basic cell biology, developmental and/or organismal biology, and clinical practice.

#37. Identification of sites of 2'-O-methylation vulnerability in human ribosomal RNAs by systematic mapping

Sunny Sharma[1], Virginie Marchand[2], Yuri Motorin[2] and Denis L.J. Lafontaine[1]

1 - RNA Molecular Biology, Fonds National de la Recherche (F.R.S./FNRS) and Université Libre de Bruxelles (ULB), BioPark campus Gosselies, Belgium
2 - Next-Generation Sequencing Core Facility, CNRS-Lorraine University, Vandoeuvre-les-Nancy, France

Ribosomal RNA modifications are important in optimizing ribosome function. Sugar 2'-O-methylation performed by fibrillarin-associated box C/D antisense guide snoRNAs impacts all steps of translation, playing a role in disease etiology (cancer). As it renders adjacent phosphodiester bonds resistant to alkaline treatment, 2'-O-methylation can be monitored qualitatively and quantitatively by applying next-generation sequencing to fragments of randomly cleaved RNA. We remapped all sites of 2'-O-methylation in human rRNAs in two isogenic diploid cell lines, one producing and one not producing the antitumor protein p53. We identified sites naturally modified only partially (confirming the existence in cells of compositionally distinct ribosomes with potentially specialized functions) and sites whose 2'-O-methylation is sensitive to p53. We mapped sites particularly vulnerable to a reduced level of the methyltransferase fibrillarin. The remarkable fact that these are largely sites of

natural hypomodification provides initial insights into the mechanism of partial RNA modification. Sites where methylation appeared vulnerable lie peripherally on the 3-D structure of the ribosomal subunits, whereas the numerous modifications present at the core of the subunits, where the functional centers lie, appeared robustly made. We suggest that vulnerable sites of 2'-O-methylation are highly likely to undergo specific regulation during normal and pathological processes.

#38. The Programmed Cell Death Protein 2 (PDCD2) protein is a novel uS5-associated protein that functions in ribosome biogenesis

Anne-Marie Landry-Voyer, Danny Bergeron, Kiersten Dionne and François Bachand

Université de Sherbrooke, Sherbrooke. Canada

We previously showed that Programmed Cell Death Protein 2-like (PDCD2L) associates with proteins involved in late maturation of the 40S ribosomal subunit, most likely via its interaction with the 40S ribosomal protein uS5. PDCD2 is a paralog of PDCD2L with 34% identity in humans. Also, it was found that the Drosophila homolog of PDCD2, Zfrp8, interacts with uS5. Consistent with an evolutionarily conserved interaction, our proteomic analyses revealed that PDCD2 forms an extra-ribosomal complex with uS5 in human cells. Bimolecular fluorescence complementation assays confirmed a direct interaction between uS5 and PDCD2 that occurs in the cytoplasm and the nucleolus. Notably, sucrose gradient analysis indicated that cells deficient for PDCD2 accumulate free 60S ribosomal subunits together with reduced levels of free 40S subunits and monosomes. Depletion of PDCD2 also affected pre-rRNA processing, as revealed by increased levels of both 43S and 26S precursors, suggesting decoupling of the cleavages at sites A0 and 1. The accumulation of 21S pre-rRNAs in PDCD2-deficient cells also suggests a role for PDCD2 in the late maturation of pre-40S particles. Interestingly, PDCD2 does not interact with other ribosomal maturation factors other than uS5. We therefore speculate that PDCD2 functions in ribosome biogenesis via its interaction with uS5. We are currently working on deciphering the mechanism by which PDCD2 controls ribosomal maturation.

#39. The GTPase activity of Nog1 is essential for the assembly of the peptidyl transferase center

Amber J. LaPeruta, Daniel M. Wilson, John L. Woolford

Carnegie Mellon University, USA

The peptidyl transferase center (PTC) is an essential and conserved functional center of the large ribosomal subunit. However, the mechanisms involved in its assembly remain elusive. Recent cryo-EM models of assembling pre-ribosomes have revealed that Nog1, a GTPase assembly factor, interacts with the rRNA of the PTC. Specifically, Nog1 splits H89 of the PTC with its N-terminal helix bundle domain and adjacent to the helix bundle, Nog1 has an Obg-like GTPase domain. How the GTPase domain functions in ribosome assembly and in the context of PTC formation is unknown. Therefore, we used biochemical and molecular genetic methods to determine how Nog1 contributes to maturation of the PTC. We assayed the effects of nog1G223A, a mutation predicted to prevent GTP hydrolysis. In this mutant, cleavage at the C2 site in the ITS2 spacer is blocked, Erb1 and Brx1 fail to be

released from pre-ribosomes, and Nog2 fails to assemble. Based on cryo-EM structures of early nucleolar assembly intermediates, Spb1 occupies a portion of the Nog2 binding site proximal to the helix bundle and GTPase domain of Nog1. Additionally, Erb1 and Brx1 are bound on top of Spb1. We hypothesize that the GTPase activity of Nog1 is essential for initiating the removal of Spb1, which destabilizes or removes Erb1 and Brx1 and allows Nog2 to bind to the PTC. This mechanism outlines a novel remodeling event leading to C2 cleavage and identifies a new function for both Nog1 and Spb1 in the assembly of the PTC. Altogether, our data suggests that Nog1 acts as a coordinator of PTC maturation.

#40. Evolution of Robustness in Ribosome Biogenesis: A Role for DEAD-box Proteins

Byron Lee and Eliezer Calo

Massachusetts Institute of Technology, USA

The DEAD-box protein family is a group of putative helicases that are involved in a wide array of RNA metabolic functions in eukaryotes, such as ribosome biogenesis, transcription, splicing, and translation. They exist in all three domains of life, and exhibit a protein family expansion from ~2-12 proteins to ~30-40 proteins correlating with the rise from prokaryotes to eukaryotes, respectively. Moreover, prokaryotic DEAD-box proteins are only essential in certain conditions, whereas most eukaryotic DEAD-box proteins are necessary in standard laboratory growth conditions, which raises the question of how and why DEAD-box proteins evolved increasingly important in roles in RNA metabolism. Here, we present our work investigating the link between the human DEAD-box helicase, DDX21, and it's ortholog in E. coli, CsdA. These proteins are highly conserved, as they cluster by phylogenetic analysis of protein sequence and share similar domain structure outside of the conserved DEAD-box domains. Furthermore, we find that expression of DDX21 can rescue growth and 50S processing in CsdA-knockout E. coli under cold conditions, suggesting that robustness in ribosome biogenesis may have been a key feature allowing both the expansion of the DEAD-box protein family and the evolution from prokaryotes to eukaryotes.

#41. Does RPL5 haploinsufficiency drive cancer?

Justine Lee, Regina Hertle, Claudia Schneider, Nicholas Watkins

Newcastle University, Newcastle, United Kingdom

Ribosome biogenesis defects are linked to disease, including >20 ribosomopathies and cancer. The 5S RNP, a large subunit (LSU) assembly intermediate (5S rRNA, RPL5 and RPL11), accumulates when ribosome biogenesis is blocked. This non-ribosomal 5S RNP binds to and suppresses MDM2, a p53 inhibitor, leading to p53 activation. RPL5 has been identified as a cancer driver in seven forms of cancer including leukaemia and breast cancer. Furthermore, RPL5 mutations are linked to Diamond Blackfan anaemia, a disease with a predisposition to cancer. We were therefore interested in determining the impact of RPL5 mutations on ribosome biogenesis and p53 signalling.

Many of the RPL5 disease mutations are either nonsense mutations or frameshifts. Mutational analysis indicated that all disease-related deletion/frameshift mutations would result in an unstable protein leading to haploinsufficiency. Smaller deletions at the N- and

C-termini do not result in an unstable protein but do impact protein function. In particular, RPL5 lacking the first 30 amino acids, which are predicted to bind the RPL5/RPL11 chaperone, does not integrate into the ribosome and is incapable of activating p53, but still associates with the 5S rRNA. Analysis of a B cell precursor cell line with a nonsense mutation in one RPL5 allele revealed that RPL5 haploinsufficiency results in a mild pre-rRNA processing defect and reduced LSU levels. Furthermore, these cells show a reduced response to actinomycin D, which blocks ribosome biogenesis, but not to drugs that cause DNA damage. Our data indicate that many disease-related RPL5 mutations would impact p53 signalling and could therefore drive carcinogenesis.

#42. The function of RPS2 in forming heterogeneous ribosomes

Chunyan Liu, Zhen Wang, Runlai Hang, Xiaofeng Cao

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

Ribosomes are ribonucleoprotein complexes comprising a large and a small subunit. In eukaryotes, the large subunit is composed of 25–28S, 5.8S and 5S rRNA together with 47 ribosomal proteins, whereas the small subunit is composed of 18S rRNA and 33 ribosomal proteins. In plants and animals, mutation in genes encoding cytoplasmic ribosomal proteins and ribosome assembly factors leads to specific developmental defects. In Arabidopsis, cytoplasmic ribosomal proteins are encoded by two to five genes. Some severe mutants are embryo lethal, but some mutants of ribosomal proteins are viable. It remains unknown why ribosomal protein mutants show different phenotypes, recent studies show that the difference may potentially result from ribosome heterogeneity, or extraribosomal functions of ribosomal proteins. Here we report that in Arabidopsis Ribosomal Protein S2 (RPS2) has four family members including RPS2A, RPS2B, RPS2C and RPS2D. In order to study the function of RPS2, we used CRISPR-Cas9 technology to knock out RPS2 family genes, to find that rps2a, rps2b, rps2c and rps2d mutants can behave differently, implying that RPS2 family members may have different functions. we hypothesis that different RPS2 family members may make up different ribosomes that can perform specific function.

#43. Engineered rDNA arrays, producing customised ribosomes, reveal the existence of NOR territories within human nucleoli

Hazel Mangan and Brian McStay

Centre for Chromosome Biology, School of Natural Sciences, National University of Ireland Galway, Ireland

Human ribosomal gene (rDNA) arrays or nucleolar organiser regions (NORs) are situated on the unsequenced p-arms of each acrocentric chromosome (HSA13, 14, 15, 21 and 22). We are only now beginning to understand their genomic architecture. After cell division, nucleoli form around individual active NORs. Subsequently, a poorly understood fusion event leads to the formation of mature nucleoli comprising multiple NORs and nucleolar-associated heterochromatin. As rDNA repeats between individual NORS are indistinguishable, how multiple NORs are organised within large mature nucleoli is unknown.

In order to address this important issue, we have developed a workflow in which rDNA repeats within a single NOR are genetically tagged. This is achieved by performing genome

editing of NORs on single human acrocentric chromosomes held within mouse A9 cells. Chromosomes with engineered NORs are then transferred to a human cell line by microcell mediated chromosome transfer (MMCT). In this context, the engineered NOR is reactivated and its products can be identified by probes that recognise the tags. Tags located within the 5' external transcribed spacer (5'ETS) and 28S rRNA encoding sequences allow us to probe where the early and late events, respectively, in ribosome biogenesis occur within large fused nucleoli. Strikingly, we observe that all the stages in the biogenesis of ribosomes derived from a single NOR occur within a distinct nucleolar territory. Establishment of this territory in underpinned by the NOR and its surrounding genomic architecture. While topologically associated domains (TADs) prevent entanglement in most of the genome, we argue that NOR territories prevent rDNA entanglement and promote rDNA genomic stability.

Finally, the work flow we have developed provides both capabilities in probing ribosome structure and production of customised ribosomes.

#44. A genome-wide RNAi screen for increased nucleolar number reveals new proteins required for ribosome biogenesis

Lisa M. Ogawa McLean, Kathleen L. McCann and Susan J. Baserga

Yale University and the Yale School of Medicine, USA

Ribosome biogenesis initiates in the nucleolus from 10 distinct genomic loci, yet, 10 nucleoli are rarely observed. In the MCF10A human breast epithelial cell line we observe on average 2-3 nucleoli per nucleus; however, intriguingly we have shown that siRNA depletion of factors required for ribosome biogenesis cause a decrease in this number from 2-3 to 1 (Freed et al., 2012). This observation led to a genome-wide siRNA screening campaign to identify novel proteins required for making ribosomes in human cells (Farley-Barnes et al., 2018). While the published work focused on proteins revealed by the one nucleolus phenotype, this screen also uncovered hits that cause an increase in nucleolar number to 5 or more (unpublished). Focused on this 5+ phenotype, we screened 18,017 genes and identified 186 significant hits. Hits were then filtered by expression and viability leaving 103 proteins, none of which overlapped with the one nucleolus hits. Of these proteins, 20% localize to the nucleolus and Ingenuity Pathway Analysis revealed functional associations with cancer, cell cycle, development, cellular assembly and organization, and DNA replication and repair. Interestingly, while ribosomal proteins were absent, the screen did identify known ribosomal DNA (rDNA) transcription-associated factors (TAF1D; SUV39H1), a 60S export factor (MDN1), and proteins identified by other screens for human ribosome biogenesis factors (ABCE1; CDCA8; TOPBP1; DYNC1H1). Preliminary data on 8 nucleolar hits reveal that while all 8 hits significantly affect global protein synthesis based on a puromycin incorporation assay ($n=3$, $p<0.05$), only 3 affect transcription of the pre-rRNA (INCENP; CDCA8; RFC1; $n=3$, $p<0.01$), and just 1 may affect pre-rRNA processing (MDN1; $n=1$). Thus, while follow-up on the one nucleolus hits revealed proteins required for pre-rRNA transcription and processing, the hits from the 5+ screen may instead be associated with other steps in ribosome biogenesis, or more broadly associated with nucleolar structure and function through repair of the rDNA, chromatin organization post-mitosis, or the structural integrity of the nucleolus itself, and therefore requires further analysis. The importance of this study thus lies in the putative identification of new proteins required for human ribosome biogenesis, but also in elucidating potential mechanisms

required to maintain nucleolar structural integrity.

#45. Tumor suppressive role of small nucleolar RNAs in the control of lipid metabolism

Mary McMahon[1], Adrian Contreras[1], Tamayo Uechi[1], Craig Forester[1], Bin Chen[2], David Quigley[3], Kate Kelley[4], John Gordan[4], Ryan Gill[5], and Davide Ruggero[1]

1 - Helen Diller Cancer Center, Department of Urology, and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94158, USA
2 - Department of Pediatrics and Institute for Computational Health Sciences, University of California, San Francisco, California 94158, USA
3 - Helen Diller Cancer Center and Department of Epidemiology and Biostatistics, University of California, San Francisco, California 94158, USA
4 - Department of Medicine (Hematology/Oncology), University of California, San Francisco, California 94158, USA
5 - Department of Pathology, University of California, San Francisco, California 94158, USA

H/ACA small nucleolar RNAs (snoRNAs) are responsible for converting hundreds of specific uridine residues to pseudouridine within the ribosome and are found altered in numerous cancers. However, it remains unknown whether H/ACA snoRNA expression and function is modulated to alter the pseudouridine landscape of the ribosome as a means to orchestrate cellular transformation. To our surprise, we have uncovered that specific subsets of H/ACA snoRNAs, guiding modifications within distinct regions of ribosomal RNA (rRNA), display marked coordinated regulation during the earliest cellular responses to oncogene activation. To address whether individual snoRNAs play a role in tumor suppression, we assessed whether a reduction in distinct H/ACA snoRNAs may alter cellular senescence, a critical barrier to oncogenic transformation. Interestingly, we observed that a reduction in distinct H/ACA snoRNAs including SNORA24, which guides pseudouridine modifications within 18S rRNA, leads to bypass of oncogene-induced senescence. Furthermore, we demonstrate that reduced expression of SNORA24 in vivo leads to bypass of senescence induced by oncogenic RAS and promotes the development of liver cancer. Histological analysis of tumor nodules revealed a dramatic increase in lipids that closely resembles human steatohepatitic hepatocellular carcinoma, a rare liver cancer variant characterized by lipid deposition. Employing pharmacological inhibitors of distinct metabolic pathways, we uncovered that SNORA24 modulates specific phospholipases thereby directly impinging on fatty acid and lipid production. Currently, we are defining the molecular mechanisms by which a single H/ACA snoRNA alters the expression and activity of genes involved in lipid metabolism at the post-transcriptional level in response to oncogenic activation. From a clinical perspective, hepatocellular carcinomas with low SNORA24 expression exhibit increased lipid content and are associated with poor patient survival. Altogether, this study provides a previously unappreciated link between H/ACA snoRNAs, lipid metabolism, and cancer and suggests that the pattern of rRNA modifications is dynamically regulated to maintain a tumor suppressor program thus providing a new layer of ribosome-mediated control in safeguarding the genome against oncogenic insult.

#46. Studying ribophagy in mammalian cells

Hannah Mende, Marc Sawatzki and Stefan Müller

Institute of Biochemistry II, Goethe University, Medical School, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

Ribosomes are the central cellular assembly lines for protein synthesis. Their production and decay has to be tightly coupled to the energy status and nutrient levels of the cell. Moreover, cells must be cleared from defective ribosomes to assure translational accuracy. In the yeast Saccharomyces cerevisiae, it has been shown that ribosomes are turned over by selective macroautophagy under nutrient starvation (Kraft et al., Nat. Cell Biol., 2008). This process, which is specific to the large and small ribosomal subunit, is termed ribophagy. In mammalian cells autophagic degradation of ribosomes under nutrient starvation was only recently described, but many aspects of this process remain elusive (An & Harper, Nat. Cell Biol., 2018). We found that distinct mammalian ribosomal trans-acting factors bind to ubiquitin-like ATG8 modifiers. Since ATG8 family members are instrumental for the formation of autophagosomes we are investigating whether these factors act as receptors of selective ribophagy. Moreover, we follow the idea that ribophagy not only functions in recycling of cellular building blocks but also contributes to the clearance of defective ribosomes during ribosome assembly. To address these issues we monitor the autophagic flux of ribosomal subunits by fusing the dichromatic Keima fluorophore to 40S and 60S ribosomal proteins. We anticipate that the elucidation of the role of selective ribophagy will provide insight into the understanding of ribosome maturation and ribosomopathies. Preliminary data on these issues will be presented.

#47. Cgr1 is a key regulator of 5S RNP rotation during maturation of the pre-60S subunit

Matthias Thoms[1], Valentin Mitterer[1], Lukas Kater[3], Laurent Falquet[2], Roland Beckmann[3], Dieter Kressler[2], Ed Hurt[1]

1 - Biochemistry Centre, University of Heidelberg, Heidelberg, Germany
2 - University of Fribourg and Swiss Institute of Bioinformatics, Fribourg, Switzerland
3 - Gene Center, University of Munich, Munich, Germany

During eukaryotic 60S biogenesis, the 5S RNP requires a large rotational movement to achieve its mature position. Cryo-EM of the Rix1-Rea1 pre-60S particle has revealed the post-rotation stage, in which a gently undulating α-helix corresponding to Cgr1 becomes wedged between Rsa4 and the relocated 5S RNP, but the purpose of this insertion was unknown. Here, we show that cgr1 deletion in yeast causes a slow-growth phenotype and reversion of the pre-60S particle to the pre-rotation stage. However, spontaneous extragenic suppressors could be isolated, which restored growth and pre-60S biogenesis in the absence of Cgr1. Whole-genome sequencing revealed that the suppressor mutations map in the Rpf2-Rrs1 module and Rpl5, which together stabilize the un-rotated stage of the 5S RNP. Thus, mutations in factors stabilizing the pre-rotation stage facilitate spontaneous 5S RNP relocation upon deletion of Cgr1, but Cgr1 itself could stabilize the post-rotation stage.

#48. Genome-wide localization of UBF at Nucleosome Free Regions stabilizes local chromatin and may protect from DNA damage, possibly explaining a novel UBF Ribosomopathy

Jean-Clement Mars[1,2], Marianne Sabourin-Felix[1,2], Michel G. Tremblay[1], Roderick T. Hori[3], Mark S. LeDoux[4] and Tom Moss[1,2]

1 - Laboratory of Growth and Development, St-Patrick Research Group in Basic Oncology, Cancer Division of the Quebec University Hospital Research Centre.
2 - Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Laval University, Québec, QC, Canada Québec, QC, Canada.
3 - Department of Molecular Sciences, University of Tennessee Health Science Center, Memphis, TN, USA
4 - Departments of Neurology and Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA

UBF is an essential RNA Polymerase I (RPI/PolI) multi-HMGB-box factor that is highly conserved across vertebrate linages. Mouse, human and other mammals display the same two splice forms of UBF. UBF1 contains 6 tandem HMGB domains, while UBF2 lacks 37 a.a from its HMG-box2. UBF2 was shown to emulate a phosphorylated form of UBF1 and to enhance transcription of a subset of RPII (PolII) genes. To better understand the genome-wide actions of UBF, we have studied the effects of its deletion on local chromatin structures and on genome stability.

UBF colocalizes with markers of active chromatin and with H2A.Z at a distinct G-C rich subset of DNase1 sensitive nucleosome-free regions (NFRs). Its loss does not affect DNase1 sensitivity, or most chromatin markers, but does enhance acetylation of H2A.Z at these sites implying that they become more dynamic. We suggest that UBF stabilizes a G-C rich subset of NFRs to enhance associated RPII promoters while also suppressing DNA damage. We further suggest that these activities may in part explain the early neurodegeneration associated with a novel UBF E210K mutation.

#49. MTR4/Exosome-interacting proteins under control of the nucleolar AAA-ATPase NVL2 in human cells

Masami Nagahama[1], Sotaro Miyao[1], Ryunosuke Nakamura[1], Nobuhiro Hiraishi[1], Kohichi Kawahara[2], Yo-ichi Ishida[1]

1 - Laboratory of Molecular and Cellular Biochemistry, Meiji Pharmaceutical University, Tokyo, Japan
2 - Department of Molecular Oncology, Kagoshima University, Kagoshima, Japan

NVL2 is a nucleolar AAA-ATPase, which acts on macromolecular complexes to stimulate the energy-dependent release of their constituents. We have shown that NVL2 interacts with an RNA processing/degradation machinery containing MTR4 and the nuclear exosome. This implicated NVL2 as a remodeling factor for the MTR4-exosome complex during ribosome biogenesis. By employing a proteomic screen, we identified WD repeat-containing WDR74 and tudor domain-containing SPF30 as proteins that are specifically dissociated from this complex depending on the NVL2 ATPase activity. WDR74 was nucleolar localized and exhibited weak sequence similarity with the yeast ribosome biogenesis factor Nsa1. Knockdown of WDR74 leads to a defect in pre-rRNA ITS1 cleavage and 60S ribosome synthesis. Interestingly, when the dissociation of WDR74 was impaired by expression of a dominant-negative mutant NVL2, the nucleolar WDR74 partly

migrated to the nucleoplasm with increased interaction to MTR4 in this compartment. By contrast, SPF30 mainly localized in the nucleoplasm and showed subtle effect on pre-rRNA processing. Additionally, PICT1 (mammalian Nop53) was shown to interact with MTR4 and exhibited functions in pre-rRNA processing during the 60S ribosome synthesis. These results suggest that MTR4 and the nuclear exosome are regulated through multiple protein interactions during ribosome biogenesis.

#50. Unraveling a novel role for RNaseE and KsgA in ribosome quality control in E. coli

Anusha Naganathan, Roxanne Keltz, and Gloria Culver

Department of Biology, University of Rochester, Rochester, NY, USA

Ribosome assembly is a remarkably complex and multi-faceted process. The post-transcriptional processing and modification of ribosomal RNA (rRNA) is a critical step in the assembly of ribosomal subunits. Several ribonucleases and accessory biogenesis factors participate in in vivo assembly to ensure the rapid synthesis of functional ribosomes. Recent studies have shed light on quality control mechanisms that identify and degrade improperly processed rRNA. However, it remains unclear how ribonucleases are differentially recruited for maturation and degradation of rRNA. Importantly, it is poorly understood how the function of ribonucleases is influenced byother members of the ribosome biogenesis pathways.

In this study, we have characterized a novel functional interaction between essential endoribonuclease, RNaseE and a universally conserved ribosome biogenesis factor, KsgA. Both factors perform known functions during the assembly of the 30S subunit. While RNaseE participates in the maturation of the 5' end of 16S rRNA, KsgA is solely responsible for the dimethylation of two adjacent adenosines (A1518 and A1519) on Helix 45 of 16S rRNA. We observed that the overexpression of RNaseE in wild-type E. coli results in the accumulation of mature 16S rRNA missing 43 nucleotides (16S(Δ43) rRNA) at its 3' end, in a region that forms Helix 45 in the 30S subunit. Interestingly, the absence of KsgA leads to reduced accumulation of 16S(Δ43) rRNA. Cells containing a catalytically inactive KsgA mutant also accumulated 16S(Δ43) rRNA to a lesser extent, indicating that the methylation of Helix 45 by KsgA is vital for the observed RNaseE-induced cleavage. Additionally, we independently identified a genetic interaction between KsgA and RNaseE, further supporting a common functional pathway between the two proteins. E. coli lacking KsgA and exoribonuclease, PNPase exhibit severe a growth defect and remarkably, a single point mutation in RNaseE (Ala55Thr) suppresses this growth defect. Further characterization of the suppressor showed alleviation of ribosome biogenesis defects and reduced cleavage of mature 16S rRNA. We predict that under some unknown conditions, RNaseE leads to synthesis of 16S(Δ43) rRNA-containing ribosomes. The absence of KsgA impedes the formation of these ribosomes. This data unravels a new role for RNaseE and KsgA in a quality control mechanism involving 16S(Δ43) rRNA.

#51. Analysis of the interactions between Nop53 and other processing factors within eukaryotic pre-60S ribosomal subunit

Felipe F. M. Bagatelli, Leidy P. Cepeda, Carla C. Oliveira

University of Sao Paulo, Sao Paulo, Brazil

In order to properly build the complex architecture of the eukaryotic 60S ribosomal subunit, a highly regulated biogenesis process takes place in which several non-ribosomal trans-acting factors are coordinately recruited and released. Recent works highlighted with growing structural detail new intermediary steps of pre-60S maturation. However, several mechanisms of protein recruitment and correct positioning still remain to be defined. The yeast nucleolar protein Nop53 has previously been identified as an assembly factor of the pre-60S complex and shown to affect 7S pre-rRNA processing through the exosome recruitment. We previously showed that Nop53 can directly interact with exosome subunits. The present work aimed at detailing the interaction between Nop53 and pre-60S processing factors, evaluating its impact on the pre-rRNA processing during ribosomal maturation. Here, we highlight new interactions, through which Nop53 would possibly modulate the exosome activity in the context of the ITS2 processing, and we show that Nop53 may be a key factor not only for recruiting, but also for the release of the exosome from the maturing ribosomal subunits.

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#52. Molecular basis for disassembly of an importin:ribosomal protein complex by the escortin Tsr2

Michaela Oplova

Institute of Medical Microbiology, University Zurich & Institute of Biochemistry, ETH Zurich, Switzerland

Disordered extensions at the termini and short internal insertions distinguish eukaryotic ribosomal proteins (r-proteins) from their anucleated archaeal counterparts. Here, we report an NMR structure of such a disordered eukaryotic specific segment (ESS) in the r-protein eS26 in complex with the escortin Tsr2. The structure reveals how ESS attracts Tsr2 specifically to importin:eS26 complexes entering the nucleus in order to trigger non-canonical RanGTP-independent disassembly. Tsr2, then sequesters the released eS26 and prevents rebinding to the importin, providing an alternative allosteric mechanism to terminate the process of nuclear import. Notably, a Diamond-Blackfan anemia associated Tsr2 mutant protein is impaired in binding to ESS, unveiling a critical role for this interaction in human hematopoiesis. We propose that eS26-ESS and Tsr2 are components of a nuclear sorting system that co-evolved with the emergence of the nucleo-cytoplasmic barrier and transport carriers.

#53. Biophysical characterization of Erb1 interactors

Lidia Orea Ordóñez, Jerónimo Bravo Sicilia

Biomedicine Institute of Valencia (IBV). Spanish National Research Council (CSIC), Spain

The mammalian PeBoW complex, composed of Pes1, Bop1 and WDR12 (or Nop-7 subcomplex: Nop7, Erb1 and Ytm1 in yeast) is critical for the processing of the 32S pre-

ribosomal RNA in ribosome biogenesis. However, the exact role of PeBoW in biogenesis is not well understood and the interactions within the complex have been poorly characterized. Erb1 is the core of the trimer and interacts with Nop7 through a region within its N-terminal domain. The exact site within Nop7 involved in binding to Erb1 in the complex is not yet known. Moreover, we have previously described the interaction of Ytm1 and Erb1 through their β-propeller domains. The most recent data show Erb1 is functionally correlated with other factors involved in 5.8Ss formation and was found part of pre-ribosomal complexes that included Drs1 and Has1 helicases, Noc2 and Nog1 by using large scale yeast two-hybrid analysis and tandem affinity purification. In this work, we focus on the association between Nop7 and Erb1 N-terminal domains and Erb1 with other interactors: Noc2, Nog1, Has1 and Drs1.

#54. Why rRNA transcription is oversensitive to DNA intercalators?

William J. Andrews[1], Swagat Ray[1,2], Tatiana Panova[1], Sherif El-Khamisy[2], Christoph Engel[3], Sameerch Alsahafi[1], Konstantin I. Panov[1]

1 - CCRCB and School of Biological Sciences, Queen's University Belfast, Belfast, UK
2 - Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, UK
3 - Dept. of Molecular Biology, MPI for Biophysical Chemistry, Göttingen, Germany

Ribosome biogenesis in eukaryotes is driven by the synthesis of the ribosomal RNA (rRNA) precursor by RNA polymerase I (Pol-I) and is tightly linked to cell growth and proliferation. The 3D-structure of the rDNA transcription unit plays an important, yet not fully understood role in regulating rRNA synthesis. This 3D architecture is maintained by Topoisomerases I and II and potentially can be affected by DNA intercalators. Here, we test the influence of commercially available intercalating compounds on Pol-I transcription in vivo and in vitro. We find that DNA intercalating agents are potent inhibitors of Pol-I specific transcription in cell based as well as in vitro assays. Notably, the tested compounds have limited effect on transcription by Pol-II and III, demonstrating their specificity. Strikingly, the synthetic ability of Pol-I is unaffected in vitro and in vivo, suggesting that that DNA intercalators primarily affect initiation of Pol-I transcription. This may be achieved by altering the 3D architecture of promoter DNA, which is well in line with the recently reported importance of biophysical rDNA promoter properties on initiation complex formation in the yeast system.

Therefore, our results indicate that DNA intercalators can have a dramatic impact on Pol-I transcription regardless of their specificity, potentially providing new directions for the development of drugs targeting ribosome biogenesis.

#55. Cytoplasmic maturation of the small ribosomal subunit: from static structure to dynamic function

Cohue Peña

ETH Zurich University of Zurich, Switzerland

Our recent cryo-EM structures of 40S pre-ribosomes have provided molecular insight into the cytoplasmic maturation pathway of the small ribosomal subunit. Here we present structure-guided functional studies aiming to uncover the dynamic mechanisms necessary for the release of late assembly factors and final processing of 20S pre-rRNA to produce mature 40S ribosomes.

#56. A cell free translation assay to study ribosome diversity

Marianna Penzo, Daniela Pollutri, Maurizio Brigotti and Lorenzo Montanaro

Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy

The traditional view of ribosomes as passive executors of translation is becoming more and more outmoded. Instead, the concept of ribosome diversity is catching up: ribosomes within cells may not be all the same, and they may actively participate in gene expression control. Because of the high complexity of ribosomal structure, diversification may arise from differences in: 1. ribosomal protein (RP) composition (including RP sequence variants), 2. RP post-translational modifications, 3. rRNA sequence or 4. rRNA post-transcriptional modifications. Changes in each of these elements can alter ribosomal structure at different levels, being responsible for alterations in translational regulation associated with physiology and disease. To tackle this hypothesis, we have developed a cell-free translation system, for evaluating the intrinsic activity of ribosomes stringently purified from human cells. This system is based on the in vitro reconstitution of the cellular translation machinery, in which a ribosome-free rabbit reticulocyte lysate is reassembled with human ribosomes and in vitro–transcribed reporter mRNAs. We have applied this system to study intrinsic functional properties (i.e. translational efficiency and fidelity) of ribosome populations characterized by alterations in RP or rRNA composition, finding that each of the studied alterations confers unique functional peculiarities to ribosomes.

#57. Striated Muscle Specific Ribosomal Protein May Be Preferentially Recruited to Sarcomeric Transcripts Via a 5'UTR Mechanism

Laura Peterson, Alexander Alimov, John McCarthy

University of Kentucky, USA

Both eukaryotes and prokaryotes require ribosomal protein L3 (RPL3) for ribosome maturation and function; however, striated muscle substitutes its own ribosomal protein, RPL3-like (MRP -Muscle Ribosomal Protein). During periods of muscle growth, such as after a hypertrophic stimulus, there is a rapid switch from MRP to RPL3 containing ribosomes. Both the robust specificity and the dynamic nature of MRP expression portend an important role in skeletal muscle. Because MRP decreases during periods of muscle growth, we hypothesize that MRP containing ribosomes are involved in the maintenance of muscle and acts as a break on muscle growth. Preliminary data from ribosome footprinting of WT and MRP knockout mice suggests that MRP containing ribosomes preferentially associate with muscle specific transcripts. Interestingly, although canonical ribosome recruitment occurs through a cap dependent mechanism, our data suggests that the preferential recruitment of MRP is via the 5' untranslated region of titin mRNA. Converse to our hypothesis, these results indicate that MRP ribosomes specialize by preferentially synthesizing proteins that are uniquely important to that tissue.

#58. Splicing of duplicated ribosomal protein genes is regulated by ohnolog specific network of transcription and splicing factors

Cyrielle Petibon and Sherif Abou Elela

Université de Sherbrooke, Sherbrooke, Canada

Ribosome biogenesis is a tightly regulated process that involves 4 ribosomal RNAs and a large number of ribosomal proteins. In yeast most ribosomal proteins are produced from two different genes generated by whole genome duplication. The expression ratio of the majority of the duplicated genes is regulated through differential splicing favoring the expression of one of the two copies. Intron deletions disrupt this expression hierarchy and reduce cell tolerance to stress underlining the importance of splicing to the regulation of duplicated genes. However, it is not clear how the splicing efficiency of each copy is determined and how it is modified under stress. In this study we show that a network of copy specific splicing and transcription factors represses splicing of the minor copy of duplicated RPGs. Using a splicing reporter screen and mass spectrometry we identified 7 proteins that differentially alter the splicing and expression of the minor form with little effect on the major copy of the small subunit protein Rps9. The newly identified proteins include transcription factors as well as a protein involved in the transcription export complex THO and a splicing factor implicated in cell cycle regulation. Chromatin immunoprecipitation indicated that the deletion of the transcription factors increases RNAPII association with the intron of RPS9 minor copy, while the deletion of splicing factor which slows the second step of splicing did not. This indicates that the repression of the splicing of the minor copy of RPS9A is achieved through the modulation of both transcription and splicing. Together our data reveal a new model of ohnolog specific regulation that involves pausing of RNAPII near the intron or the attenuation of the second step of splicing.

#59. Characterization of the Molecular Crosstalk Within the Essential Grc3/Las1 pre-rRNA Processing Complex

Monica C. Pillon, Mack Sobhany, Mario J. Borgnia, Jason G. Williams, Lalith Perera and Robin E. Stanley

National Institute of Environmental Health Sciences (NIEHS/NIH), USA

Las1 is a recently discovered endoribonuclease that cooperates with Grc3-Rat1-Rai1 to process precursor ribosomal RNA (pre-rRNA), yet its mechanism of action remains unknown. Disruption of the mammalian Las1 gene has been linked to congenital lethal motor neuron disease and X-linked intellectual disability disorders, thus highlighting the necessity to understand Las1 regulation and function. We report that the essential Las1 endoribonuclease displays weak RNase activity, however in the presence of the polynucleotide kinase Grc3, Las1 is reprogrammed for efficient and specific RNA cleavage both in vitro and in Saccharomyces cerevisiae. Biophysical techniques reveal that Las1 and Grc3 assemble into a tetrameric complex that is required for competent rRNA processing. A comprehensive mutational analysis of conserved residues found at the Grc3 kinase active site uncover an intricate molecular crosstalk that ensures coupling of RNA cleavage and phosphorylation during pre-rRNA processing. The tetrameric Grc3/Las1 crosstalk draws unexpected parallels to endoribonucleases RNase L and Ire1, and establishes Grc3/Las1 as a novel member of the RNase L/Ire1 RNA Splicing Family.

#60. Protein crosstalk that facilitates ribosome assembly visualized by Cryo-Electron Microscopy

Aida Razi, Dushyant Jahagirdar, Jingyu Sun, Dr. Joaquin Ortega

McGill University, Montreal, Canada

The ribosome is the enzyme responsible for protein synthesis in all cells. In Escherichia coli the ribosome is made from more than 50 different components organized into the small (30S) and large (50S) ribosomal subunits. Understanding how the components of the bacterial ribosome come together and organize themselves still remains a daunting challenge. In spite of its complexity, each bacterial cell assembles more than 20,000 ribosomes in less than 30 minutes. Cells are able to maintain this assembly rate because a number of auxiliary factors make the process extremely efficient. My work focuses YjeQ and Era, two of the protein factors that facilitate ribosome biogenesis. Recent work indicates that both factors assist at the late stages of the 30S subunit assembly.

#61. A novel interaction partner guarding ribosomal protein S6

Ingrid Rössler, Julia Embacher, Brigitte Pertschy

Institute of Molecular Biosciences, University of Graz, Austria

All cellular proteins are synthesized by the ribosome in the cytoplasm of the eukaryotic cell. In addition, this machine is assembled as well as matured in a complex and highly conserved process during which most ribosomal proteins have to be transported from the cytoplasm to the nucleus and incorporated into the pre-ribosomal particles. On their way through the cell, specific chaperones and importins protect ribosomal proteins from aggregation and guard them through the Nuclear Pore Complexes into the nucleus.

Heretofore, only a few dedicated chaperones for ribosomal proteins have been identified. Hence, we are systematically searching for unknown specific chaperones, importins and other non-ribosomal interaction partners of ribosomal proteins to gain new insights into the mechanisms of their import. Here, we present a potential chaperone which escorts Rps6.

#62. Maternal ribosomes are sufficient for tissue diversification during embryonic development in C. elegans

Elif Sarinay Cenik, Xuefeng Meng, Ngang Heok Tang, Richard Nelson Hall, Joshua Arribere, Yishi Jin, Andrew Fire

Stanford University, Mountain View, California, USA

Caenorhabditis elegans provides an amenable system to explore whether the synthesis of new ribosomal species is required to progress through development. Despite the complex pattern of tissues that are formed during embryonic development, we found that C. elegans null homozygotes lacking any of six different ribosomal proteins (RP) can produce fully functional first stage larvae, with similar developmental competence seen upon complete deletion of the multi-copy ribosomal RNA locus. These animals, relying on maternal but not zygotic contribution of ribosomal components, are capable of completing embryogenesis and of fully differentiating the entire collection of tissue types in the complex organism. These observations indicate that all diversity required of the translational machinery during

functional tissue diversification can derive from the pool of maternal ribosomal RNAs, RP transcripts, protein components and ribosomes present in the oocyte. While a fully differentiated and functional larva can be formed in the absence of new ribosomal components, the resulting animals are arrested before progression from the first larval stage and fail in two assays for postembryonic plasticity of neuronal structure. Mosaic analyses in which organism-wide arrest is observed in larvae that are a mixture of ribosome-competent and non-competent cells suggest a global regulatory mechanism in which ribosomal insufficiency in a subset of cells triggers organism-wide growth arrest.

#63. Functional analysis of Pol5 in the production of the small and large ribosomal subunits

Christina Schmidt[1], Katherine E. Sloan[2], Dietmar Sperling[1], Michael Brummer[1], Markus T. Bohnsack[2,3] and Jorge Perez-Fernandez[1]

1 - University of Regensburg Biochemie-Zentrum Regensburg (BZR), Department of Biochemistry III, D-93053, Regensburg, Germany
2 - University Medical Centre Göttingen, Department of Molecular Biology, D-37073, Göttingen, Germany
3 - University of Göttingen, Göttingen Center for Molecular Biosciences, D-37077, Göttingen, Germany

The synthesis of ribosomes is an essential process in all growing cells that assures the assembly of the small and the large ribosomal subunits (SSU and LSU respectively). Imbalance in the production of the two ribosomal subunits alters translation but only little is known about how cells coordinate SSU and LSU synthesis. On the one hand, SSU formation requires the transient association of more than 50 assembly factors (AFs) with the nascent RNA to form the SSU-processome. On the other hand, initial formation of the LSU requires the recruitment of a plethora of AFs and the endonucleolytic cleavage that separates the pre-SSU and pre-LSU particles.

Some AFs have been found to form large protein complexes not engaged in ribosome assembly. Among them, the UTP-A/t-UTP complex has been characterized as the primary binder of the nascent rRNA. Interestingly, the UTP-A/t-UTP complex has been defined with two different protein compositions, either lacking or containing Pol5. Pol5 has been proposed to be important for Pol I transcription and ribosome biogenesis but so far, its precise functions have remained unclear and it has not been identified in any SSU or LSU pre-ribosomal particles.

We present a detailed analysis of the functions of Pol5 in pre-rRNA processing and during the assembly of the pre-ribosomal subunits. Our results suggest key roles of Pol5 in the early biogenesis steps of the large subunit as well as in the disassembly of the SSU processome. We discuss possible functions of Pol5 in recycling the UTP-A complex and coordinating the production of the small and large subunits.

#64. Visualizing the functional role of RbgA during 50S subunit maturation

Amal Seffouh[1], Robert Britton[2] and Joaquin Ortega[1]

1 - Department of Anatomy and Cell Biology. McGill University, Montreal QC H3A 0C7, Canada.
2 - Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA.

The rapid emergence of antibiotic-resistant bacteria, poses a growing threat to public health. One of the most significant obstacles in developing new antibiotics is the limited understanding of the structure and mechanisms underlying new targets. Thus, it is crucial to understand structure and function of key cellular processes in bacteria to identify a new therapeutic targets and drugs for the treatment of infectious diseases. Research in our laboratory aligns with this idea and aims to provide detailed structural and functional characterization of new targets in the ribosome assembly process. Ribosomes synthesize every protein in the cell and are the main target of current clinical antibiotics. However, the ribosome assembly pathway has not been exploited as an antimicrobial target. My project focuses on identifying the precise function of RbgA (ribosome biogenesis GTPase A protein) in the assembly of the 50S subunit and provide a clear picture for the molecular rearrangements associated with its function. Recently, it has been shown that the mutation that affect GTPase activity result in the accumulation of the immature 50S particle in Bacillus1. In this study, we measured the binding affinity of RbgA to the large ribosomal subunit using microscale thermophoresis. Kinetics and affinity analysis should guide our assembly approach to form the complex between RbgA and the large ribosomal subunit for its analysis using Cryo-electron microscopy. The structures at atomic resolution of the accumulated large subunit and its complexes with mutant RbgA should inform us about the mechanism by which RbgA facilitate the last steps of 50S subunit assembly. Furthermore, it will provide insights regarding any potential interplay between ribosomal proteins and RbgA.

1 Megha Gulati and all 2014.

#65. Establishing the function of specialized ribosomes during the maternal-zygotic transition

Arish Shah and Eliezer Calo

Massachusetts Institute of Technology, USA

During development, the information encoded in the genome must be properly interpreted to generate a distinct array of cell types and tissues. At the translational level, global protein synthesis rates and translation of specific mRNAs are highly specialized in different cell types. This exquisite translational regulation is thought to be achieved through the action of specialized ribosomes. Specialization of ribosomes can occur at the protein level, where ancillary factors or the ribosomal proteins themselves modulate the functional activity of ribosomes in diverse biological processes allowing cells to rapidly respond and adapt to stress responses, developmental cues, and signaling environments. Another layer of ribosome specialization is encoded by the ribosomal RNA (rRNA). However, very little is known about how the rRNA scaffold facilitates the assembly of specialized ribosomes. I have investigated the sequence composition, structure, and post-transcriptional

modifications of the rRNAs during the early development of the zebrafish embryo and uncovered that the composition of the rRNA in maternally deposited ribosomes and those in the zygote differ. No previous work has tied the idea of specialized ribosomes to the maternal-zygotic-transition in early development. Also, the tissue-specific incorporation of unique rDNA into the nucleolus for ribosome biogenesis is a novel system to probe rDNA genomic regulation. Using a variety of biochemical and transgenic approaches, I am characterizing these two forms of ribosomes and will determine if they impart different functionalities in the context of early development.

#66. Functional analysis of yeast Kre33 C-terminal extension

Sophie Sleiman, Erlinda Fernandez Diaz, and François Dragon

Université du Quebec à Montreal, Montreal, Canada

RNA acetyltransferase Kre33 is essential for yeast viability and plays a critical role in formation of the small ribosomal subunit as it is a component of the SSU processome. Kre33 modifies 18S rRNA and tRNAs, but acetylation of tRNAs requires the assistance of the adaptor protein Tan1. From bacteria to humans, all homologues of Kre33 contain conserved domains such as ATPase/RNA helicase, N-acetyltransferase and tRNA-binding; our bioinformatics searches revealed that in eukaryotes, this “catalytic core” is flanked by N- and C-terminal extensions that harbor unique signature motifs. In particular, the C-terminal extension (C-ter) contains a putative coiled-coil motif (CC) and a putative nuclear localisation signal (NLS). In this study we generated deletion mutants lacking the C-ter or one of the putative motifs. Complementation assays with Kre33 mutants ΔC-ter and ΔNLS led to reduced growth at 30°C, and these phenotypes were more pronounced at lower temperatures. Fluorescence microscopy analyses showed that the absence of the C-ter or NLS impaired optimal nucleolar localization of Kre33. In addition, we found that SSU processome component Bfr2 strongly interacted with Kre33 in a yeast two-hybrid assay, and depletion of Bfr2 altered the distribution of Kre33 in sucrose density gradients. Immunoprecipitation experiments (IPs) with Kre33 deletion mutants indicated that absence of the C-ter, and specifically the CC motif, perturbed the interaction with Bfr2. We also investigated the relationship between Kre33 and Tan1: IPs and sucrose gradient analyses revealed that Kre33 and Tan1 interact in vivo and co-sediment in a complex of ~20S.

#67. Analysis of RNA helicase functions in human ribosome biogenesis

Priyanka Choudhury, Philipp Hackert, Katherine E. Sloan, Markus T. Bohnsack

University Medical Centre Goettingen, Germany

During their maturation, pre-ribosomal complexes undergo many structural transitions before they achieve the final architecture present in mature complexes. RNA helicases, which are best known for their functions in the NTP-dependent unwinding of RNA duplexes but can also displace proteins from RNAs and act as RNA clamps, have emerged as important regulators of pre-ribosome remodelling events. In yeast, 21 RNA helicases are required for ribosome biogenesis and diverse functions, such as mediating the release of small nucleolar RNAs (snoRNAs) and enabling the pre-ribosomal access of RNA

methyltransferases and endonucleases, have been described for these proteins. While many yeast RNA helicases are evolutionarily conserved, RNAi-based screens suggest that some may have different or additional functions during human ribosome assembly. Furthermore, several metazoan-specific RNA helicases have recently been implicated in this pathway and the roles of many human RNA helicases in ribosome assembly remain largely uncharacterised. Here, we show that the nucleolar RNA helicase DHX37 is required for biogenesis of the small ribosomal subunit and that lack of DHX37 leads to turnover of early pre-ribosomal particles. Depletion of DHX37 also inhibits the conversion of the 21S pre-rRNA to 18SE and expression of catalytically inactive DHX37 impairs A' processing. Interestingly, UV crosslinking and analysis of cDNA (CRAC) reveals binding of DHX37 to the hinge region of the U3 snoRNA that basepairs with the 5' external transcribed spacer (5'ETS) in proximity of the A' cleavage site and we show that depletion of DHX37 causes accumulation of the U3 snoRNA on pre-ribosomes. Together, our data imply that dissociation of the U3 snoRNA, facilitated by DHX37, is an important early pre-ribosome remodelling event that licences downstream steps in human ribosome assembly.

#68. Functional interplay between YjeQ and Era in 30s subunit

Jingyu Sun, Dushyant Jahagirdar, Aida Razi, Joaquin Ortega

University of Mcgill, Montreal, Canada

Era, RimM, RbfA and YjeQ are assembly factors which assist the folding of the decoding center during the late stages of assembly of the 30s subunit. These factors can not only assist the assembly of 30s subunit, but also interact with each other while assisting the assembly of the subunit. Previous studies have demonstrated the existence of a functional interplay between YjeQ and RbfA, showing that YjeQ facilitates the release of RbfA once the maturation of 30s subunit is completed. Additionally, the binding sites of YjeQ and Era to 30s are not overlapping, so there is a possibility that a functional interplay may also exist between Era and YjeQ when binding to 30s subunit. To test this hypothesis, we are using cryo-EM to image the Era+YjeQ+30s complex. We are also performing microscale thermophoresis assays to measure the binding affinity of Era and YjeQ with the 30s subunit. Our results so far show that YjeQ is unable to bind to the 30S subunit in the presence of Era. This work indicates that simultaneous binding of YjeQ and Era to the 30S subunit does not occur.

#69. Structural and Functional Studies on the Role of Noc3p for Large Ribosomal Subunit Maturation in Saccharomyces cerevisiae

Fabian Teubl, Thomas Hierlmeier, Jan Linnemann, Gisela Pöll, Joachim Griesenbeck, Herbert Tschochner and Philipp Milkereit

University of Regensburg Biochemistry III AG Milkereit, Germany

Ribosomes are complex and highly conserved ribonucleoprotein particles that translate in cells of all domains of life messenger RNA into proteins. During ribosome production ribosomal RNA (rRNA) precursors undergo extensive processing and folding events and assemble with a large number of ribosomal proteins (r-protein) in a highly dynamic and defined manner. A multitude of small nucleolar RNAs (snoRNA) and biogenesis factors transiently interact with the maturing eukaryotic pre-ribosomal particles. Most of these

factors are conserved from yeast to human and play important roles in ribosome maturation.

Examples are the “Noc-proteins” Noc1p and Noc3p which contain a common homology domain and which are essential in yeast for early to intermediate steps of large ribosomal subunit rRNA maturation.

Here, we analyzed the effect of Noc3 in vivo depletion on the assembly of yeast large ribosomal subunit precursors. Furthermore, we investigated how the r-protein assembly state influences pre-ribosomal recruitment and release of Noc3p. Our findings indicate that Noc3p coordinates rRNA processing with subunit assembly events in intermediate large ribosomal subunit precursors. We are currently studying by tertiary structure probing approaches possible structural transitions accompanying these Noc3p mediated maturation events and will report on the results.

#70. The heterodimeric RNA polymerase I subcomplex Rpa34.5/Rpa49 and its roles in transcription initiation and elongation

Philipp Merkl[2], Michael Pilsl[1], Virginia Babl[1], Jorge Perez-Fernandez[1], Olivier Gadal[3], Gernot Längst[1], Philipp Milkereit[1], Joachim Griesenbeck[1] and Herbert Tschochner[1]

1 - Universität Regensburg, Biochemie-Zentrum Regensburg (BZR), Lehrstuhl Biochemie III, 93053 Regensburg, Germany
2 - present address Dept. of Microbiology and Immunobiology, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA
3 - Laboratoire de Biologie Moléculaire Eucaryote, Université Paul Sabatier, Toulouse III, France

Eukaryotic RNA polymerases contain a conserved core. In addition, the core of RNA polymerase I (Pol I) contains a heterodimeric subomplex consisting of subunits Rpa34.5 and Rpa49. It shares homology with the RNA polymerase II (Pol II) transcription factor TFIIF and TFIIE and with the RNA polymerase III (Pol III) heterodimeric subcomplex Rpc37/Rpc53. The heterodimer Rpa34.5/Rpa49 of the yeast S. cerevisiae supports Pol I transcription initiation and elongation. Its dimerisation module consists of Rpa34.5 and the N-terminal part of Rpa49 (Rpa34.5/Rpa49NT). The dimerisation module is a constitutive part of the Pol I lobe and stimulates Pol I-dependent RNA cleavage, whereas the C-terminal part of Rpa49 (Rpa49CT) enhances DNA binding and recruitment of Pol I to the promoter region. Using in vitro approaches, we attribute further functional properties to the two modules. Cooperation between the two domains influenced transcription fidelity, elongation speed, Pol I processivity and transcription through nucleosomes. Under similar transcriptional conditions purified core enzymes of Pol I which contained the Rpa34.5/Rpa49 heterodimer, and of Pol III, but not of Pol II were able to transcribe a nucleosomal template. Furthermore, based on in vivo studies, we discuss the role of Rpa49CT in the fast opening of chromatin at the ribosomal DNA locus when cells restore growth from stationary phase.

#71. AtPRMT3 promotes proper pre-rRNA processing by regulating the function of Ribosomal Protein S2 (RPS2)

Runlai Hang, Zhen Wang, Chunyan Liu and Xiaofeng Cao

Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

Ribosome biogenesis a fundamental and essential cellular process, including rDNA transcription, processing, modification and assembly with ribosomal proteins, several hundred factors are involved to ensure the proper process. A variety of human diseases called ribosomopathies have been shown caused by defects in ribosome biogenesis. Compared with human and yeast, much remains unknown in plants, in arabidopsis, mutants in genes encoding essential ribosome biogenesis factors can cause embryo lethal or other severe developmental defects.

In our previous work, we identifeid that atprmt3 mutants showed pleotropic developmental defects like retarded growth and pointed first true leaves. AtPRMT3 is a member of protein araginine methyltransferase, it functions in ribosome biogenesis by regulating proper pre-rRNA processing, however the molecular mechanism remains unknown. Here we identified that 40S ribosomal protein S2 (RPS2) could interact with AtPRMT3. In arabidopsis, RPS2 has four highly conserved family members including RPS2A, RPS2B, RPS2C and RPS2D. By using CRISPR-Cas9 we created rps2a2b mutants, finding that rps2a2b mutants show similar developmental phenotypes and pre-rRNA processing defects to atprmt3. We also identified that the triple mutants of rps2a2b and atprmt3 mutants are embryo lethal, implying their essential genetic relationship. Furthermore, we identified that RPS2A2B can bind to pre-rRNA, so it can regulate processing directly. Above all, our findings reveal a molecular mechanism by which AtPRMT3 and RPS2A2B regulate pre-rRNA processing.

#72. Role of the GTPase RbgA in the Assembly of 50S Ribosomal Subunit in Bacillus subtilis

Tanzila Wasi[1], Amal Seffouh[1], Robert Britton[2] and Joaquin Ortega[1]

1 - McGill University, Montreal, Canada
2 - Baylor College of Medicine, USA

Ribosome biogenesis GTPaseA (RbgA) assists in the late stages of assembly of the 50S ribosomal subunit of Bacillus subtilis. It binds to a 50S intermediate called 45S particle and hydrolyses GTP. RbgA-F6A expression prevents 50S maturation due to its lower GTPase activity, which leads to slow growth rate of bacteria. Suppressors spontaneously arising from this mutant partially corrects the growth defect. These suppressors have additional mutation in L6 ribosomal protein and accumulate 44S rather than 45S particles. Thus, L6 mutation allows more efficient 50S maturation and bypasses the requirement of fully functional RbgA. This suggests functional interplay between RbgA and L6 in wild type bacteria. To understand the role of RbgA in 50S maturation one approach is to visualize the events following the binding of RbgA to 45S particles. However, in order to understand the functional interplay of RbgA with L6, we want to visualize the events following the binding of RbgA-F6A with 44S particles. Surprisingly, L6 is present in low to high occupancy in 44S particles depending on the different L6 mutations. I am interested in characterizing the 44S particles with L6-R3C mutation that has high occupancy of L6. Two classes of the 44S 3D structures are obtained so far using cryo-EM single particle analysis. 44S particles are

more immature and flexible than the 45S particles which may make the binding of late ribosomal proteins more energy efficient. Preliminary binding assay using microscale thermophoresis shows that RbgA-F6A binds to 44S particles.

#73. A novel regulatory mechanism that controls ribosome production by repressing ribosomal subunit maturation

Archana Prusty[1], Matthew Eastham[2], Lars Schoenemann[1], Raji Meduri[1], Andria Pelava[2], Andreas Schlosser[1], Nicholas James Watkins[2], Utz Fischer[1]

1 - University of Wuertzburg, Germany
2 - Newcastle University, United Kingdom

Many stresses, signalling pathways and stimuli are known to impact ribosome production. Ribosomal proteins are produced in excess in mammalian cells and it has been proposed that ribosomal (r)RNA levels are rate-limiting for ribosome production. While regulation at the level of rRNA transcription has been described, it is unclear whether a mechanism exists that controls ribosomal subunit maturation.

Here, we describe C8ORF33 (UPF488), a novel inhibitor of ribosome biogenesis that is not found in yeast. C8ORF33 directly binds GRWD1, the ribosomal protein L3 (RPL3) chaperone. We show that GRWD1 is needed for the production of both ribosomal subunits, blocking pre-rRNA processing early in the nucleolus. Both GRWD1 and C8ORF33 are present in early pre-ribosomes, together with the U3 snoRNP, and before the 5S rRNA is recruited. Furthermore, the C-terminus of C8ORF33 binds to GRWD1 and this interaction blocks GRWD1 function. Depletion of C8ORF33 resulted in a two-fold increase in production of mature ribosome subunits without increasing rRNA transcription, while overexpression mimicked GRWD1 depletion. Pre-ribosomes associated with C8ORF33, which contain poly-adenylated pre-rRNA, are likely destined to be turned over.

Our data suggest that both the rRNA and ribosomal proteins are produced in excess and neither are rate-limiting. Instead, we propose that ribosome production is controlled at the level of ribosomal subunit maturation by C8ORF33 in higher eukaryotes.

#74. Depletion of factors involved in all aspects of ribosome biogenesis activates p53 via the 5S RNP

Andria Pelava, Graeme Wells, Matthew Eastham, Maria Deichner, Joshua Stewart, Regina Hertle, Nicholas Watkins, Claudia Schneider

ICaMB, Newcastle University, United Kingdom

Defective ribosome biogenesis is associated with a number of genetic diseases, known as ribosomopathies, as well as several forms of cancer. Disruption of ribosome production leads to activation of the tumour suppressor p53, via the 5S RNP, a large subunit (LSU) assembly intermediate. Excess 5S RNP binds to and inhibits the p53 inhibitor MDM2 resulting in p53 activation, a process often referred to as nucleolar stress. However, given that defects in all stages of ribosome production are linked to disease, it is unclear if 5S RNP-mediated p53 activation is limited to the nucleolar steps.

Here, we show that depletion of individual ribosomal proteins involved in different stages of

the production of large (LSU) and small (SSU) subunits leads to 5S RNP-dependent activation of p53 in multiple human cell lines. No additive p53 activation was observed upon simultaneous depletion of LSU and SSU ribosomal proteins. Knockdown of ribosomal proteins from either subunit also resulted in cell cycle arrest. Importantly, increased p53 protein levels and pre-rRNA processing defects were seen after just 3-6 hours following siRNA or ASO silencer treatment. However, at these short time-points the ribosome biogenesis defect had no impact on mature rRNA levels. Furthermore, depletion of ribosome biogenesis factors required for early (RRP5, BMS1, BOP1, PICT1, BXDC1), middle (ENP1, PNO1, ABCF2) and late/cytoplasmic (RIO2, LSG1) stages of both LSU and SSU production also activated p53 in a 5S RNP-dependent manner. Our data therefore suggest that the term nucleolar stress is misleading since all aspects of ribosome biogenesis are coupled to p53 signalling.

#75. The C-terminus of Nog1 facilitates rRNA folding of the polypeptide exit tunnel

Daniel Musa Wilson and John L Woolford

Carnegie Mellon University, USA

The polypeptide exit tunnel (PET) is an essential, conserved functional center of the large ribosomal subunit that functions in protein synthesis. However, the mechanisms involved in assembly of this functional center have remained elusive. Recent cryo-EM models of assembling pre-ribosomes purified from the yeast S. cerevisiae have revealed that Nog1, a GTPase assembly factor (AF), interacts with the PET. Specifically, Nog1 probes the rRNA the PET with its C-terminus. Interestingly, following exit of Nog1 from pre-ribosomes, cryo-EM models show that two additional AFs, Rei1 and then Reh1, occupy the PET in ways strikingly similar to Nog1. How the C-termini of these three AFs function in ribosome assembly and in the context of the PET remains unknown. We used biochemical and molecular genetic methods to answer this question. Truncation of the last 52 amino acids from the C-termini of all three tunnel-occupying AFs caused slow growth and cold-sensitive defects. This truncation was sufficient to cause a cold-sensitive phenotype and accumulation of 27SB pre-rRNA. Despite all three AFs being truncated, we found that nog1D595-647 was sufficient to cause all observed molecular phenotypes. Furthermore, the export adaptor Arx1 fails to associate with pre-ribosomes in nog1D595-647 mutants. Surprisingly, we did not observe any effects on the assembly of proteins required for 27SB pre-rRNA processing. We hypothesize that these defects we observe are caused by a failure to fold rRNA helices in domains II and V that make up part of the mature PET. Cryo-EM shows that these helices adopt immature conformations just prior to insertion of the Nog1 C-terminus. Altogether, our data suggest that Nog1 acts as a coordinator of maturation events in two functional centers in the large subunit, the PTC and the PET.

#76. Translational changes associated to dyskerin downregulation in breast cancer cells

Zacchini F, Penzo M, Rizzo D, Inga A, Dassì E, Montanaro L.

University of Bologna, Italy

The human pseudouridine synthase dyskerin (DKC1) mediates the site specific pseudouridylation in rRNA. Experimental reduction of DKC1 levels alters ribosomal function affecting mRNA translation. Importantly, in breast cancer cells a link between DKC1 expression, rRNA pseudouridyation and translational control has been observed. However, an unbiased approach aimed to identify which mRNAs are differentially translated when DKC1 function is reduced has never been performed in breast cancer cells. Therefore, we characterized the context of translational changes associated to DKC1 downregulation by high-throughput massive sequencing of the mRNAs recruited to polysomes. A limited number of DKC1 translational targets mainly involved in the control of apopotosis, protein synthesis and immune response was identified. In parallel, to have information on the rRNA pesudouridylation sites involved by DKC1 downregulation, in the same experimental models we comprehensively evaluated changes in H/ACA box snoRNA levels. Obtained results showed a complex re-modulation of selected H/ACA box snoRNAs, including some predicted to guide pseudouridylation at functionally relevant ribosomal sites. Altogether, these results provide information concerning the molecular mechanisms underlying the biological effects of DKC1 downregulation in breast cancer cells.

#77. Rps10 loop mediated Tigecycline resistance in Escherichia coli

Gernot Zarfel[1], Clemens Kittinger[1], Daniela Toplitsch[1], Valentina Percher[2], Brigitte Pertschy[1]

1 - University of Graz, Autria
2 - Carinthian University of Applied Sciences, Austria

Tigecycline is a last line antibiotic belonging to the tetracyline antibiotic family, which are potent inhibitors of translation elongation. Tigecycline binds bacterial 30S ribosomal subunits at the A-site, where it prevents delivery of aminoacyl-tRNA.

Several mutations have been described to mediate resistance to tigecycline, including allelic versions of the rpsJ gene, coding for the ribosomal protein Rps10 (uS10). While the largest part of Rps10 is positioned on the solvent exposed side of 30S subunits, two beta strands connected by an unstructured loop dive deeply into the ribosomal RNA, reaching close to the A site on the intersubunit side of the 30S subunit. All amino acid exchanges known to cause tigecycline resistance lie in the loop region of Rps10. Notably, Tigecycline does not directly bind to the Rps10 loop, but its binding site is ~10 Å apart in helix 31 of the 16S rRNA. This raises the question how mutations at a physically distant site can cause resistance to tigecycline.

In this project, we are functionally characterizing mutants in the Rps10 loop in order to gain a better understanding of the effects of Rps10 loop mutation on tigecycline resistance, as well as ribosome biogenesis and function.

List of Attendees

Last name	First name	email
Abou Elela	Sherif	sherif.abou.elela@usherbrooke.ca
Aguilar	Lisbeth	lisbethcarolina.aguilar@ircm.qc.ca
Ahmed	Yasar	yasar.ahmed@bzh.uni-heidelberg.de
Ameismeier	Michael	ameismeier@genzentrum.lmu.de
Angulo Ibanez	Maria	mangulo@stanford.edu
Aravin	Alexei	aravin@caltech.edu
Arya	Dev	dev.arya@nubadllc.com
Bagatelli	Felipe	felipe.bagatelli@usp.br
Baker	Nicholas	nicholas.baker@einstein.yu.edu
Beckmann	Roland	beckmann@genzentrum.lmu.de
Bellofatto	Vivian	bellofat@njms.rutgers.edu
Bergler	Helmut	helmut.bergler@uni-graz.at
Beringer	Daniel	beringed@bc.edu
Bertrand	Edouard	edouard.bertrand@igmm.cnrs.fr
Bhutada	Priya	priya.bhutada@uni-graz.at
Biedka	Stephanie	sbiedka@andrew.cmu.edu
Black	Joshua	jjblack@utexas.edu
Bohnsack	Markus	markus.bohnsack@med.uni-goettingen.de
Boivin	Vincent	vincent.boivin2@usherbrooke.ca
Bonham-Smith	Peta	peta.bonhams@usask.ca
Bouchard-Bourelle	Philia	philia.bouchard-bourelle@usherbrooke.ca
Brangwynne	Clifford	cbrangwy@princeton.edu
Britton	Robert	robert.britton@bcm.edu
Buskiewicz	Iwona	ibuskiew@uvm.edu
Bustelo	Xosé	xbustelo@usal.es
Cao	Xiaofeng	xfcao@genetics.ac.cn
Charette	Michael	michaeljcharette@gmail.com
Cheng	Jingdong	jcheng@genzentrum.lmu.de
Cluff	James	charettem@brandonu.ca

Corey	Seth	coreylab@yahoo.com
Correll	Carl	carl.correll@rosalindfranklin.edu
Cruz	Victor	victor.cruzruiz@utsouthwestern.edu
Cunningham	Rebecca	r.j.cunningham2@ncl.ac.uk
DaDalt	Ashley	dadalta@uwindsor.ca
De Keersmaecker	Kim	kim.dekeersmaecker@kuleuven.be
de la Cruz	Jesus	jdlcd@us.es
Deliu	Lisa	lpdeliu@ucalgary.ca
Denicourt	Catherine	catherine.denicourt@uth.tmc.edu
Diaz	Jean-Jacques	jean-jacques.diaz@lyon.unicancer.fr
Dosil	Mercedes	mdosil@usal.es
Dragon	François	dragon.francois@uqam.ca
Duss	Olivier	oduss@scripps.edu
Eastham	Matthew	m.eastham2@ncl.ac.uk
Ellis	Steven	janice.burkett@louisville.edu
Entian	Karl-Dieter	kdentian@srd-biotec.de
Erdmann	Philipp	stawski@biochem.mpg.de
Ernst	Felix	felix.ernst@ulb.ac.be
Faucher-Giguère	Laurence	laurence.faucher.giguere@usherbrooke.ca
Fefelova	Elena	il.tardigrado@gmail.com
Fequino	Leandro	leandro.fequino@usherbrooke.ca
Ferreira-Cerca	Sébastien	sebastien.ferreira-cerca@ur.de
Fitzgerald	Fiona	ffitzger@andrew.cmu.edu
Fredrick	Kurt	fredrick.5@osu.edu
Gadal	Olivier	olivier.gadal@ibcg.biotoul.fr
Garcia	Ivelitza	igarcia@allegheny.edu
George	Amee	amee.george@anu.edu.au
Germain	Devon	devon.germain@meduniwien.ac.at
Gibbs	Michelle	gibbs.285@osu.edu
Gleizes	Pierre-Emmanuel	gleizes@ibcg.biotoul.fr
Gómez Gaspar	Sonia	soniiagomez@usal.es
Hannan	Kate	kate.hannan@anu.edu.au
Henras	Anthony	henras@biotoul.fr

Heurgué-Hamard	Valérie	valerie.heurgue@ibpc.fr
Hurt	Ed	ed.hurt@bzh.uni-heidelberg.de
Iben	Sebastian	sebastian.iben@uni-ulm.de
Ishiguro	Kensuke	ken5533@home.email.ne.jp
Jahagirdar	Dushyant	dushyant.jahagirdar@mail.mcgill.ca
Jain	Chaitanya	cjain@miami.edu
Jain	Nikhil	nikhilj@bcm.edu
Jaremko	Daniel	danielja@buffalo.edu
Johnson	Arlen	arlen@austin.utexas.edu
Karbstein	Katrin	kkarbst@scripps.edu
Kater	Lukas	kater@genzentrum.lmu.de
Kiparaki	Marianthi	kiparaki.marianthi@gmail.com
Klinge	Sebastian	klinge@rockefeller.edu
Knueppel	Robert	robert.knueppel@ur.de
Kofler	Lisa	lisa.kofler@uni-graz.at
Kothe	Ute	ute.kothe@uleth.ca
Kressler	Dieter	dieter.kressler@unifr.ch
Kunz	Kathrin	k.kunz@med.uni-frankfurt.de
Lacoux	Caroline	caroline.lacoux@ibpc.fr
Lafontaine	Denis	denis.lafontaine@ulb.ac.be
Landry-Voyer	Anne-Marie	anne-marie.landry-voyer@usherbrooke.ca
LaPeruta	Amber	alaperut@andrew.cmu.edu
Lebaron	Simon	lebaron@ibcg.biotoul.fr
Lee	Byron	byronlee@mit.edu
Lee	Justine	j.k.lee2@ncl.ac.uk
Lin	Chi-Wang	linchiwang@gmail.com
Lindahl	Lasse	lindahl@umbc.edu
Liu	Chunyan	cyliu@genetics.ac.cn
Lyons	Shawn	smlyons@bwh.harvard.edu
Mangan	Hazel	hazel.mangan@nuigalway.ie
Martens	Elena	e.martens@erasmusmc.nl
McAlear	Michael	mmcalear@wesleyan.edu
McLean	Lisa	lisa.ogawa@yale.edu

McMahon	Mary	mary.mcmahon@ucsf.edu
McNutt	Zakkary	mcnutt.58@osu.edu
Meier	Tom	tom.meier@einstein.yu.edu
Mende	Hannah	mende@med.uni-frankfurt.de
Meyer	Michelle	m.meyer@bc.edu
Mitterer	Valentin	valentin.mitterer@bzh.uni-heidelberg.de
Montanaro	Lorenzo	lorenzo.montanaro@unibo.it
Montellese	Christian	christian.montellese@bc.biol.ethz.ch
Morais	Pedro	pmorais@proqr.com
Moss	Thomas (Tom)	tom.moss@crhdq.ulaval.ca
Musalgaonkar	Sharmishtha	sharmishthamusalgaonkar@gmail.com
Mustafa	Malik ghulam	mustafa.malik.ghulam@usherbrooke.ca
Nagahama	Masami	nagahama@my-pharm.ac.jp
Naganathan	Anusha	anaganat@ur.rochester.edu
Oeffinger	Marlene	marlene.oeffinger@ircm.qc.ca
Oliveira	Carla	ccoliv@iq.usp.br
Oplova	Michaela	michaela.oplova@bc.biol.ethz.ch
Orea Ordóñez	Lidia	lorea@ibv.csic.es
Ortega	Joaquin	joaquin.ortega@mcgill.ca
Panov	Konstantin	k.panov@qub.ac.uk
Panse	Vikram	vpanse@imm.uzh.ch
Parker	Melissa	mparker@scripps.edu
Peddada	Nagesh	nagesh.peddada@utsouthwestern.edu
Pellegrino	Simone	simone.pellegrino@igbmc.fr
Peña	Cohue	cpena@imm.uzh.ch
Penzo	Marianna	marianna.penzo@unibo.it
Perez-Fernandez	Jorge	jpfernandez@ur.de
Pertschy	Brigitte	brigitte.pertschy@uni-graz.at
Peterson	Laura	laura.peterson@uky.edu
Petibon	Cyrielle	cyrielle.petibon@usherbrooke.ca
Pikaard	Craig	cpikaard@indiana.edu
Pillet	Benjamin	benjamin.pillet@unifr.ch
Pillon	Monica	monica.pillon@nih.gov

Plisson-Chastang	Celia	celia.plisson@ibcg.biotoul.fr
Pollutri	Daniela	danielapollutri@hotmail.it
Razi	Aida	aida.razi@mail.mcgill.ca
Rink	Constance	cirink@buffalo.edu
Romeo	Yves	romeo@biotoul.fr
Rössler	Ingrid	ingrid.roessler@uni-graz.at
Sailer	Carolin	carolin.sailer@uni-konstanz.de
Saraf	Kritika	kritika.saraf@ulb.ac.be
Sarinay-Cenik	Elif	elif.sarinay@gmail.com
Savage	Sharon	savagesh@mail.nih.gov
Schmidt	Christina	christina1.schmidt@vkl.uni-regensburg.de
Schmidt	Richard	christina_schmidt31@t-online.de
Schneider	Claudia	claudia.schneider@ncl.ac.uk
Scott	Michelle	michelle.scott@usherbrooke.ca
Seffouh	Amal	amal.seffouh@mcgill.ca
Shah	Arish	arish@mit.edu
Shore	David	david.shore@unige.ch
Singh	Raja	rsingh@genomealberta.ca
sleiman	sophie	sophiesleimane@gmail.com
Sloan	Katherine	katherine.sloan@med.uni-goettingen.de
Stanley	Robin	robin.stanley@nih.gov
Stasinopoulos	Andreas	kiparakimarianthi@gmail.com
Stolyarenko	Anastasia	molbiola@yandex.ru
Sulima	Sergey	sergey.sulima@kuleuven.be
Sun	Jingyu	jingyu.sun@mail.mcgill.ca
Teubl	Fabian	fabian.teubl@ur.de
Thomson	Emma	e.thomson@sheffield.ac.uk
Trahan	Christian	christian.trahan@ircm.qc.ca
Tschochner	Herbert	h.tschochner@mac.com
Varshney	Umesh	varshney@iisc.ac.in
Wang	Zhen	z.wang@genetics.ac.cn
Ware	Vassie	vcw0@lehigh.edu
Warner	Jonathan	jon.warner@einstein.yu.edu

Warren	Alan	ajw1000@cam.ac.uk
Wasi	Tanzila	tanzila.wasi@mail.mcgill.ca
Watkins	Nicholas	nick.watkins@ncl.ac.uk
Wells	Graeme	graeme.wells@ncl.ac.uk
Williams	Noreen	nw1@acsu.buffalo.edu
Williamson	James	katrinas@scripps.edu
Wilson	Daniel	dmwilson@andrew.cmu.edu
Woodson	Sarah	swoodson@jhu.edu
Woolford	John	jw17@andrew.cmu.edu
Ye	Keqiong	yekeqiong@ibp.ac.cn
Yu	Yi-Tao	yitao_yu@urmc.rochester.edu
Zacchini	Federico	federico.zacchini3@unibo.it
Zarfel	Gernot	gernot.zarfel@medunigraz.at
Zemp	Ivo	ivo.zemp@bc.biol.ethz.ch

Author Index

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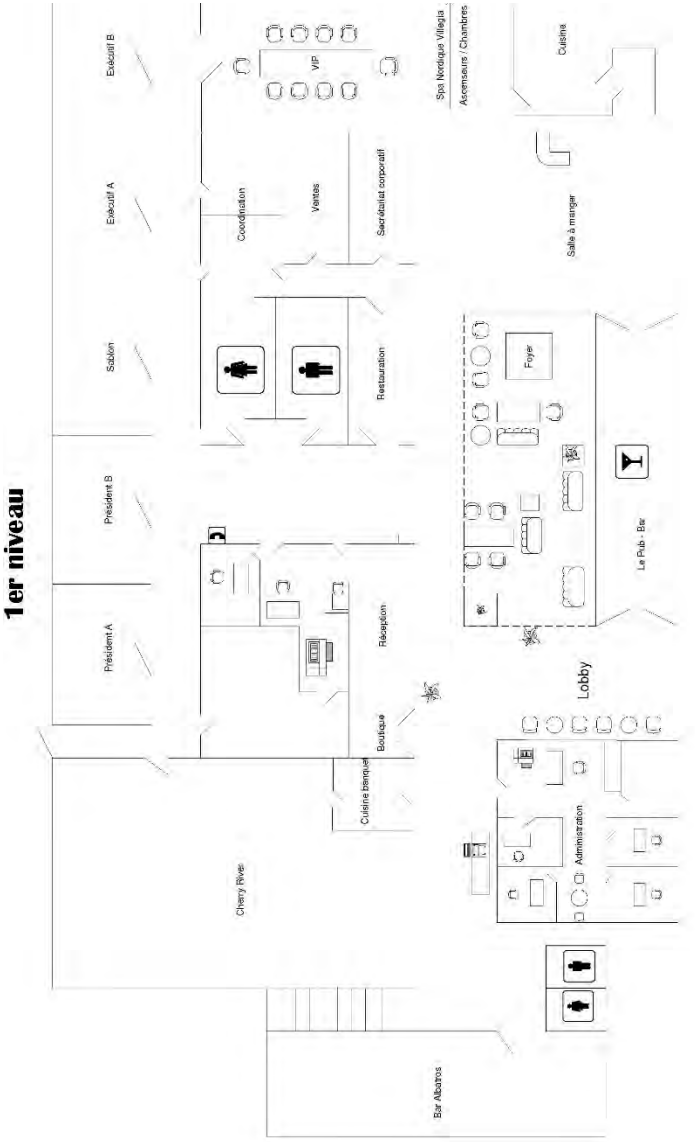
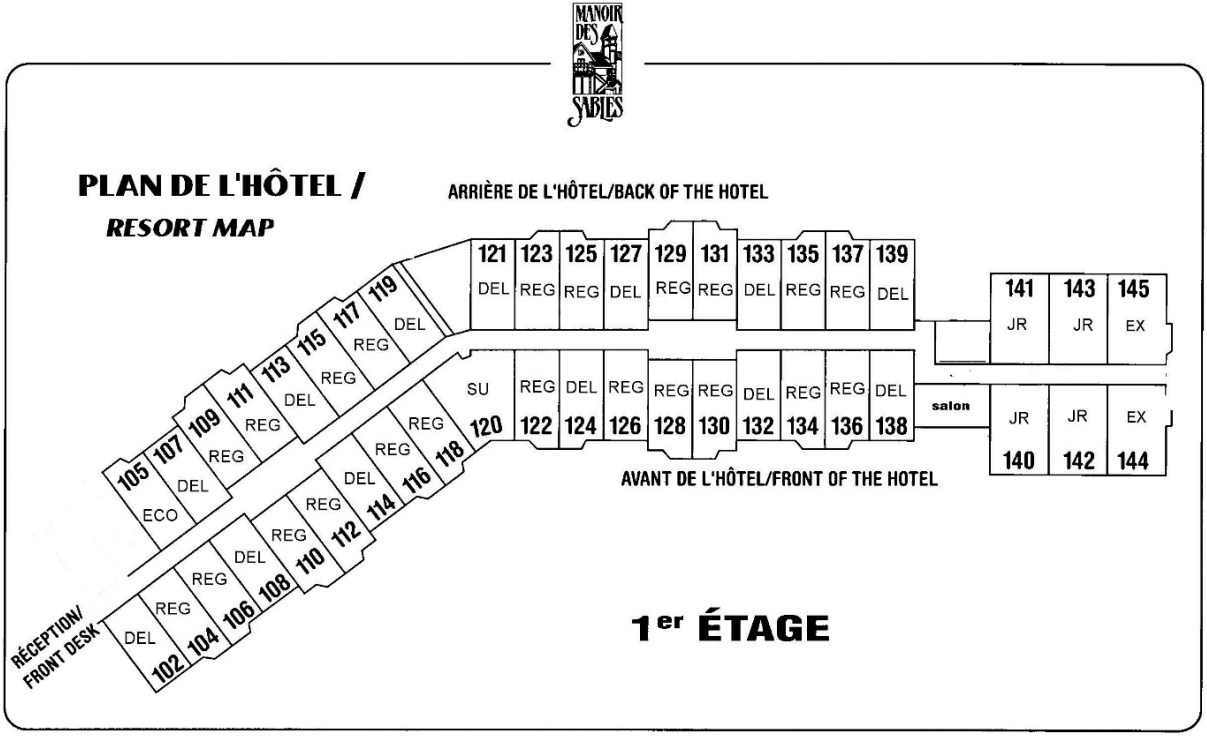
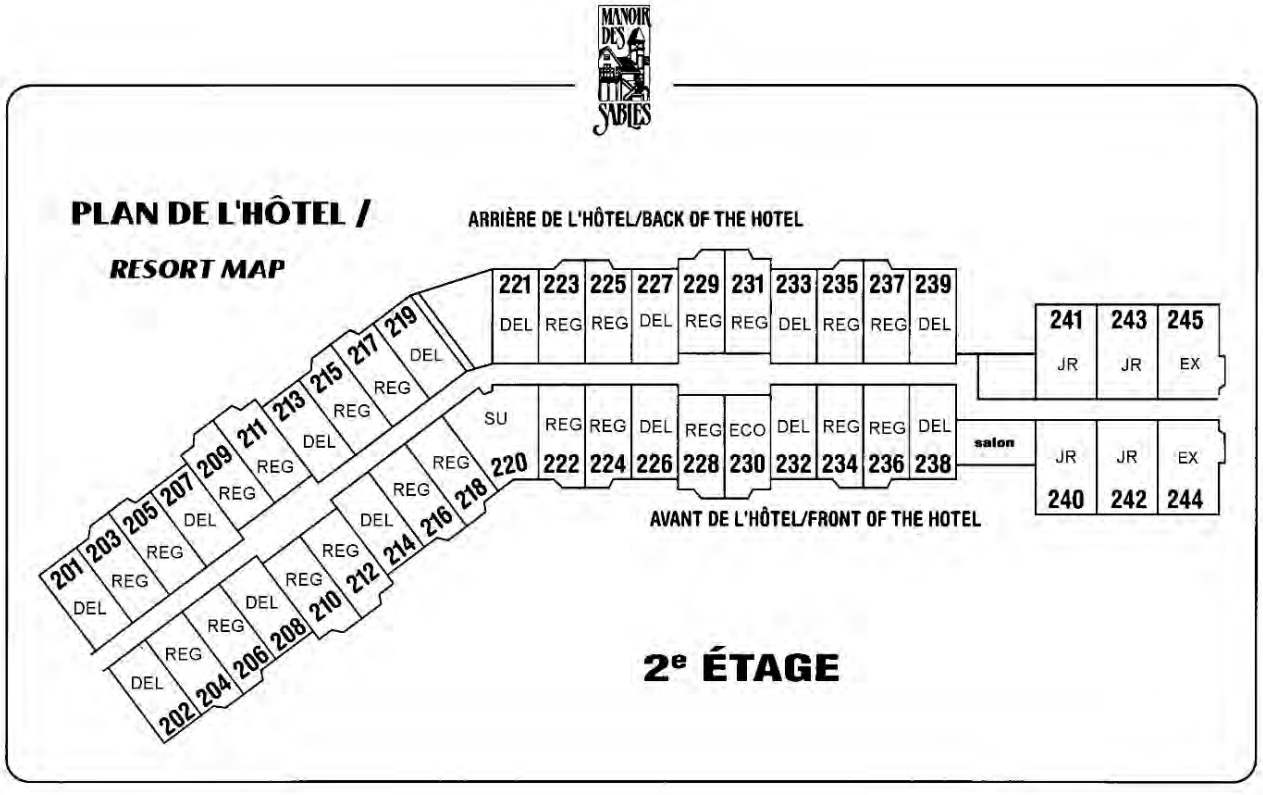
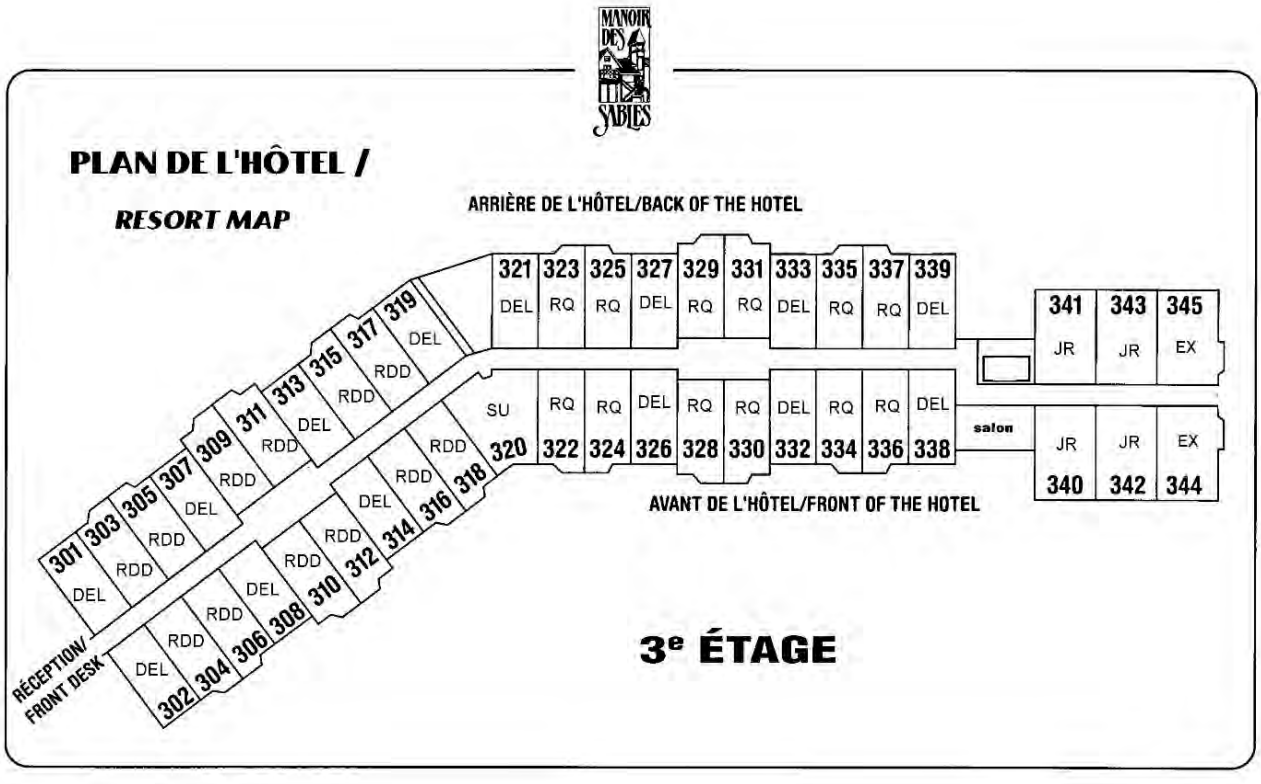
Hotel floor plan




MANOIR DES SABLES
PLAN DE L'HÔTEL /
RESORT MAP
ARRIÈRE DE L'HÔTEL/BACK OF THE HOTEL
121 DEL
123 REG
125 REG
127 DEL
129 REG
131 REG
133 DEL
135 REG
137 REG
139 DEL
141 JR
143 JR
145 EX
119 DEL
117 REG
115 REG
113 DEL
111 REG
109 REG
107 DEL
105 ECO
SU 120
122 REG
124 DEL
126 REG
128 REG
130 REG
132 DEL
134 REG
136 REG
138 DEL
salon
140 JR
142 JR
144 EX
118 REG
116 REG
114 DEL
112 REG
110 REG
108 DEL
106 REG
104 REG
102 DEL
RÉCEPTION/
FRONT DESK
AVANT DE L'HÔTEL/FRONT OF THE HOTEL
1er ÉTAGE


MANOIR DES SABLES
PLAN DE L'HÔTEL /
RESORT MAP
ARRIÈRE DE L'HÔTEL/BACK OF THE HOTEL
221 DEL
223 REG
225 REG
227 DEL
229 REG
231 REG
233 DEL
235 REG
237 REG
239 DEL
241 JR
243 JR
245 EX
219 DEL
217 REG
215 REG
213 DEL
211 REG
209 REG
207 DEL
205 REG
203 REG
201 DEL
SU 220
222 REG
224 REG
226 DEL
228 REG
230 ECO
232 DEL
234 REG
236 REG
238 DEL
salon
240 JR
242 JR
244 EX
218 REG
216 REG
214 DEL
212 REG
210 REG
208 DEL
206 REG
204 REG
202 DEL
AVANT DE L'HÔTEL/FRONT OF THE HOTEL
2e ÉTAGE


MANOIR DES SABLES
PLAN DE L'HÔTEL /
RESORT MAP
ARRIÈRE DE L'HÔTEL/BACK OF THE HOTEL
321 DEL
323 RQ
325 RQ
327 DEL
329 RQ
331 RQ
333 DEL
335 RQ
337 RQ
339 DEL
341 JR
343 JR
345 EX
319 DEL
317 RDD
315 RDD
313 DEL
311 RDD
309 RDD
307 DEL
305 RDD
303 RDD
301 DEL
SU 320
RQ 322
RQ 324
DEL 326
RQ 328
RQ 330
DEL 332
RQ 334
RQ 336
DEL 338
salon
JR 340
JR 342
EX 344
RDD 318
DEL 316
RDD 314
RDD 312
DEL 310
RDD 308
RDD 306
DEL 304
RDD 302
RÉCEPTION/ FRONT DESK
AVANT DE L'HÔTEL/FRONT OF THE HOTEL
3e ÉTAGE

PROGRAM SCHEDULE

August 1st

17:00 – 17:10	Opening notes
17:10 – 18:30	Session 1: Nucleolar organization and dynamics
18:30 – 20:00	Dinner (dining room)
20:00 – 21:05	Keynote Lecture I
21:05 – 01:00	Get Together (Salle Albatros)

August 2nd

08:30 – 10:20	Session 2: Ribosomal RNA Synthesis
10:20 – 10:50	Coffee break
10:50 – 12:25	Session 3: Subunit Assembly I
12:30 – 14:15	Networking Lunch (dining room)
14:15 – 15:35	Session 4: Ribosomal RNA processing
15:35 – 16:05	Coffee break
16:05 – 17:10	Session 5: Prokaryotic and Organelle Ribosome Assembly I
17:10 – 17:25	Break
17:25 – 18:30	Keynote Lecture II
18:30 – 20:00	Dinner (dining room)
20:00 – 22:30	Poster Session I (Salle Cherry River)
22:30 – 01:00	Get Together (Salle Albatros)

August 3rd

08:30 – 10:05	Session 6: snoRNP biogenesis and RNA modification
10:05 – 10:35	Coffee break
10:35 – 12:10	Session 7: Subunit Assembly II
12:15 – 14:00	Lunch (dining room)
13:30 – 14:00	Business meeting
14:00 – 18:00	Free afternoon
18:00 – 20:00	BBQ Dinner
20:00 – 22:30	Poster Session II (Salle Cherry River)
22:30 – 01:00	Get Together (Salle Albatros)

August 4th

08:30 – 10:05	Session 8: CryoEM and other advances
10:05 – 10:35	Coffee Break
10:35 – 11:55	Session 9: Ribosomopathies I
12:00 – 14:00	Lunch (dining room)
14:00 – 15:10	Session 10: Ribosome variants and alternative biogenesis pathways
15:10 – 15:25	Break
15:25 – 16:30	Session 11: Ribosomopathies II
16:30 – 17:00	Coffee Break
17:00 – 17:10	Poster Awards
17:10 – 18:15	Keynote Lecture III
18:15 – 20:00	Cocktail and Music Show (lobby)
20:00 – 22:30	Banquet (Salle Champêtre)
22:30 – 01:00	Dance (Salle Albatros)

August 5th

09:30 – 10:45	Session 12: Prokaryotic and Organelle Ribosome Assembly II
11:00	Departure