HANDBOOK

EMBO Workshop

Ribosome Synthesis: from Mechanisms to Therapy

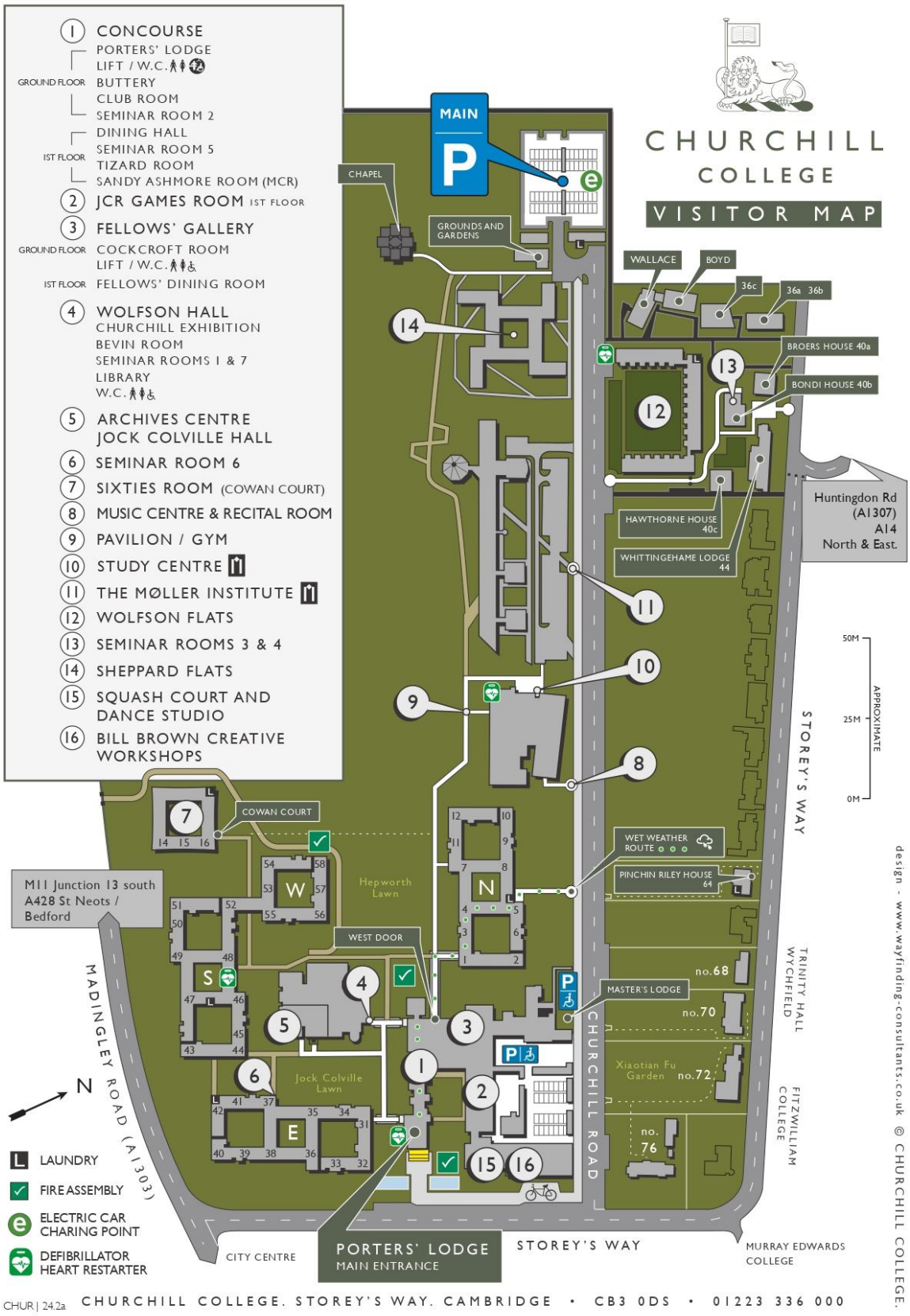
Churchill College, Cambridge, UK

Sunday 7th - Friday 12th September, 2025

EMBO | EMBO Workshop

EMBO-funded event

Churchill College Plan



Contents

Information for Delegates

Fire Assembly Points

On the site plan of Churchill College, there are three assembly points which are shown as boxes with ticks inside. On hearing the alarm, delegates should proceed to whichever is nearest to them at the time. Residents should familiarise themselves with escape routes before retiring to bed.

Security and Interruption

Please note that conference badges must be worn at all times (access may be denied if not worn). Please ensure that your phone is switched off or in silent mode during the presentations.

Smoking

Smoking is not permitted onsite at Churchill College, including all bedrooms and the conference location, and residents should not tamper with smoke detectors in their bedrooms.

Internet Access

Free wi-fi is available in all public areas, meeting rooms and bedrooms by connecting to the Cambridge University Network. Wi-Fi is available via the UniOfCam-Guest network.

Poster Presenters

Please refer to the author letter you would have received confirming your successful abstract. There are two dedicated poster sessions in the programme. All odd-numbered posters will be presented on Monday 8th September at 20:00, and all even-numbered posters will be presented on Tuesday 9th September at 20:00.

Drinks Vouchers

Inside your name badge there will be a drinks voucher for Sunday, Monday and Tuesday evening. The drinks voucher allows you to receive 2 complimentary drinks on these evenings. For the Monday and Tuesday evening, these drinks can be received during the poster sessions. Please go to the bar to use the drinks vouchers.

Gala Dinner

If you have booked to attend the gala dinner, this will take place on Thursday 11th September at St John's College. You will find a gala dinner ticket in your name badge which you will need to bring with you. Your name badge for the gala dinner should be visible throughout the evening. If you have a dietary requirement, your dietary card will be in your conference badge, once seated for the conference dinner please place your dietary card in front of you, then the waiting staff will serve you the appropriate meal.

Bedrooms at Churchill College

For delegates that have booked the accommodation package, check-in and key collection are at the Porters' Lodge which is situated at the main entrance to the College. Rooms will be available from 15:00 on the arrival date.

Rooms to be vacated by 10:00 on the morning of departure and room keys should be returned to the Porters' Lodge.

Parking

Parking is free at the College *subject to availability*. There are two places you can park: either along Churchill Road (the College's private road) or in the main car park at the end of the road. Permits are not required.

The College has eight charging points for electric cars which guests are welcome to use. Users should book their car in at the Porters Lodge on arrival, making plans to relocate it and freeing up the space for the next user, after 4 hours.

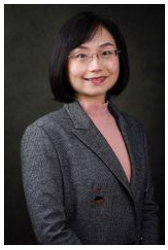
Taxis

Here are a couple taxi suggestions:

Cambridge City Taxis	01223 832832 https://cambridgecitytaxis.co.uk/
Veezu	01223 715715 https://www.veezu.co.uk/cambridge

The College is 2.7 miles from Cambridge railway station, and 32 miles from Stansted airport (40 minutes). There are also plenty of public transport options – see https://www.chu.cam.ac.uk/ourlocation/find-us/, including the U-bus to the station which costs about £2, journey time 30 minutes.

Organising Committee



Ling-Ling Chen
CAS Center for Excellence in Molecular Cell Science (CEMS), Shanghai Institute of Biochemistry and Cell Biology (SIBCB), Chinese Academy of Sciences (CAS)

Jeffrey Lipton
Feinstein Institutes for Medical Research, Zucker School of Medicine at Hofstra/Northwell

Michal Minczuk
MRC Mitochondrial Biology Unit, University of Cambridge

Joaquin Ortega
Department of Anatomy and Cell Biology McGill University, Montreal, QC, CA

Robin Stanley
National Institute of Environmental Health Sciences (NIEHS) National Institutes of Health (NIH), Research Triangle Park, NC, USA

Alan Warren
Cambridge Institute of Medical Research, University of Cambridge

Programme

Sunday 7th September 2025

16:00	Registration Opens – Concourse
17:00	Welcome Address – Wolfson Hall
17:10	Venki Ramakrishnan **Initiation of translation by the ribosome**
17:55	Katrin Karbstein **Maintaining Ribosome Homeostasis During and After Assembly**
19:00	Welcome Dinner (Networking) – Jack Colville Lawn

Monday 8th September 2025

Session #1 - Emerging Themes in Nucleolar Dynamics – Wolfson Hall **Chair:** Ling-Ling Chen **Fellow co-chair:** Sofia Quinodoz	
9:00 - 9:25	Xin Zhang **Micropolarity govern structural organization of the multilayered nucleolus**
9:25 - 9:40	Lin Shan **Pre-rRNA spatial distribution and functional organization of the nucleolus**
9:40- 9:55	Sofia Quinodoz **Mapping and engineering RNA-driven architecture of the multiphase nucleolus**
9:55 - 10:20	Eliezer Calo **Nucleolar Biophysical Homeostasis in Health and Disease**
10:20 -10:35	Herbert Tschochner **Why RNA polymerase I can make christmas trees**
10:35 – 11:00	Tea/Coffee Break – The Buttery
Session #2 -Regulation of rDNA copy number and rRNA Transcription – Wolfson Hall **Chair:** Vardhman Rakyan **Fellow co-chair:** Marianthi Kiparaki	
11:00 - 11:25	Takehiko Kobayashi **Unequal distribution of histone modifications that regulates rDNA stability rejuvenates yeast daughter cells**
11:25 - 11:40	Dorthe Larsen **TIN2 regulates nucleolar function and rDNA integrity**
11:40 - 11:55	Vardhman Rakyan **Germline sequence variation within the ribosomal DNA is associated with human complex traits**
11:55 - 12:10	Marianthi Kiparaki **RpS12-mediated induction of the Xrp1short isoform links ribosomal protein mutations to cell competition**

12:10 - 12:25	Shajahan Anver **rRNA transcription and subsequent ribosome biogenesis attenuation in the gut promotes longevity**
12:30 – 14:00	Buffet Lunch (Meet the Speaker Networking) – Dining Hall
Session #3A - Translacore Session, Mechanisms of Eukaryotic Ribosome Assembly – Wolfson Hall **Chair:** Alan Warren **Fellow co-chair:** Gabriella Lopez-Perez	
14:00 - 14:05	Overview of Translacore (Alan)
14:05 - 14:50	Keynote Speaker: Denis Lafontaine **Making Ribosomes One Nucleotide at a Time**
14:50 - 15:05	Taylor Ayers **RNA helicase Drs1 localizes onto pre-ribosomes via protein-protein and protein-RNA interactions**
15:05 - 15:20	Jacob Gordon **A structural atlas of multi-enzyme integration and regulation within the human rixosome complex**
15:20 - 15:35	Helmut Bergler **A comprehensive view on r-protein binding and rRNA domain structuring during early eukaryotic ribosome formation**
15:35 – 16:00	Tea/Coffee Break – The Buttery
Session #3B - Translacore Session, Mechanisms of Eukaryotic Ribosome Assembly – Wolfson Hall **Chair:** Brigitte Pertschy **Fellow co-chair:** Taylor Ayers	
16:00 - 16:25	Brigitte Pertschy **A dual role for the snR37 H/ACA snoRNP in pseudouridylation and rRNA scaffolding during pre-60S maturation**
16:25 - 16:40	Matthias Thoms **Modular folding of the 18S rRNA central domain guided by the snR30 H/ACA snoRNP**
16:40 - 16:55	Helgo Schmidt **Structure and Mechanism of the Ribosome Maturation Factor Rea1**
16:55 - 17:10	Sebastian Klinge **Helicase-mediated mechanism of SSU processome maturation and disassembly**
17:10 - 17:25	Justina Chu **Investigating real-time spatio-temporal progression of ribosome biogenesis using single-molecule resolution microscopy**
17:25 - 17:45	Poster Flash #1 (6 x 3 min Flash Talks from Poster Session #1). 1. Savannah Seely. 2. Vinithra Iyer. 3. Sheetanshu Saproo. 4. Mengjie Wu. 5. Kamil Flipek. 6. Isabella Lawrence
18:00	Mentoring Dinner (Meatless Monday, Assigned Seats) – Dining Hall
20:00	Poster Session #1 – The Buttery Odd-numbered Posters

Tuesday 9th September 2025

Session #4 - Translacore, Dysregulation of Ribosome Synthesis and Human Disease – Wolfson Hall **Chair:** Deena Iskander **Fellow co-chair:** Amee George	
9:00 - 9:25	Deena Iskander **Modelling genotype-phenotype correlations in Diamond-Blackfan anaemia syndrome**
9:25 - 9:40	Amee George **HEATR3 and novel effectors of the nucleolar surveillance pathway: uncovering links between ribosome biogenesis and disease**
9:40- 9:55	Sebastian Iben **ER stress and Alzheimer proteins shapen the quality of ribosomal protein synthesis in aging human fibroblasts**
9:55 - 10:20	Michael Buszczak **Late steps in pre-60S maturation impact early human brain development**
10:20 - 10:35	Mercedes Dosil **Preribosome proteostasis stress between nucleolus and nucleoplasm in 40S ribosomal protein deficiencies**
10:35 – 11:00	Tea/ Coffee Break – The Buttery
Session #5 - Translacore Session, Novel Approaches for Targeting Ribosome Synthesis in Cancer – Wolfson Hall **Chair:** John Knight **Fellow co-chair:** Holly Guo	
11:00 - 11:25	John Knight **Oxaliplatin and 5FU damage RNAs by multiple different mechanisms**
11:25 - 11:40	Faraz Mardakheh **A pre-rRNA-dependent positive feedback loop drives malignant ribosome biogenesis downstream of RAS oncogene**
11:40-11:55	Holly Guo **A small molecule inhibitor of NVL suppresses tumor growth by blocking ribosome biogenesis**
11:55 - 12:20	Julie Aspden **Conserved mechanism of uORF-dependent translational control by specialised ribosomes**
12:20 - 12:35	Marianna Penzo **Ribosomal protein L5 (RPL5/uL18) I60V mutation is associated to increased translation and modulates drug sensitivity in T-cell acute lymphoblastic leukemia cells**
12:35 – 14:00	Buffet Lunch (Meet the Speaker Networking) – Dining Hall
Session #6 - Emerging Therapies for Ribosomopathies- Wolfson Hall **Chair:** Jeff Lipton **Fellow co-chair:** Faraz Mardakheh	
14:00 - 14:25	Manuel Palacios **Gene Therapy and Gene Editing Strategies for the Treatment of Diamond-Blackfan Anemia syndrome**
14:25 - 14:50	Ross Hannan

	In vivo functions of UBF1 and UBF2 isoforms and The novel RNA polymerase I transcription inhibitor PMR-116 exploits a critical therapeutic vulnerability in a broad-spectrum of high MYC-malignancies.
14:50 - 15:05	Elaine Sanij **Targeting the nucleoli as a strategy to treat ovarian cancer**
15:05 - 15:20	Adrianna Vlachos **The International Diamond Blackfan Anemia Syndrome Cancer Consortium: Preliminary Results**
15:20 - 15:35	Lionel Blanc **Ribosomal Subunits RPS19 and RPL5 Differentially Orchestrate Fetal Hematopoiesis**
15:35 – 16:00	Tea/ Coffee Break – The Buttery
Session #7 - Pre-rRNA Processing and Folding – Wolfson Hall **Chair:** Jan Erzberger **Fellow co-chair:** Jacob Gordon	
16:00 - 16:25	Jan Erzberger **RNA Shape-Shifting: DEAD-box ATPases Drive Structural Transitions to Assemble the Large Ribosomal Subunit**
16:25 - 16:40	Sarah Woodson **Molecular interactions with nucleolar proteins and helicases chaperone pre-rRNA folding**
16:40 - 16:55	Valentin Mitterer **RNA helicases in SSU processome formation and maturation**
16:55 - 17:20	Margaret Rodgers **Early steps of eukaryotic ribosome assembly revealed by single-molecule microscopy**
17:20 - 17:35	Shuyi Zhao **A UTP3-dependent nucleolar translocation pathway facilitates pre-rRNA 5'ETS processing**
17:35 - 17:55	Poster Flash #2 (5 x 3 min Flash Talks from Poster Session #2) 1. Federico Zacchini. 2. Diu Nguyen. 3. Konstantin Panov. 4. Nuria Sanchez Puig. 5. Bilal Hafeez
18:00	Networking Dinner (Outdoor BBQ) – Jack Colville Lawn
20:00	Poster Session #2 – The Buttery Even numbered posters

Wednesday 10th September 2025

Session #8 - Non-eukaryotic ribosome assembly and advances in antibiotic development – Wolfson Hall **Chair:** Joaquin Ortega **Fellow co-chair:** Anastasiia Chaban	
9:00 - 9:25	Joey Davis **Visualizing ribosomes in near native conditions through cryoEM and machine learning**
9:25 - 9:40	Sébastien Ferreira-Cerca **Characterization of *cis*- and *trans*-acting elements required for ribosome synthesis in archaea**

9:40- 9:55	Noa Avidan **Autonomous synthesis and assembly of early and late stage intermediates of the Escherichia coli 50S subunit in a minimal system**
9:55 - 10:20	Kurt Fredrick **Role of the leader-trailer helix of pre-16S rRNA in biogenesis of the 30S subunit**
10:20 - 10:35	Anastasiia Chaban **Functional coupling between ribosomal RNA transcription and early processing**
10:35 – 11:00	Tea/ Coffee Break – The Buttery
Session #9 - Regulation of Mitoribosome Biogenesis and Non-Eukaryotic Ribosome Assembly – Wolfson Hall **Chair:** Michal Minczuk **Fellow co-chair:** Amal Seffouh	
11:00 - 11:15	Amal Seffouh **YsxC is a placeholder for ribosomal protein uL2 during 50S ribosomal subunit assembly.**
11:15 - 11:30	Gabriella Lopez-Perez **A ribosome biogenesis associated RNA helicase impacts r-protein production**
11:30-11:55	Hauke Hillen **Mechanisms of human mitochondrial RNA biogenesis and mitoribosome synthesis**
11:55 - 12:10	Alexey Amunts **Integration of experimental data with model prediction and simulation reveals how Mettl15-Mettl17 modulates pre-mitoribosome**
12:10-12:35	Joanna Rorbach **Mammalian mitochondrial ribosome assembly: insights from recent structural studies**
12:35 – 13:30	Early Career Session - This activity will support the professional growth of all members of our communities and provide an open forum for discussion and mentoring. ERC Representative - Maria Siomos and Biochemical Society Presentation
13:30	Free Afternoon/Evening

Thursday 11th September 2025

Session #10 - Specialized Ribosomes, Regulation, and Quality Control – Wolfson Hall **Chair:** William Faller **Fellow co-chair:** Bulat Fatkhullin	
9:00 - 9:25	William Faller **Loss of different ribosomal proteins have distinct effects on antigen processing and presentation**
9:25 - 9:40	Vikram Paralkar **Regulation of RNA Polymerase I activity and rRNA transcription by cell-type-specific transcription factor CEBPA**
9:40- 9:55	Kim De Keersmaecker **Translatome and translation dynamics analysis reveals mechanistic diversity of**

	frequent ribosomal protein mutations in cancer, with profound changes for RPS15-mutations.
9:55 - 10:20	Vassie Ware **Ribosomal Protein Paralogues of the eRpL22 family as Drivers of Developmental Changes in Spermatogenesis in *Drosophila***
10:20 - 10:35	Marlene Oeffinger **Compositionally distinct 5.8S-L rRNA ribosomes link differential mRNA translation to selective cell fitness in *S. cerevisiae***
10:35 – 11:00	Tea/ Coffee Break – The Buttery
Session #11 - New Developments in the Ribosome Epitranscriptome – Wolfson Hall **Chair:** Sandra Blanco **Fellow co-chair:** Vikram Paralkar	
11:00 - 11:25	Sandra Blanco **Differential rRNA Methylation Orchestrates Ribosome Specialization to Fine-Tune Translation During the Cell Cycle**
11:25 - 11:40	Gleizes Pierre-Emmanuel **Cytoplasmic uridylation monitors maturation of the human 18S rRNA 3' end**
11:40-12:05	Anders Lund **Assigning functions to ribosomal 2'-O-methylations**
12:05 - 12:20	Merav Socolovsky **A key erythroid transcriptional switch coincides with an EpoR/Stat5-driven spike in ribosome biogenesis and protein synthesis**
12:20-12:35	U. Thomas Meier **Nucleolar integration and molecular mechanism of ribosomal RNA modification by liquid-liquid phase separation**
12:35 – 14:00	Buffet Lunch (Meet the Speaker Networking) – Dining Hall
Session #12A - Hot Topics and Technological Innovations in Ribosome Assembly – Wolfson Hall **Chair:** Felipe Karam Teixeira **Fellow co-chair:** Lin Shan	
14:00 - 14:25	Mark Bruce **Advancing Ribosome Research through Direct RNA Sequencing with Oxford Nanopore Technologies**
14:25 - 14:40	Xiaohan Zhao **Investigate nucleolar pre-ribosome assembly in situ**
14:40 - 15:05	Juliette Fedry **Visualization of translation reorganization upon persistent ribosome collision stress in mammalian cells**
15:05 - 15:20	Shirley Daube **Towards autonomous cell-free biogenesis of the central dogma of biology**
15:20 - 15:35	Ximena Zottig **Mechanistic Insights into Non-AUG Codon Stringency**
15:35 – 16:00	Tea/ Coffee Break – The Buttery
Session #12B Hot Topics and Technological Innovations in Ribosome Assembly – Wolfson Hall **Chair:** Juliette Fedry	

16:00 - 16:30	**Sponsored talk from Thermo Fisher - Presented by Rebecca Thompson**	
16:30-17:00	Felipe Karam Teixeira **Young EMBO Lecture**	
17:00 - 17:30	Business Meeting and Awards	Entire Planning Committee
19:00	Gala Dinner – St John's College	

Speaker Biographies

Julie Aspden
University of Leeds

Julie is an Associate Professor in RNA Biology in the Faculty of Biological Sciences at the University of Leeds, UK. She read Biochemistry at the University of Oxford before undertaking a PhD in Biochemistry at the University of Cambridge on the initiation of mRNA translation. During her first postdoc at the University of California, Berkeley, her work focused on alternative mRNA splicing in Drosophila. Her second postdoc was at the University of Sussex, where she became interested in long non-coding RNAs and their potential translation. She discovered the translations of 100s novel ORFs from lncRNAs in Drosophila. In 2015 Julie established her independent research group at Leeds. Her group addresses questions on the regulation of mRNA translation, ribosome heterogeneity, non-coding RNA function and the role of specific RNA-protein complexes. They combine biochemistry, genomics, molecular biology and genetics to study RNAs in Drosophila melanogaster and mammalian tissue culture, including human neuronal cells. Julie has 19 years of experience and expertise in RNA biology, and an active member of the RNA Society. Julie is academic co-lead of LeedsOmics, the virtual omics institute at the University of Leeds. She has previously led gender equality initiatives in her faculty and is passionate about creating a supportive and inclusive research culture. Julie now leads a large interdisciplinary team "RiboCode" to understand how changes in ribosome composition impact translation and how this occurs across different organisms. Her group's work has been funded by both MRC and BBSRC.

Sandra Blanco
Spanish Research Council, Cancer Research Center, Salamanca Spain

Dr Sandra Blanco is group leader at the Epitranscriptomics and Cancer Lab, in the Cancer Research Centre (National Council Research and University of Salamanca) and associate researcher at the Biomedicine Research Institute of Salamanca (Spain). The IP's research has focused since her postdoc in deciphering the role of RNA modifications (epitranscriptome) in stem cell biology, homeostasis and disease.

Michael Buszczark
UT Southwestern Medical Center

Michael Buszczak is the Lillian B. and Tom B. Rhodes Professor in Stem Cell Research at the University of Texas Southwestern Medical Center, where he also serves as Associate Director for the Hamon Center for Regenerative Science and Medicine. He has spent over fifteen years studying how ribosomes and mRNA translation influence development and disease.

Eliezar Calo
MIT

Eliezer Calo was born and raised in Puerto Rico. He earned a bachelor's degree in Chemistry from the University of Puerto Rico, Río Piedras Campus, and a Ph.D. in Biology from the Massachusetts

Institute of Technology. For his postdoctoral training, he attended Stanford University, where he worked with Dr. Joanna Wysocka and was awarded the Helen Hay Whitney Postdoctoral Fellowship. Currently, he is an associate professor in the Department of Biology at the Massachusetts Institute of Technology. His research focuses on the mechanisms underlying ribosome assembly and function, as well as how these processes influence developmental disorders and cancer. Dr. Calo is the recipient of the Basil O'Connor Starter Scholar Award and the Charles H. Hood Foundation Child Health Research Award and was named a 2019 Pew Scholar.

Joey Davis
MIT

Joey is the Whitehead Associate Professor of Biology at the Massachusetts Institute of Technology where his lab develops and deploys software tools to study the structure and dynamics of macromolecular complexes, including the ribosome. He completed his undergraduate studies at UC Berkeley, with dual degrees in Computer Science and Biological Engineering before performing his doctoral work in Biology at MIT under the direction of Professors Bob Sauer and Tania Baker. In 2010, Joey helped start the synthetic biology company Ginkgo BioWorks as the first employee before returning to Academia as a Jane Coffin Childs' post-doctoral scholar in Jamie Williamson's group at Scripps Research in 2012. Joey started his lab as an Assistant Professor at MIT in 2018, where his group has developed machine learning based frameworks for analyzing cryoEM and cryoET datasets, including the tools cryoDRGN and tomoDRGN.

Jan Erzberger
UT Southwestern Medical Center

Jan Erzberger earned his A.B. in Biochemical Sciences from Harvard University, followed by a Ph.D. in the lab of James Berger at UC Berkeley. He completed postdoctoral research in Nenad Ban’s lab at ETH Zurich before establishing his own lab at UT Southwestern, where he is now an Associate Professor of Biophysics. His research focuses on the mechanistic basis of large ribosomal subunit biogenesis, with particular emphasis on the roles of nucleotide triphosphatases and methyltransferases. His lab employs an integrated strategy, combining cryo-electron microscopy with genetic, proteomic, and cell biological methods to unravel the complex molecular choreography of ribosome assembly and its connections to cellular stress responses, quality control mechanisms, and cancer.

William Faller
Netherlands Cancer Institute

William studied for his BSc at the National University of Ireland, Galway, and graduated in 2003. He followed this with a PhD under the supervision of Prof. William Gallagher at the UCD Conway Institute in Dublin, where his project involved the study of DNA methylation in melanoma cells. He continued his focus on cancer in his Post Doctoral studies with Prof. Owen Sansom at the CRUK Beatson Institute in Glasgow. During this time he began to work with mouse models of colorectal cancer, primarily focusing on mTOR signaling and mRNA translation. In 2017 he became a Group Leader at the NKI in Amsterdam, and in 2025 he joined the University of Bristol. SInce starting his

lab, his work has focused on “non-canonical” forms and functions of the ribosome, including both ribosome specialization, and the ribosome stress response.

Juliette Fedry
University of Cambridge

Juliette Fedry used cryo electron tomography and subtomogam averaging during her postdoc to provide subnanometer visualization of protein translocation at the mammalian Endoplasmic Reticulum membrane. This work allowed the visualisation of the different ribosome translocon complexes, as well as their quantification and spatial analysis in native membranes. Juliette further implemented Focused Ion Beam milling approaches to study mRNA translation and its reorganization upon ribosome collision stress in intact mammalian cells. This study revealed a couple of bottlenecks in collision stress clearance and provides a framework for quantitative analysis of translation dynamics in situ. Juliette started her lab at the MRC-LMB 2 years ago, further investigating translation regulation in mammalian cells.

Kurt Fredrick
The Ohio State University

I grew up in Minnesota and attended Gustavus Adolphus College, earning a BA Biology degree in 1992. I did my graduate work at Cornell University with John D. Helmann. There, I studied RNA polymerase and flagellar gene expression in Bacillus subtilis, earning my PhD in Microbiology in 1997. I then moved on to the University of California Santa Cruz for postdoctoral studies with Harry F. Noller. At UCSC, I studied ribosome structure and function, focusing primarily on the mechanism of translocation. In 2003, I joined the faculty of Microbiology at Ohio State University (OSU) and established my own research program, focused on ribosome biogenesis and function. I earned tenure in 2009 and was promoted to Full Professor in 2013. In 2021, I became Chair of the Department.

Hauke Hillen
University Medical Center Göttingen

Hauke studied biochemistry in Tübingen (Germany) and earned his PhD in structural biology working on mitochondrial transcription with Patrick Cramer at the Ludwigs Maximilians University in Munich. After a short postdoc, he became project leader at the Max Planck Institute for Biophysical Chemistry in Göttingen (Germany), where he studied the structural basis of mitochondrial and viral gene expression. In 2020, he became a professor at the University Medical Center in Göttingen and group leader at the Max Planck Institute for Multidisciplinary Sciences in Göttingen. His group combines cutting-edge structural biology methods with biochemical, biophysical and cellular approaches to decipher the molecular mechanisms of organellar and viral gene expression.

Deena Iskander
Imperial College London

Dr Deena Iskander is a Clinical Lecturer at Imperial and an Honorary Peadiatric Hameatologist at St Mary's Hospital London. She completed a Blood Cancer UK Clinical Research Fellowshp in 2018 and has received 4 fellowships from the National Institute of Health Research, allowing her to establish a National Clinical Registry for Diamond Blackfan anaemia and decipher disease mechanisms underpinning genotype phenotype correlations in this rare ribosomopathy.

Katrin Karbstein
Vanderbilt University

Professor Karbstein received her Ph.D. from Stanford University, where she studied catalysis and conformational changes in RNA enzymes in the laboratory of Dan Herschlag. During her postdoc in Nobel Laureate Jennifer Doudna's lab in Berkeley, she developed a biochemical system to study ribosome assembly. In 2006, she joined the faculty at the University of Michigan in Ann Arbor and in 2010 moved to Scripps Florida. At Scripps Florida, she rose through the ranks to become a Full Professor at The Scripps Research Institute in 2020. In the summer of 2024, she joined the Biochemistry Department at Vanderbilt University and became a co-leader in the cancer cell biology program at VICC.

Dr. Karbstein's laboratory studies how cells maintain both the correct number and composition of ribosomes, and how failure of this process leads to the development and progression of cancers. Her work is funded by the National Institutes of Health, and has been recognized with an NSF CAREER award, an HHMI Faculty Scholar Award, and a UF Research Foundation professorship.

Takehiko Kobayashi
University of Tokyo

In 1992, Takehiko Kobayashi obtained his Ph.D. from Kyushu University, studying replication termination in E. coli. He then spent three years as a postdoctoral researcher with Dr. Melvine DePamphilis at the National Institutes of Health in Maryland, USA, investigating DNA replication initiation in mammals. Upon returning to Japan, he was appointed as an associate professor at the National Institute for Basic Biology in Okazaki, where he studied DNA amplification of rDNA. In 2006, he became a full professor at the National Institute of Genetics in Mishima. In 2015, he moved to the University of Tokyo. His research focuses on the relationship between rDNA stability, cellular senescence, and rejuvenation.

John Knight
University of Manchester

John is a lecturer in cancer biology at the University of Manchester where his group studies the roles of protein and RNA synthesis in colorectal cancer biology. He completed his PhD at the University of York with Jo Milner then did post-docs with Anne Willis and Owen Sansom.

Denis Lafontaine
Universite libre de Bruxelles

Denis L.J. Lafontaine received a research MSc (1991) and a PhD (1995) in Molecular Biology & Genetics from the University of Namur, Belgium, under the supervision of the late Prof Jean Vandenhaute. He engaged in postdoctoral training (1995-2001) with Prof David Tollervey within the Gene Expression program at the European Molecular Biology Laboratory in Heidelberg, Germany, and The Wellcome Trust Center for Cell Biology at the University of Edinburgh, United Kingdom. Denis was recruited in 2001 to a permanent position with the Belgian Fund for Scientific Research (F.R.S./FNRS). Since then he has worked as Principal Investigator at the Institute of Molecular Biology & Medicine at the University of Brussels (ULB). He is currently a Research Director with the F.R.S./FNRS and a Professor at the University of Brussels. In 2010, after 15 years of work on fundamental aspects of ribosome biogenesis, including RNA processing and RNA modification, in budding yeasts, the Lafontaine team turned towards human cells, performing multiple large-scale screens leading to identification of hundreds of novel ribosome assembly factors and essential contributors to nucleolar structure maintenance. Notably, each of the eighty ribosomal proteins was tested individually for a role in precursor ribosomal RNA processing, mature rRNA accumulation, nucleolar structure, and homeostasis of the anti-tumor protein p53 (See Www.RibosomalProteins.Com). A key finding was that only two ribosomal proteins are really very important for nucleolar structure maintenance: uL5 (RPL11) and uL18 (RPL5). These two ribosomal proteins are incorporated at a late stage of maturation, together with the 5S ribosomal RNA, into the maturing large ribosomal subunit, to form a functionally important architectural landmark of the ribosome: the central protuberance. When these ribosomal proteins are not assembled, for example in cells that cannot make their ribosomes well, they accumulate outside the ribosome to capture Hdm2. This leads to p53 stabilization and to killing of the deficient cell in a regulatory loop known as nucleolar surveillance. The long-term goal of the Lafontaine Lab is to understand the molecular bases and tissue-specific origins of human diseases caused by ribosome biogenesis dysfunction. The nucleolus, where the initial steps of ribosome biogenesis occur, has recently been redefined has a biomolecular condensate formed by liquid-liquid phase separation. The team is also interested in developing new tools to probe the liquid nature of the nucleolus and the importance of macromolecular condensation in ribosome biogenesis. Denis Lafontaine co-organized the international “Ribosome Synthesis Meeting” in 2012 (Banff, Canada), 2015 (Brussels, Belgium – his home town), 2018 (Magog, Canada), and 2022 (Engelberg, Switzerland). Between 2017 and 2021, he acted as Dissemination Officer for the European COST action Epitran (CA16120) on Epitranscriptomics and he is now involved in the COST action TRANSLACORE (CA21154) on the importance of translation in cancer. He also gives lectures to students working towards Bachelor’s or Master’s degrees in Belgium (U. Brussels, U. Charleroi, U. Namur), France (U. Lorraine), and Japan (University of Tokyo). Denis Lafontaine acts as a referee for journals in the fields of human genetics, molecular and cell biology, and RNA biochemistry and for numerous international funding agencies: American NSF, Austrian FWF, Belgian F.R.S/FNRS, British Wellcome Trust, Cancer Research UK (CRUK), Czech GACR, French ANR and INSERM, European Research Council, German DFG, Hong Kong Research Grants Council (RGC), Human Frontier Science Organization, Israel Science Foundation, Polish National Science Centre, Swiss NSF, etc.. He is also a HUGO Gene Nomenclature Committee (HGNC) specialist advisor [Ribosomal biogenesis factors]. Denis is an Associate Editor at RNA Biology (Taylor & Francis).

Anders Lund
University of Cambridge

Anders H. Lund graduated from the University of Aarhus, Denmark, in 1996. He trained as a postdoc at the University of Aarhus (1996-1999) and the Netherlands Cancer Institute (1999-2004) where he worked on the identification of novel oncogenes. He was appointed associate professor at the Biotech Research and Innovation Centre (BRIC), University of Copenhagen in 2004, became full professor in 2009, and was appointed Director of BRIC in 2019. With a focus on RNA modifications, translation and specialized ribosomes, the aim of the laboratory is to unveil fundamental biological mechanisms and understand how these become perturbed during diseases.

Venki Ramakrishman
Laboratory of Molecular Biology, Cambridge UK

Group leader at the MRC Laboratory of Molecular Biology, working on the structure and function of ribosomes, translational initiaiton, and regulation of translation

Vassie Ware
Lehigh University

Vassie C. Ware is a professor of molecular biology in the Department of Biological Sciences at Lehigh University where her laboratory investigates functional diversification of ribosomal protein (Rp) paralogues of the eRpL22 family and the impact of ribosome heterogeneity in Drosophila melanogaster germline development. Her work in the ribosome field started as a postdoctoral associate in Susan Gerbi's laboratory at Brown University where she determined the first primary and secondary structures for a multicellular organismal 28S rRNA (Xenopus laevis), defined eukaryotic-specific expansion segments, and mapped species-specific processing sites within Drosophila and Sciara 28S rRNA expansion segments. Her current work seeks to understand mechanisms governing translation specificities of paralogue-specific ribosomes in spermatogenesis to define specialized ribosomes with unique roles and/or to define new roles for eRpL22 paralogues in extra-ribosomal pathways. She received a B.A. degree in human biology from Brown University and M.Phil. and Ph.D. degrees in biology from Yale University.

Xin Zhang
Westlake University

Xin Zhang is the Professor of Chemistry at the Westlake University, Hangzhou. Prior to joining the faculty at Westlake in 2021, Zhang was the Paul & Mildred Berg Early Career Professor and associate professor of chemistry and of biochemistry and molecular biology at the Pennsylvania State University. Zhang was a Helen Hay Whitney postdoctoral fellow at the Scripps Research Institute, California. He earned a doctoral degree at the California Institute of Technology, a master's degree at the Dalian Institute of Chemical Physics of the Chinese Academy of Sciences, and a bachelor's degree at the University of Science and Technology of China. Zhang's research is focused on the chemistry of biological aggregates formed by proteins and RNAs under physiological and pathological conditions. Zhang's independent work has received multiple honors and awards, including Priestley Prize for undergraduate teaching in chemistry, Kavli Fellow of the National Academy of Sciences USA, CAPA Distinguished Junior Faculty Award, NSF CAREER award, NIGMS MIRA, Pew Scholar in the Biomedical Sciences, Scialog Fellowship, Sloan Research Fellowship, the Lloyd and Dottie Huck Early Career Award, the Burroughs Wellcome Fund Career Award at the Scientific Interface.

Speaker Abstracts

Sunday 7th September 2025

17.10
Initiation of translation by the ribosome
Venki Ramakrishnan
MRC Laboratory of Molecular Biology, Cambridge, UK

Initiation of mRNA translation is a key regulatory step in gene expression as well as the target of viruses. In bacteria, initiation involves three protein factors and a special initiator tRNA, which together assemble the small ribosomal subunit positioned over the start codon. In eukaryotes, the process if far more complex, and involves over a dozen factors which recruit the small ribosomal subunit to the 5' end of mRNA.

I will discuss our work on the structure of eukaryotic initiation complexes without and with a start codon, and its implications for the mechanism of initiation. I will also show how certain core features are conserved across kingdoms despite the enormous evolutionary divergence in initiation.

17.55
Maintaining Ribosome Homeostasis During and After Assembly
Katrin Karbstein
Vanderbilt University, Tennessee, USA

To catalyze protein synthesis, ribosomes must interpret the information within the mRNA to produce the right amount of the correct protein. In addition, ribosome collisions mediate mRNA quality control. Thus, misassembled ribosomes affect the sequence and abundance of proteins *and* the integrity of mRNAs, thereby globally disrupting protein homeostasis. Moreover, translation initiation and ribosome collisions are sensitive to perturbations in ribosome concentration, which can affect protein synthesis in an mRNA-specific manner. As a result, a number of diseases arise from defects in ribosome assembly, including neurodegenerative disorders and cancer. I will present studies into how cells maintain the correct number and composition of ribosomes

Monday 8th September 2025

Session #1 - Emerging Themes in Nucleolar Dynamics

9.00

Micropolarity govern structural organization of the multilayered nucleolus

Xin Zhang
Department of Chemistry, Westlake University, 600 Dunyu Road, Hangzhou 310030, Zhejiang, China

Nucleolus is a representative multilayered condensate, wherein the organization of the layered structures is critical to its function in ribosome biosynthesis. How microenvironments of biomolecular condensates affect their structure and function remains unknown. In this talk, I will present novel chemical tools that are developed to reveal hidden features of bimolecular condensates. These tools allow us to show that the arrangements and partitioning of biomolecules are dictated by the differences between the micropolarity of each subcompartment. Sufficient difference in micropolarity results in layered structures with the exterior shell presenting a more polar microenvironment than the interior core. Accordingly, micropolarity inversion is accompanied by conversions of the layered structures. These findings demonstrated the central role of the previously overlooked microenvironment in regulating the structural organization and function of membraneless organelles.

09.25

Pre-rRNA spatial distribution and functional organization of the nucleolus

Yu-Hang Pan[1], **Lin Shan**[1], Yu-Yao Zhang[2], Zheng-Hu Yang[1,3], Yuan Zhang[1], Shi-Meng Cao[1], Xiao-Qi Liu[1], Jun Zhang[1], Li Yang[2], Ling-Ling Chen[1,3,4]
[1]State Key Laboratory of RNA Innovation, Science and Engineering, CAS Center for Excellence in Molecular Cell Science, Shanghai, China
[2]Center for Molecular Medicine, Children's Hospital, Fudan University, Shanghai, China
[3]School of Life Science and Technology, ShanghaiTech University, Shanghai, China
[4]Shanghai Academy of Natural Sciences, Shanghai, China

The self-organized nucleolus is where the pre-ribosomal RNA (pre-rRNA) processing and pre-ribosomal ribonucleoprotein (RNP) assembly take place. Here we present the spatiotemporal distribution of pre-rRNA intermediates in the human nucleolus, revealing a segregated spatial distribution of small subunit (SSU) and large subunit (LSU) precursors. Notably, the 5' external transcribed spacer (5' ETS)-containing SSU pre-rRNAs are retained across FC-DFC-PDFC regions, while the internal transcribed spacer 2 (ITS2)-containing LSU pre-rRNAs move to PDFC-GC regions for processing. Inhibiting 5' ETS processing impairs the SSU pre-rRNA distribution and the FC/DFC sub-nucleolar structure. Cells in amniotes possess a multi-layered nucleolus, whereas anamniotes have only a bipartite structure with a merged FC/DFC. Kinetic labeling of pre-rRNA outflow shows a 7-fold higher pace in tetrapartite nucleoli over that in bipartite nucleoli, indicating that the emergence of the nested FC/DFC may facilitate an efficient SSU pre-rRNA processing over the course of evolution. Collectively, depicting the spatiotemporal distribution of pre-rRNAs reveals a key role of processing steps in organizing the multi-layered nucleolus and suggests a possible evolutionary advantage of the multi-layered structure in amniotes.

9.40

Mapping and engineering RNA-driven architecture of the multiphase nucleolus

Sofia Quinodoz[1,11*], Lifei Jiang[2*], Aya Abu-Alfa[2], Troy Comi[3], Hongbo Zhao[1,3], Qiwei Yu[4], Lennard Wiesner[1], Jordy Botello[2], Anita Donlic[1], Elizabeth Soehalim[3], Prashant Bhat[5, 6], Christiane Zorbas[7], Ludivine Wacheul[7], Andrej Kosmrlj[8, 9], Denis Lafontaine[7], Sebastian Klinge[10], Clifford Brangwynne[1,2,3,4,9,11]

[1]Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA
[2]Molecular Biology, Princeton University, Princeton, NJ, USA
[3]Omenn-Darling Bioengineering Institute, Princeton University, Princeton, NJ, USA
[4]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA
[5]Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA
[6]David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA
[7]RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S./FNRS), Université libre de Bruxelles (ULB), Biopark campus, Gosselies, Belgium
[8]Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ, USA
[9]Princeton Materials Institute, Princeton University, Princeton, NJ, USA
[10]Laboratory of Protein and Nucleic Acid Chemistry, The Rockefeller University, New York, NY, USA

Biomolecular condensates are key features of intracellular compartmentalization. As the most prominent nuclear condensate in eukaryotes, the nucleolus is a multiphase liquid-like structure where ribosomal RNAs (rRNAs) are transcribed and processed, undergoing multiple maturation steps to form the small and large ribosomal subunits (SSU and LSU). However, how rRNA processing is coupled to the nucleolus' layered organization is poorly understood due to a lack of tools to precisely monitor and perturb nucleolar rRNA processing dynamics. Here, we developed two complementary approaches to spatiotemporally map rRNA processing and engineer *de novo* nucleoli. Using sequencing in parallel with imaging, we found that rRNA processing steps are spatially segregated, with sequential maturation of rRNA required for its outward movement through nucleolar phases. By generating synthetic nucleoli in cells through an engineered rDNA plasmid system, we show that defects in SSU processing can alter the ordering of nucleolar phases, resulting in inside-out nucleoli and preventing rRNA outflux, while LSU precursors are necessary to build the outermost layer of the nucleolus. These findings demonstrate how rRNA is both a scaffold and substrate for the nucleolus, with rRNA acting as a programmable blueprint for the multiphase architecture that facilitates assembly of an essential molecular machine.

9.55

Nucleolar Biophysical Homeostasis in Health and Disease

Eliezer Calo
Massachusetts Institute of Technology**,** Massachusetts, USA

Ribosome assembly is an essential and highly conserved process in all cells. Disruptions in ribosome production can cause disproportionately severe effects, often manifesting as distinct syndromes driven by stress responses originating from defects during ribosome synthesis. Ribosome assembly occurs within the nucleolus—a dynamic biomolecular condensate in the cell nucleus—whose physical and chemical properties are critical for its function. Recent work from our laboratory and others has revealed that the nucleolus is an acidic condensate capable of maintaining a distinct pH gradient, a feature that may be central to its role in coordinating ribosome assembly. I will discuss how regulation of nucleolar biophysical homeostasis—particularly pH maintenance—drives ribosome

biogenesis, how its disruption underlies tissue-selective vulnerability in ribosome assembly disorders, and how these mechanisms extend to broader conditions such as cancer and aging.

10.20

Why RNA polymerase I can make christmas trees

Katrin Schwank[1], Catharina Schmid[1], Tobias Fremter[1], Olivier Gadal[2], Felix Grünberger[3], Christoph Engel[1], Philipp Milkereit[1], Joachim Griesenbeck[1], **Herbert Tschochner**[1]
[1]Regensburg Center of Biochemistry (RCB), University of Regensburg, Regensburg, Germany
[2]Molecular, Cellular and Developmental Biology Unit (MCD), Centre de Biologie Intégrativ (CBI), Université de Toulouse, CNRS, Toulouse, France
[3]Microbiology, Regensburg Center of Biochemistry (RCB), University of Regensburg, Regensburg, Germany

A significant difference between RNA polymerase II (Pol II) and RNA polymerase I (Pol I) transcribed genes is the dense packing of Pol I molecules on its gene which ensures fast and efficient rRNA synthesis. Between 60 and 100 Pol I molecules per gene can be detected in electron micrographs constituting Christmas tree-like structures. In such an arrangement frequent pausing, which includes backtracking and RNA cleavage might be disadvantageous. Pol I and Pol II differ also in the number and features of subunits which bind to the lobe structure of the core enzyme. In Pol II only subunit Rpb9 is bound to the lobe whereas in Pol I the subunits Rpa34.5 which dimerizes with Rpa49 and the RNA cleaving subunit Rpa12.2 are associated to the lobe. Using reconstituted transcription assays, we analyzed the mutual dependency of the different domains of the lobe binding subunits to optimize the elongation process. We found that the tandem winged helix domain of Rpa49 including the linker region promotes forward movement and NTP misincorporation and inhibits cleavage when Pol I is in elongation mode. In contrast, the dimerization domains of Rpa34.5 and Rpa49 support cleavage and transcription fidelity. Furthermore, an *in vivo* phosphorylated serine on subunit Rpa190, which interferes with the dynamics of subunit Rpa12.2, supports Pol I in its forward movement mode, but impairs proofreading. We present a model, how the dynamic interaction of lobe binding domains and Pol I phosphorylation mediate optimal RNA synthesis adapted to different transcription conditions.

Session #2 -Regulation of rDNA copy number and rRNA Transcription

11.00

Unequal distribution of histone modifications that regulates rDNA stability rejuvenates yeast daughter cells

Takehiko Kobayashi
Institute for Quantitative Biosciences (IQB), The University of Tokyo

The budding yeast is one of the most valuable model organisms for aging research, owing to its finite replicative lifespan (~20 cell divisions) and the distinct aging phenotypes that emerge toward the end of its life cycle. Notably, as a mother cell undergoes successive divisions (a process known as "budding"), each daughter cell is rejuvenated, regaining the capacity to divide approximately 20 times. This rejuvenation mechanism is essential for the continuity of life across diverse organisms. In humans, a comparable process occurs through generational alternation, ensuring that offspring always begin life at age zero, regardless of the mother’s age.

In budding yeast, lifespan is closely linked to the stability of the ribosomal RNA gene (rDNA), a highly unstable genomic region. In aged mother cells, the rDNA becomes increasingly unstable. Interestingly, this instability is reversed in daughter cells derived from old mothers, indicating that rDNA instability is asymmetrically segregated during cell division. However, the mechanism underlying this biased segregation has remained unclear. Without such asymmetry, daughter cells would inherit unstable rDNA, leading to accelerated aging.

In our study, we identified that histone modifications around the replication fork barrier (RFB) site within the rDNA play a critical role in mediating this asymmetric segregation. This finding provides a key mechanistic insight into how rDNA stability is restored in daughter cells and represents a significant breakthrough in our understanding of cellular rejuvenation and longevity.

Reference:

Hori, Y., Engel, C., and Kobayashi, T. (2023) Regulation of ribosomal RNA-gene transcription, copy number and nucleolus organization in eukaryotes. *Nature Reviews Molecular Cell Biology.* 24:414-429. https://doi.org/10.1038/s41580-022-00573-9

11.25

TIN2 regulates nucleolar function and rDNA integrity

Stavroula Boukoura[1], Kezia Oxe[1], Ekaterina Dulina[1], Oliver Quevedo[1], Huixin Xu[2], Yonglun Luo[2], Maria A Blasco[3], **Dorthe Helena Larsen**[1]

[1]Danish Cancer Institute, Danish Cancer Society, Copenhagen, Denmark

[2]Department of Biomedicine, Aarhus University, Aarhus, Denmark

[3]Molecular Oncology Programme, Spanish National Cancer Research Center, Madrid, Spain

The ribosomal RNA genes (rDNA) cluster in the nucleolus where rRNA is transcribed and incorporated into ribosomal subunits, required for protein translation. The rDNA is one of the most unstable genomic regions and its instability can be further exacerbated in conditions such as cancer. However, the link between rDNA instability and human diseases remains understudied, due to the incompatibility of rDNA with commonly used high-throughput methods. Our research focuses on how cells maintain the stability of rDNA and the cellular consequences of rDNA destabilization. Upon DNA damage in rDNA, a specialized nucleolar DNA damage response (n-DDR) is activated, leading to transcriptional inhibition and nucleolar restructuring, promoting rDNA repair. We conducted a high throughput protein-depletion screen combined with rDNA targeted double-strand breaks and identified the protein TIN2 as n-DDR regulator. We find that absence of TIN2 compromise DNA damage signalling, inhibition of rRNA transcription, nucleolar restructuring and cap formation. We also observe an increased micronucleation and decreased cellular survival in response to rDNA damage. TIN2 is known to function in maintenance of telomeres and TIN2 mutations cause the bone marrow disorder Dyskeratosis congenita (DC) but no nucleolar function has been reported. Notably, defects in the nucleolar DNA damage response cannot be rescued by point mutations found in DC patients. We propose a model of how TIN2 functions to maintain the integrity of the nucleolus and rDNA, and how this novel function of TIN2 potentially contribute to the development of DC.

11.40

Germline sequence variation within the ribosomal DNA is associated with human complex traits

Francisco Rodriguez-Algarra[1], Maia Cooper[2], Faraz Mardakheh[2], David Evans[3], **Vardhman Rakyan**[1]

[1]Blizard Institute, Queen Mary University of London, London, United Kingdom

[2]Biochemistry, University of Oxford, Oxford, United Kingdom
[3]Institute for Molecular Bioscience, Queensland, Brisbane, Australia

Despite its highly conserved function, ribosomal DNA displays substantial genetic variation within all species analysed to date. This variation comprises both inter-individual differences in total copy number (CN) as well as inter- and intragenomic sequence variation in the form of single nucleotide variants (SNV) and insertions/deletions (INDELs) across rDNA copies. Whether germline variation of rDNA sequence associates with phenotypic traits in humans is, to date, unknown. Using the UK Biobank whole genome sequencing data, we have derived a high confidence list of rDNA-associated SNVs and INDELs that are validated in multiple ways. Using this list, we show that specific rDNA variants associate with several human traits. In particular, traits associated with body size appear enriched in variants within the Expansion Segment 15L region in the 28S rRNA. The strength of these associations does not diminish when accounting for the total rDNA CN of each individual. Our work represents the first large-scale association analysis of human traits with germline sequence variation in the rDNA, a source of human complex trait-relevant genetic variation that has thus far been largely ignored.

11.55

RpS12-mediated induction of the Xrp1^{short} isoform links ribosomal protein mutations to cell competition

Myrto Potiri[1,3,*], Eleni Tsakiri[1*], Kyriaki Kontogiannidi[1,4], Maria Loizou[1], Efthimios Skoulakis[1], Martina Samiotaki[2], Panagiota Kafasla[1], **Marianthi Kiparaki**[1]
[1]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center “Alexander Fleming”, Vari, Greece
[2]Institute for Bioinnovation, Biomedical Sciences Research Center “Alexander Fleming”, Vari, Greece
[3]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
[4]School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Cell competition, a universal yet enigmatic phenomenon, eliminates less-fit cells via interactions with their neighbors. It was originally described in *Drosophila* mosaics, where heterozygous ribosomal protein *(Rp$^{+/-}$)* mutant cells are eliminated by wild-type neighbors. The transcription factor Xrp1 mediates most of the *Rp$^{+/-}$*-associated phenotypes, including reduced competitiveness and translation. Although RpS12 is required for Xrp1 induction in *Rp$^{+/-}$* cells, the mechanism remained unresolved. We demonstrate that RpS12, via alternative splicing, induces the Xrp1 short (Xrp1^{short}) isoform expression in *Rp$^{+/-}$*cells, which is both necessary and sufficient for their elimination. Strikingly, RpS12 overexpression in wild-type cells is sufficient to induce Xrp1^{short} expression and confer a “loser” phenotype. While Xrp1^{long} isoform is not required in *Rp$^{+/-}$*cells, expression of either Xrp1 isoform is sufficient to promote the loser status in wild-type cells. We further identify an RNA-binding protein reduced in *Rp$^{+/-}$* cells, as a critical Xrp1 suppressor; its depletion in wild-type cells activates Xrp1-dependent competition. Our findings establish RpS12’s specialized function in Xrp1^{short} promotion, not proteotoxic stress, as the primary driver in *Rp$^{+/-}$* cells, providing new perspectives that challenge prevailing models. Our work contributes in long-standing questions about ribosomal protein-linked fitness surveillance and provides insights into ribosomopathy pathologies.

12.10

rRNA transcription and subsequent ribosome biogenesis attenuation in the gut promotes longevity

Shajahan Anver, Aisyah Alizan, Dekai Kai, Haocheng Shou, Yuting Ren, Guillermo Martínez Corrales, Danny Filer, Tatiana Svermova, Nazif Alic

[1]Genetics, Evolution and Environment and the UCL Institute of Healthy Ageing, University College London, London, United Kingdom

RNA polymerase (Pol) I is a highly conserved enzyme which is responsible for the transcription of rRNAs. rRNAs are crucial for ribosome biogenesis and subsequent protein translation, which are strong drivers of cellular and organismal ageing. We found that the partial inhibition of Pol I extends lifespan in the fruit fly. The adult midgut specific knockdown of a Pol I subunit in the enterocytes or intestinal stem cells (ISCs) is sufficient to recapitulate this effect. Reduction in Pol I activity reduces age-associated hyperplasia in the ISCs and delays the loss of gut barrier function improving overall gut health. Moreover, age-induced decrease in walking and climbing ability were delayed in these flies suggesting a delay in the neuromuscular system ageing. Considering this together with the observed improvements in age-related gut function, rRNA transcription emerges as a common driver of several, apparently unrelated, age-induced deficits in multiple organ systems. More recently, we have characterized the consequences for ribosome biogenesis of this partial Pol I loss of function in the fruit fly gut. As expected, pre- and mature-rRNA levels were reduced in partially Pol I depleted fly guts, while proteomic analysis identified a reduction in ribosomal proteins. Consequently, we find that the protein synthetic capacity is also reduced in these fly guts. We are now exploring how these molecular changes drive systemic health improvement in older age.

Session #3A - Translacore Session, Mechanisms of Eukaryotic Ribosome Assembly

14.05

Making Ribosomes One Nucleotide at a Time

Denis L.J. Lafontaine

RNA Molecular Biology, Université libre de Bruxelles, Fonds de la Recherche Scientifique, Belgium

Ribosome biogenesis relies on the sequential processing of polycistronic precursor rRNAs, which are cleaved to generate the mature 5’ and 3’ ends of rRNAs. In parallel, nascent rRNAs undergo extensive covalent modification, including ribose methylation, pseudouridylation, and diverse base modifications. These processing and modification events provide powerful readouts of ribosome assembly: they reveal where and when biogenesis is perturbed, serve as distinctive biomarker signatures of ribosomopathies and cancer, and even offer molecular fingerprints with the potential to trace tissue of origin and disease grade.

For decades, pre-rRNA processing and modification were interrogated using classical biochemical methods such as northern blotting, pulse–chase labeling, HPLC, and primer extension. While extremely robust and quantitative, these approaches demand large RNA inputs, limiting clinical applicability, and remain relatively low in resolution and sensitivity.

In this keynote, I will present our ongoing efforts to overcome these limitations by developing next-generation approaches for high-resolution, highly sensitive mapping of RNA processing and RNA modification, leveraging both short- and long-read sequencing technologies. I will also discuss how these methodological advances are beginning to uncover the blueprint of the nucleolus, bringing us closer to understanding how ribosomes are made, one nucleotide at a time.

14.50

RNA helicase Drs1 localizes onto pre-ribosomes via protein-protein and protein-RNA interactions

Taylor Ayers[1], Fiona Fitzgerald[2], John Woolford[1]

[1]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, USA

[2]Department of Neurology, University of Pittsburgh, Pittsburgh, USA

Near-atomic resolution cryo-EM structures of nucleolar large ribosomal subunit assembly intermediates provided a glimpse into the rRNA folding pathway, by revealing that the 25S rRNA domains I, II, and VI compact prior to III, IV, and V. Yet, the mechanisms driving rRNA folding into its three-dimensional, catalytically competent structure remain ill-defined. In *Saccharomyces cerevisiae,* nineteen phylogenetically conserved RNA helicases have been implicated in mediating rRNA transitions during ribosome biogenesis, but the function of many of these helicases is unexplored. We identified that the DEAD-box helicase (DBH) Drs1 is required for compaction of 25S domain III rRNA. DBHs contain a conserved catalytic core that recognizes RNA in a sequence-independent manner, flanked by unique auxiliary domains that are conventionally understood to direct DBHs to their specific substrates. Our molecular genetic and biochemical data suggest that *both* its C-terminal domain *and* its core recruit Drs1 to pre-ribosomes. We identified that a patch of residues on the assembly factor Erb1, which crosslinks to the Drs1 core, is critical for recruiting Drs1 onto the pre-ribosome. Mutating these residues on Erb1 (*erb1-1*) reduces the presence of Drs1 on assembling particles, impairs direct interactions between Erb1 and Drs1, and results in a slow-growth phenotype. Furthermore, truncating the basic, intrinsically disordered C-terminal tail of Drs1, that is predicted to bind RNA, in combination with the *erb1-1* mutations, result in synthetic lethality and largely abolish the presence of Drs1 on assembling particles. Together, this work illustrates that DBHs rely upon protein-protein and protein-RNA interactions to properly locate their substrates.

15.05

A structural atlas of multi-enzyme integration and regulation within the human rixosome complex

Jacob Gordon[1], Andrea Kaminski[2], Alan Warren[3], Robin Stanley[1]

[1]Molecular and Cellular Biology Laboratory, National Institutes of Environmental Health Sciences, Research Triangle Park, USA

[2]Genome Integrity and Structural Biology Laboratory, National Institutes of Environmental Health Sciences, Research Triangle Park, USA

[3]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom

Many molecular machines function in concert to properly build the ribosome. The Rixosome is a large and conserved protein complex that coordinates multiple enzymes required for 60S synthesis. These include, 1) endoribonuclease-kinase complex RNase PNK (LAS1L-NOL9) which initiates ITS2 pre-rRNA spacer degradation, 2) MDN1 AAA+-ATPase motor which irreversibly remodels pre-60S particles, and 3) SENP3 SUMO protease which regulates SUMOylation status of several 60S biogenesis factors. PELP1-WDR18-TEX10 form the structural core of the Rixosome and facilitate complex recruitment to the pre-60S. How does the Rixosome coordinate these diverse molecular machines to perform distinct steps of 60S ribosome synthesis? We have determined the overall architecture of the human Rixosome complex using *in vivo* reconstitution and co-purification, cryo-EM, X-ray crystallography, and *in silico* modeling. Notably, we revealed how large intrinsically disordered regions (IDRs) in the Rixosome scaffold PELP1 modularly bind 60S biogenesis enzymes MDN1 and SENP3. We hypothesized that these enzymes are functionally regulated by binding PELP1. To test this, we produced recombinant SENP3 protease domain +/- the PELP1 binding region to

assess SENP3 protease activity. SUMO protease activity of SENP3 was significantly increased when bound to PELP1 compared to SENP3 alone. We next solved an X-ray structure of SENP3-PELP1 revealing how PELP1 allosterically binds SENP3. Our data provide an atlas for how several essential 60S biogenesis enzymes are integrated within the human Rixosome. We also provide evidence of direct enzyme regulation coordinated by the Rixosome via PELP1. This begins to elucidate how the Rixosome coordinates multiple maturation steps in 60S synthesis.

15.20

A comprehensive view on r-protein binding and rRNA domain structuring during early eukaryotic ribosome formation

Magdalena Gerhalter[1,4], Michael Prattes[1], Lorenz E. Grundmann[2], Irina Grishkovskaya[2], Enrico F. Semeraro[1], Gertrude Zisser[1], Juliane Merl-Pham[3], Stefanie M. Hauck[3], David Haselbach[2], **Helmut Bergler**[1]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria
[2]Research Institute of Molecular Pathology (IMP), Vienna BioCenter, Vienna, Austria
[3]Metabolomics and Proteomics Core, Helmholtz Center Munich, Munich, Germany
[4]Laboratoire de Biologie Structurale de la Cellule (BIOC), Ecole polytechnique, Institut Polytechnique de Paris, Palaiseau, France

Formation of the eukaryotic ribosomal subunits follows a strict regime to assemble ribosomal proteins (r-protein) with ribosomal RNAs (rRNA) while removing internal (ITS) and external (ETS) transcribed rRNA spacers. During early stages of large subunit (LSU) formation, ITS2 together with six assembly factors forms the characteristic foot structure of early nuclear pre-LSU particles. Here, we address the function of this foot structure during early stages of ribosome assembly. We present cryo-EM structures from wild-type cells and cells depleted for the foot structure factor Rlp7. We show that compaction of domain I of the 25S rRNA is strictly dependent on the presence of foot factors, while domain II folds independently. Furthermore, Rlp7-depletion accumulated small subunit (SSU) processome intermediates prior to A1 cleavage and compaction of the individual domains of the 18S rRNA, providing also novel insights into the SSU-assembly process. SILAC labeling and affinity purification of co-transcriptional assembled pre-ribosomes enabled us to resolve the assembly line of the r-proteins step by step. This showed that incorporation of r-proteins in eukaryotes neither follows the bacterial regime nor a strict linear co-transcriptional mode. Instead, seed r-proteins might structurally define the individual rRNA domains before their compaction and fixation in the context of the SSU processome.

Session #3B - Translacore Session, Mechanisms of Eukaryotic Ribosome Assembly

16.00

A dual role for the snR37 H/ACA snoRNP in pseudouridylation and rRNA scaffolding during pre-60S maturation

Jutta Hafner[1], Matthias Thoms[2], Hussein Hamze[3], Anna Forstner[1], Annika Hausharter[1], Sébastien Favre[4], Ismaël Alidou-D'Anjou[5], Hafiza Hebbachi[5], Priya Bhutada[1], Timo Denk[2], Katharina Schlick[1], Katharina Schindlmaier[1], Simon Lebaron[3], Sherif Ismail[6], Ed Hurt[6], Dieter Kressler[4], Yves Henry[3], François Dragon[5], Roland Beckmann[2], Anthony K. Henras[3], **Brigitte Pertschy[1]**

[1]Institute of Molecular Biosciences, University of Graz, Austria.

[2] Gene Center, University of Munich, Germany.
[3] Molecular, Cellular and Developmental Biology Unit (MCD), Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, France.
[4] Unit of Biochemistry, Department of Biology, University of Fribourg, Switzerland.
[5] Centre d'Excellence en Recherche sur les Maladies Orphelines – Fondation Courtois, Département des Sciences Biologiques, Université du Québec à Montréal, Canada
[6] Biochemistry Center, University of Heidelberg, Germany

Early pre-60S ribosomal precursors are among the least characterized intermediates in ribosome biogenesis. These particles are intrinsically flexible and undergo extensive rRNA modifications mediated by small nucleolar ribonucleoprotein particles (snoRNPs). Here we identify Upa1 and Upa2, two SPOUT domain-containing assembly factors, as well as their interaction partner, the 25S rRNA domain VI binding protein Rbp95, as stable components of one particular H/ACA snoRNP, snR37. We show that Upa1 and Upa2 are required for efficient recruitment of snR37 to pre-60S particles and for pseudouridylation at U2944 in 25S rRNA, whereas base-pairing of snR37 with its rRNA target in domain V is essential for pseudouridylation but dispensable for snoRNP recruitment. We further present the 2.8 Å cryo-EM structure of the snR37 snoRNP, revealing its unique architecture and identifying snR37-specific RNA elements as binding platform for the Upa1-Upa2 heterodimer. We further show that the snR37 snoRNP is genetically and physically linked to the Npa1 complex. An snR37 variant incapable of pseudouridylation can fully rescue synthetic growth defects caused by deletion of snR37, uncovering functions of the snR37 snoRNP beyond rRNA modification. We propose that next to its role in pseudouridylation, the unconventional snR37 snoRNP functions as an rRNA scaffolding factor in early 60S biogenesis, tethering domains V and VI together, hence contributing to the structural organization of the earliest pre-60S intermediates.

16.25

Modular folding of the 18S rRNA central domain guided by the snR30 H/ACA snoRNP

Matthias Thoms[1], Paulina Fischer[2], Benjamin Lau[2], Timo Denk[1], Maria Kuvshinova[2], Otto Berninghausen[1], Dirk Flemming[2], Ed Hurt[2], Roland Beckmann[1]
[1] Department of Biochemistry, Gene Center, University of Munich, Munich, Germany.
[2] Biochemistry Center, Heidelberg University, Heidelberg, Germany.

The early stages of eukaryotic ribosome assembly, and in particular the role of small nucleolar RNAs (snoRNAs) in guiding pre-rRNA modification and folding, remain poorly understood. A major challenge is the structural visualization of these transient and dynamic early intermediates. Thus, despite advances in cryo-electron microscopy (cryo-EM), many snoRNA-mediated mechanisms remain elusive. A prime example is snR30 (U17/SNORA73 in humans), the only essential H/ACA snoRNA in yeast, whose precise role in 40S subunit biogenesis has long been uncertain due to the lack of an associated rRNA base modification. Using cryo-EM in combination with a dominant negative Krr1 trapping mutant, we reveal the structure of snR30 bound to the H/ACA core proteins (Cbf5-Gar1-Nop10-Nhp2) and to a subdomain of the pre-18S rRNA on a stalled 90S particle. This domain includes the central domain helices h20-h23 and part of expansion segment 6. Chaperoned by the snR30 snoRNP, the central domain remains externalized from the developing 90S core. In addition, the snoRNP contains the assembly factors (Krr1, Utp23, Kri1) and ribosomal proteins (uS11, uS15), forming a bimodular complex comprising the H/ACA core and the 18S platform module. Krr1-mediated dissociation of snR30 allows subsequent integration of the domain into the maturing pre-ribosome. Our study reveals the essential role of snR30 in chaperoning the formation of the central domain as a discrete assembly

unit. The combination of cryo-EM and biochemical trapping may provide new approaches to capture and visualize otherwise inaccessible assembly intermediates.

16.40

Structure and Mechanism of the Ribosome Maturation Factor Rea1

Helgo Schmidt[1], Deepika Velayudhan[1], Geraldine Koenig[1], Yves Henry[2], Anthony Henras[2], Carine Dominique[2], Benjamin Albert[2]

[1]Integrated Structural Biology , Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France

[2]Molecular, Cellular and Developmental Biology Unit (MCD), Université de Toulouse, Toulouse, France

The ribosome maturation factor Rea1 (or Midasin) catalyses the removal of assembly factors from large ribosomal subunit precursors and promotes their export from the nucleus to the cytosol. Rea1 consists of nearly 5000 amino-acid residues and belongs to the AAA+ protein family. It consists of a ring of six AAA+ domains from which the ≈ 1700 amino-acid residue linker emerges that is subdivided into stem, middle and top domains. A flexible and unstructured D/E rich region connects the linker top to a MIDAS (metal ion dependent adhesion site) domain, which is able to bind the assembly factor substrates. Despite its key importance for ribosome maturation, the mechanism driving assembly factor removal by Rea1 is still poorly understood. Here we demonstrate that the Rea1 linker is essential for assembly factor removal. It rotates and swings towards the AAA+ ring following a complex remodelling scheme involving nucleotide independent as well as nucleotide dependent steps. ATP-hydrolysis is required to engage the linker with the AAA+ ring and ultimately with the AAA+ ring docked MIDAS domain. The interaction between the linker top and the MIDAS domain allows direct force transmission for assembly factor removal.

16.55

Helicase-mediated mechanism of SSU processome maturation and disassembly

Olga Buzovetsky, **Sebastian Klinge**

[1]Laboratory of Protein and Nucleic Acid Chemistry, The Rockefeller University, New York City, USA

Eukaryotic ribosomal small subunit (SSU) assembly requires the SSU processome, a nucleolar precursor containing the RNA chaperone U3 snoRNA. The underlying molecular mechanisms of SSU processome maturation, remodeling, disassembly, RNA quality control, and the transitions between states remain elusive due to a paucity of intermediates. Here we report 16 native SSU processome structures alongside genetic data, revealing how two helicases, the Mtr4-exosome and Dhr1, are controlled for accurate and unidirectional ribosome biogenesis. Our data show how irreversible pre-ribosomal RNA degradation by the redundantly tethered RNA exosome couples the transformation of the SSU processome into a pre-40S particle during which Utp14 can probe evolving surfaces, ultimately positioning and activating Dhr1 to unwind the U3 snoRNA and initiate nucleolar pre-40S release. This study highlights a paradigm for large dynamic RNA-protein complexes where irreversible RNA degradation drives compositional changes and communicates these changes to govern enzyme activity while maintaining overall quality control.

17.10 (P12)

Investigating real-time spatio-temporal progression of ribosome biogenesis using single-molecule resolution microscopy

Justina Chu[1], François Simon[2], Marlene Oeffinger[1, 3], Lucien E. Weiss[2], Daniel Zenklusen[1]
[1]Département de Biochimie et Médecine Moléculaire, Université de Montréal, Montréal, Canada, [2]Département de Génie Physique, Polytechnique Montréal, Montréal, Canada, [3]RNP Biochemistry Lab, Institut de recherches cliniques de Montréal, Montréal, Canada

Ribosome biogenesis requires the interplay of hundreds of ribosome biogenesis factors (RBFs), ribosomal proteins (RPs) and rRNAs, and occurs predominantly in the nucleolus, a layered multiphase condensate. Despite extensive research, various aspects of ribosome biogenesis, in particular with respect to its spatio-temporal progression, are still not fully understood, in part due to technical limitations in measuring the fast-changing dynamics occurring across the nucleolar subcompartments, which are difficult to resolve using conventional light microscopy approaches.

To overcome some of these limitations, we applied live-cell single-molecule microscopy to elucidate the spatial progression of ribosome biogenesis in real-time. Using Halo-tagged RPs/RBFs as proxies for the progression of maturing subunits, coupled with fast image acquisition, enabled us to monitor the dynamic behaviour of single proteins within nucleolar subcompartments at a resolution of approximately 50 nm. Tracking analysis allowed us to distinguish free from pre-ribosome-bound RBFs/RPs and correlate their molecular states to their localization within the nucleolar subcompartments.

Single-particle movement analysis of pre-ribosome-bound RPs did not show a directed radial movement towards the nucleolar periphery. Instead, we observed a confined dwelling behaviour and lateral movement of these RPs within nucleolar subcompartments. This suggests that maturing pre-ribosomal subunits do not follow a uniform diffusion pattern as they traverse the nucleolus and that their molecular behaviour is not solely due to their biophysical properties or those of the subcompartments. Taken together, live-cell single-molecule microscopy provides a useful approach to revisit some long-standing questions regarding the relation of spatial-temporal behaviour of maturing subunits and their dynamic progression through the nucleolus.

Tuesday 9th September

Session #4 - Translacore, Dysregulation of Ribosome Synthesis and Human Disease

9.00

Modelling genotype-phenotype correlations in Diamond-Blackfan anaemia syndrome

Deena Iskander MBChB PhD MRCP FRCPath
Centre for Haematology, Department of Immunology & Inflammation, Imperial College London, UK
Department of Paediatric Haematology, St Mary's Hospital, Imperial College Healthcare Trust, UK

Ribosome dysfunction underlies the pathogenesis of many cancers and heritable ribosomopathies. Our work interrogates how mutations in either ribosomal protein large (RPL) or ribosomal protein small (RPS) subunit genes selectively affect erythroid progenitor development and clinical phenotypes in Diamond Blackfan anaemia syndrome (DBAS), a rare ribosomopathy with limited therapeutic options. Using single-cell assays of patient-derived bone marrow, we previously delineated two distinct cellular trajectories segregating with RP genotypes: almost complete loss of erythroid specification in RPS-DBAS, contrasting with relative preservation of qualitatively abnormal 'stressed' erythroid progenitors in RPL-DBAS, that undergo accelerated differentiation. Concordant

with these cellular differences, patients with RPL-DBAS exhibit later onset of anaemia and more durable glucocorticoid responses.

To define the cellular and molecular mechanisms underpinning these genotype phenotype differences, we have established three-dimensional organoid models of DBAS bone marrow, generated from healthy induced pluripotent stem cells, engrafted with patient stem/progenitor cells. These recapitulate the distinct defects in RPS- versus RPL-DBAS and demonstrate anaemia rescue by glucocorticoids, representing a new human ex vivo model to facilitate therapeutic target discovery in rare ribosomopathies.

9.25

HEATR3 and novel effectors of the nucleolar surveillance pathway: uncovering links between ribosome biogenesis and disease

Olivia Delfino[1], Jasmina Frawley[1], Nadine Hein[1], Rita Ferreira[1], Kira Wysoke[1], Eric Kusnadi[2], Nicholas Watkins[3], Katherine Hannan[1], Ross Hannan[1], **Amee George**[1]
[1]The John Curtin School of Medical Research, The Australian National University, Canberra, Australia
[2]Research Division, Peter MacCallum Cancer Centre, Parkville, Australia
[3]Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom

The Nucleolar Surveillance Pathway (NSP) is a critical quality control mechanism that couples ribosome biogenesis with cell cycle control by stabilising p53 in response to nucleolar stress. This occurs through ribosomal protein–mediated inhibition of the E3 ubiquitin ligase MDM2. While dysregulation of the NSP contributes to the pathogenesis of ribosomopathies, its therapeutic activation is also being explored as a strategy to treat malignancy. To dissect the molecular drivers of NSP activation, we conducted genome-wide RNAi high-throughput screens (Hannan *et al.*, Cell Reports, 2022), identifying a core set of ribosome biogenesis factors whose depletion stabilises p53. Among several novel components, we highlight HEATR3, a protein implicated in 5S ribonucleoprotein (5S-RNP) biogenesis, as a key regulator of NSP function. Variants in HEATR3 have recently been formally recognised in the latest Diamond-Blackfan Anaemia Syndrome (DBAS) International Consensus Statement, underscoring its clinical relevance. We will present data from *in vitro* and *in vivo* models demonstrating that HEATR3 loss perturbs ribosome assembly, impairs cell proliferation, and alters the bone marrow microenvironment. Leveraging these models, we are pursuing RNA-based therapeutic approaches to target the molecular defects underlying ribosome biogenesis disorders. These studies underscore the NSP as a central effector of p53-mediated responses to ribosomal stress and reveal novel avenues for intervention in NSP-related pathologies.

9.40

ER stress and Alzheimer proteins shape the quality of ribosomal protein synthesis in aging human fibroblasts

Max Hartmann, Zhouli Cao, Amy Schug, Yinqiu Li, Karin Scharffetter-Kochanek, **Sebastian Iben**
[1]Dermatology, Ulm University, Ulm, Germany

The accuracy of protein synthesis by the ribosome, translational fidelity, co-evolved with longevity on one side and is on the other side a parameter that influences protein homeostasis (proteostasis). Here we present evidence that in a human aging model, fibroblasts from young and old donors, respective in vitro aged fibroblasts, ribosomal fidelity increases with donor age at the price of a reduced overall proteins synthesis. Translational fidelity is not a fixed parameter but is adapted to

cellular ER stress levels that are mirroring the load of misfolded proteins and the buffering capacity of the ER. With aging we observe a decrease in the main ER stress inhibitor, GRP78, and an increase in the amount and activity of the ER-stress kinase PERK. This leads to an elevated level of phoshpho-eIF2alpha that reduces translation re-initiation, ribosomal biogenesis and increases fidelity of translation. Interestingly the Alzheimer proteins amyloid-beta precursor protein (APP) and presenilin 1 (PSEN1) are involved in the regulation of translational fidelity of the ribosome. The knockdown of APP leads to reduced GRP78 levels and elevated PERK signaling resulting in less errors by the ribosome, whereas the loss of PSEN1 leads to more GRP78, more errors but a stimulated overall protein synthesis. The reduction of the error-rate of translation with age was not observed in fibroblasts from young and old C57BL/6 mice suggesting that this adaptation might be of relevance for long-living species like humans that need to preserve proteostasis for the maintenance of susceptible organs like the post-mitotic central nervous system.

9.55

Late steps in pre-60S maturation impact early human brain development

Michael Buszczak
Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Although features of ribosome assembly are shared between species, our understanding of the complexity, dynamics, and regulation of ribosome production in multicellular organisms remains incomplete. To gain insights into ribosome biogenesis in human cells, we perform a genome-wide loss-of-function screen combined with differential labeling of pre-existing and newly assembled ribosomes. These efforts identify two functionally uncharacterized genes, C1orf109 and SPATA5. We provide evidence that these factors, together with CINP and SPATA5L1, control a late step of human pre-60S maturation in the cytoplasm. Allelic variants in all four complex components are primarily associated with neurodevelopmental disorders. Using human cerebral organoids in combination with proteomic, single-cell RNA-seq, and single-organoid translation analyses, we identify a previously unappreciated drop in protein production during early brain development. We find ribosome levels decrease during neuroepithelial differentiation, making differentiating cells particularly vulnerable to perturbations in ribosome biogenesis during this time. Reduced ribosome availability more profoundly impacts the translation of specific transcripts, disrupting both survival and cell fate commitment of transitioning neuroepithelia. Enhancing mTOR activity suppresses the growth and developmental defects associated with complex variants. This work provides evidence for the functional importance of regulated changes in global protein synthesis capacity during cellular differentiation.

10.20

Preribosome proteostasis stress between nucleolus and nucleoplasm in 40S ribosomal protein deficiencies

Sonia G. Gaspar[1], Miguel Sánchez-Álvarez[2], Rosa María Ramírez-Cota[1], Blanca Nieto[1], Marta Flujas[1], Miguel Á. del Pozo[3], Xosé R. Bustelo[1,4], and **Mercedes Dosil[1,4]**
[1]Centro de Investigación del Cáncer, CSIC and Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain
[2]Instituto de Investigaciones Biomédicas “Sols-Morreale”, CSIC and Universidad Autónoma de Madrid, 28029 Madrid, Spain
[3]Centro Nacional de Investigaciones Cardiovasculares, 28029 Madrid, Spain

[4]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), CSIC and Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

Diamond-Blackfan anemia (DBA) is caused by haploinsufficiency of one of 20 ribosomal proteins, with *RPS19* being the most frequently mutated gene. The diversity of causal mutations indicates that DBA can arise after the generation of different types of abortive preribosomes, including immature or near-mature 40S or 60S subunit precursors, formed at different subcellular locations (nucleolus, nucleoplasm or cytoplasm). Consequently, malformed preribosomes have been largely overlooked as a unifying pathogenic mechanism or as a possible explanation for the high prevalence of *RPS19* mutations. However, the biochemical properties, subcellular dynamics, and relative accumulation of aberrant preribosomes in ribosomal protein deficiencies remain poorly characterized. Here, we combined differential preribosome extraction, conventional microscopy, and high-throughput automated imaging screens with siRNAs targeting individual 40S ribosomal proteins (RPSs) or transacting ribosome biogenesis factors (RBFs) to systematically track the formation and localization of abortive preribosomes resulting from each depletion. We found that all RPS deficiencies, including those affecting nucleolar or cytoplasmic steps of 40S subunit maturation, lead to large accumulations of malformed preribosomes in the nucleoplasm. These aberrant particles originate in the nucleolus and include condensation-prone preribosomes with altered biophysical properties, as well as free subcomplexes that get dispersed in a diffuse manner throughout the nucleoplasm. Notably, RPS19 deficiency produces a particularly severe and distinct phenotype. Together, our findings classify RPS deficiencies into clusters based on the patterns of disrupted preribosome dynamics within the nucleus, and identify alterations shared by DBA-associated deficiencies, providing new insights into the early pathogenic events in ribosomopathies.

Session #5 - Translacore Session, Novel Approaches for Targeting Ribosome Synthesis in Cancer

11.00

Oxaliplatin and 5FU damage RNAs by multiple different mechanisms

Zijian Zhang[1], Zornitsa Vasileva Kotopanova[1], Kexin Dang[1], Xiangxu Kong[1], Nicole Simms[1], Michael Braun[1,2], Mark Saunders[1,2], David Thornton[3,4], **John Knight**[1]

[1]Division of Cancer Sciences, University of Manchester, Manchester, United Kingdom

[2]Colorectal and Peritoneal Oncology Centre, The Christie NHS Foundation Trust, Manchester, United Kingdom

[3]Manchester Cell Matrix Centre, University of Manchester, Manchester, United Kingdom

[4]Lydia Becker Institute of Immunology and Inflammation, University of Manchester, Manchester, United Kingdom

Combination chemotherapy is the mainstay for treatment of advanced cancers. Colorectal cancer is among the tumour types treated with 5FU in combination with oxaliplatin, termed FOLFOX. Worldwide, over 2 million people are treated with one or both drugs each year, yet we still do not understand everything that these compounds do at the molecular level. RNA, and not DNA, has recently emerged as a key molecular target for both 5FU and oxaliplatin, and has been linked to their cytotoxicity. Here we aim to understand the molecular effects of both drugs using RNA-centric methods, enabling us to reveal multiple modes of RNA damage that are specific for both compounds. Complementary biochemical and imaging-based approaches were developed to analyse 5FU-induced RNA damage. While two orthogonal biochemical methods were developed to analyse oxaliplatin-

induced RNA damage. These methods allowed us to measure direct covalent damage of RNA by these compounds. Leveraging this, we show that RNA damage follows different kinetics for each drug, which has implications for the sequential drug delivery used clinically. Upon covalent RNA modification by either drug, we observe specific molecular activities of these RNA:drug molecules that give rise to covalent RNA:protein crosslinks by divergent mechanisms. To understand this in more detail we developed an unbiased proteomic approach to identify crosslinked proteins. This led us to identify oxaliplatin-induced RNA:protein crosslinks in the pre-90S ribosome, while revealing 5FU to preferentially crosslink non-rRNA transcripts. Together our work reveals the multifaceted effects of RNA damage by chemotherapies, which likely contribute to their cytotoxicity.

11.25

A pre-rRNA-dependent positive feedback loop drives malignant ribosome biogenesis downstream of RAS oncogene

Emilie Alard[1, 2], Martin Dodel[1], Sam Wallis[2], Stefan Stricker[3], **Faraz Mardakheh**[1]
[1]Department of Biochemistry, University of Oxford, Oxford, UK
[2]Barts Cancer Institute, Queen Mary University of London, London, UK
[3]Helmholtz Zentrum, LMU München, Munich, Germany

Alterations to the nucleolar morphology and function are well-established hallmarks of cancer, yet their molecular underpinnings remain poorly understood. Using a RAS-inducible mouse model of pancreatic ductal adenocarcinoma (PDAC), we performed a spatially resolved proteomic and phospho-proteomic profiling of the nucleolus, following oncogene activation. Our analysis identified a RAS-driven phosphorylation programme, orchestrated through relocation of Casein Kinase 2 (CK2) holoenzyme to the nucleolus. This programme predominantly targets rDNA transcription and early rRNA processing factors, resulting in hyperactivation of rRNA synthesis and ribosome biogenesis. Disrupting the nucleolar localisation of CK2 blocks RAS-induced rRNA synthesis, while constitutive trapping of CK2 in the nucleolus is sufficient to activate rRNA synthesis independent of RAS. Mechanistically, nucleolar CK2 accumulation is mediated through its direct binding to the pre-rRNA, creating a positive feedback loop that further amplifies rRNA synthesis. Examination of tissues from diverse human cancers revealed nucleolar CK2 accumulation as a broadly conserved cancer-associated feature. Importantly, disrupting this feedback loop selectively inhibits RAS-expressing cancer cells, and blocks PDAC tumourigenesis. Our findings reveal an RNA-dependent positive feedback loop involving CK2 that is a critical driver of malignant ribosome biogenesis, and highlights a specific cancer vulnerability that could be exploited through disrupting this pathway.

11.40

A small molecule inhibitor of NVL suppresses tumor growth by blocking ribosome biogenesis

Holly Guo[1], Ye Tao[1], Victor Cruz[3], Min Fang[1], Vishal Khivansara[1], Shanhai Xie[1], Ashley Leach[1], Divya Reddy[1], Johann Peterson[1], Jiwoong Kim[4], Noelle Williams[2], Arin Aurora[1], Jan Erzberger[3], Jef De Brabander[1], Deepak Nijhawan[1]
[1]Department of Radiation Oncology, Section in Molecular Medicine, University of Texas Southwestern Medical Center, Dallas, USA
[2]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, USA
[3]Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, USA

[4]Quantitative Biomedical Research Center, Department of Clinical Sciences, University of Texas Southwestern Medical Center, Dallas, USA

A longstanding hypothesis, stemming from the enlarged nucleoli typical of cancer cells, posits ribosome production as a selective cancer liability. Certain genotoxic chemotherapies work partly by disrupting ribosome biogenesis, highlighting the need for selective inhibitors of this pathway. Using forward genetics, we identified mutations in the essential 60S ribosomal subunit assembly factor NVL that confer resistance to MM17, a dibenzothiazepinone with anticancer activity. Cryo-EM reconstructions of the NVL hexameric assembly reveal two MM17 docking sites adjacent to resistance mutations. NVL inhibition by MM17 arrests 60S biogenesis in the nucleolus and induces cell cycle arrest or apoptosis through both MDM2/p53-dependent and p53-independent pathways, without causing DNA damage. A bioavailable analog, MM927, suppresses tumor growth in mouse models of leukemia and colorectal cancer without observable toxicity. These findings establish NVL inhibitors as a promising new class of targeted therapeutics and validate ribosome biogenesis as a cancer-specific vulnerability.

11.55

Conserved mechanism of uORF-dependent translational control by specialised ribosomes

Nan Zhao, Veronica Thuburn, Elton J. R. Vasconcelos, Karl Norris, Carey Metheringham, Joanne Cunningham, Bulat Fatkhullin, Ella Dimascio, Tayah S. Hopes, Alex Goncalves, Barry Causier, Alan J. S. Beavan, Alison Wood, Tessa Chan, Alison Wood, Chalmers Chau, Chris West, Paolo Actis, Mary J. O'Connell, Anton Calabrese, Gordon Simpson, Adrian Whitehouse, Juan Fontana, Brendan Davies, Emma Thomson, **Julie L. Aspden**

The composition of ribosomes varies across tissues, during development and in response to stress signals. Such changes can result in specialised ribosome populations, which regulate the translation of specific ORFs (translons) or transcripts. We have previously characterised specialised ribosomes induced during lytic infection by Kaposi's sarcoma-associated herpesvirus (KSHV). These ribosomes have increased association with BUD23, which leads to reduced uORF translation and increased translation of viral main ORFs (Murphy et al Nat Coms 2023).

BUD23, which is highly conserved across eukaryotes, methylates a conserved 18S rRNA G in *S. cerevisiae* and *H. sapiens*. Bud23 mutants in *Arabidopsis thaliana*, have revealed that BUD23 is essential for viability, proper growth, and 40S ribosome biogenesis. Direct-RNA sequencing confirmed that the conserved G1581 in *Arabidopsis* is methylated by BUD23. Analysis in *Arabidopsis* and *Drosophila* suggests that this conserved site is methylated to a similar extent across different eukaryotes. Additionally, reporter assays demonstrate that BUD23 also regulates the translation of uORFs, and their associated main ORFs in *Arabidopsis*, highlighting BUD23-mediated methylation as a conserved mechanism for translational control. Work is underway to dissect the molecular mechanisms by which BUD23 regulates uORF translation and its role in stress response.

12.20

Ribosomal protein L5 (RPL5/uL18) I60V mutation is associated to increased translation and modulates drug sensitivity in T-cell acute lymphoblastic leukemia cells

Lorenza Bacci[1,§], Daniela Pollutri[1,2,§], Israt Jahan Ripa[1,§], Guglielmo Rambaldelli[1], Virginie Marchand[4], Yuri Motorin[4,5], Anne-Marie Hesse[3], Yohann Couté[3], Kamil Filipek[1], **Marianna Penzo**[1,2]

[1]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy
[2]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[3]Univ. Grenoble Alpes, INSERM, CEA, UA13 BGE, CNRS, CEA, UAR2048, Grenoble, France
[4]UAR2008 IBSLor CNRS-INSERM, Université de Lorraine, BioPole, Nancy, France
[5]UMR7365 IMoPA, CNRS, Université de Lorraine, BioPole, Nancy, France

Somatic mutations in ribosomal protein L5 (RPL5/uL18) contribute to pediatric T-cell Acute Lymphoblastic Leukemia (T-ALL), occurring in ~2% of cases without defined mutational hotspots. Mutant ribosomal proteins (RPs) can disrupt ribosome function, promoting malignancy. Here, we investigate the RPL5-I60V mutation's effects on translation and drug response in T-ALL.
Using CRISPR-Cas9, we engineered a homozygous RPL5-I60V knock-in in Jurkat cells and assessed ribosome biogenesis. We observed both quantitative and qualitative changes in large subunit production. Ribosomes harboring mutant RPL5 displayed intrinsically enhanced global translation and altered expression of specific mRNA targets, as revealed by polysome profiling. Functionally, mutant cells showed increased proliferation and resistance to apoptosis under both steady-state and pro-apoptotic conditions.
We next tested sensitivity to compounds targeting protein synthesis—MNK1 inhibitor, metformin, silvestrol, homoharringtonine, anisomycin, resveratrol, and hygromycin B—as well as cytarabine, a standard T-ALL therapy. The RPL5-I60V mutation conferred heightened sensitivity to most agents, except hygromycin B.
Our findings shed light on how oncoribosomes drive leukemia progression and suggest that targeting aberrant ribosome function may offer a novel therapeutic angle. This work supports further exploration of ribosome-directed strategies in precision oncology.

Session #6 - Emerging Therapies for Ribosomopathies

14.00

Gene Therapy and Gene Editing Strategies for the Treatment of Diamond-Blackfan Anemia syndrome

Susana Navarro, **Manuel Palacios Perez**
[1]Biomedical Innovation, CIEMAT/CIBERER/IIS-FJD, UAM, Madrid, España

Diamond-Blackfan anemia syndrome is a rare congenital bone marrow failure disorder characterized by erythroblastopenia, congenital anomalies, and increased cancer risk. Affecting 5–10 per million live births, around 40% of patients depend on chronic transfusions, while hematopoietic stem cell transplantation (HSCT) remains the only curative option, with increased toxicity risks in older patients. Most DBA cases involve autosomal dominant mutations in ribosomal protein genes, particularly RPS19 (25%). To address this, a lentiviral (LV) gene therapy approach was developed targeting RPS19-deficient cells. A clinically applicable lentiviral vector with a codon optimized version of RPS19 under the control of the phospoglicerate kinase promoter was developed. In RPS19-deficient K562 cells, this LV restored ribosomal function and in primary CD34+ cells from DBA patients, enhanced erythroid colony formation, and increased CD71+/CD235+ mature erythroid output. Transduced cells repopulated immunodeficient mice with improved erythropoiesis and a healthy polyclonal hematopoietic profile. This Orphan Drug designated medicine is undergoing clinical development. Additionally, between 7–11% of DBA cases involve RPL5 mutations, requiring tight expression control due to its interaction with MDM2/p53. A homologous recombination (HR)-based gene editing (GE) strategy was designed using CRISPR/Cas9 and AAV6 vectors to insert a

codon-optimized RPL5 cDNA (CoRPL5). In cord blood-derived CD34+ cells, self-complementary AAV6 donors achieved up to 84% edited alleles. This strategy rescued the erythroid defect in RPL5-knockout cells, enhancing erythroid clonogenicity and differentiation. HR analyses confirmed effective GE, with 75% of edited colonies showing biallelic correction. These findings support GE as a promising therapy for RPL5-related DBA.

14.25

In vivo functions of UBF1 and UBF2 isoforms

Ross Hannan[1, 2], Jiayi Guo[1], Joshua McRae[1], Dominik Spensberger[3], Nadine Hein[1], Rita Ferreira[1]
[1]Genome Science and Cancer Division, John Curtin School of Medical Research, Australian National University, Canberra, Australia
[2]Division of Cancer Research, Peter MacCallum Cancer Centre, Melbourne, Australia
[3]ANU Phenogenomics Targeting Facility, Australian National University, Canberra, Australia

Upstream Binding Factor (UBF) is a key architectural transcription factor required for RNA Polymerase I (Pol I)-mediated transcription of ribosomal RNA (rRNA) genes. UBF binds across both regulatory and coding regions of rDNA in a sequence-independent manner, maintaining an open chromatin state that facilitates active transcription. The number of UBF-bound loci correlates with the number of transcriptionally active rDNA repeats. Complete loss of UBF in mice results in pre-implantation lethality (e3.5), reflecting its essential role in ribosome biogenesis and early development. Mutations in UBF have also been associated with neuro-regression syndromes and cancer. UBF exists in two isoforms generated via alternative splicing: UBF1 (retaining exon 8) and UBF2 (lacking exon 8). UBF1 predominantly binds rDNA regulatory regions, while UBF2 is enriched at coding regions and may regulate highly expressed Pol II-dependent genes, such as histone clusters. This has led to the hypothesis that UBF1 plays a more prominent role in Pol I transcription; however, their functional distinctions remain poorly defined. To address this, we generated mouse models expressing either UBF1 or UBF2 exclusively. Ubf2 knockout mice are viable and develop normally, with no alteration in UBF1 binding or Pol I activity. In contrast, Ubf1 knockout embryos develop normally until e9.5, when lethality occurs. This suggests that UBF2 can sustain Pol I transcription and ribosome biogenesis in early embryogenesis, while UBF1 is essential for later developmental processes, potentially beyond ribosome production. Further characterisation will clarify the unique and overlapping functions of UBF isoforms in development and disease.

The novel RNA polymerase I transcription inhibitor PMR-116 exploits a critical therapeutic vulnerability in a broad-spectrum of high *MYC*-malignancies.

Nadine Hein[1], Rita Ferreira[1], Kate Hannan[1], Konstantine Panov[2], Luc Furic[3], Mustapha Haddach[4], **Ross Hannan[1,3]**
[1]Genome Science and Cancer Division, ANU, Acton, Australia
[2]Patrick G Johnston Centre for Cancer Research and School of Biological Scie, Queen`s University, Belfast, United Kingdom
[3]Division of Cancer Research, Peter MacCallum Cancer Centre, Melbourne, Australia
[4]Pimera Inc, San Diego, USA

The first step in the synthesis of new ribosomes is the transcription of the ribosomal RNA (rRNA) genes (rDNA) by RNA Polymerase I (Pol I) a process tightly regulated by tumour suppressor proteins such as Rb and p53 to control cell growth and proliferation. However, in cancer cells Pol I transcription is highly elevated due to the activation of oncogenes such as MYC. MYC amplifies RiBi to support uncontrolled cell growth, presenting a critical downstream vulnerability. Although first-

generation RiBi inhibitors such as CX-5461 showed clinical potential, their off-target effects, the induction of DNA damage via TOP2alpha inhibition, have raised concerns whether CX-5461 anti-cancer activity can be attributed to the direct inhibition of RiBi. To validate RiBi as therapeutic target for cancer treatment we evaluated PMR-116, a second-generation RiBi inhibitor with improved pharmacological properties and reduced genotoxicity for its therapeutic efficacy. PMR-116 inhibits Pol I transcription by stalling the Pol I at the rDNA promoter preventing promoter escape and exhibits potent anti-tumour activity across diverse preclinical models of haematological and solid tumours, particularly in those with MYC dependency. PMR-116 efficacy is achieved though activation of nucleolar surveillance pathways but most importantly, without activation of global DNA damage signalling. These findings validate RiBi as a druggable vulnerability and based on its potent efficacy, favorable pharmacological profile, and non-genotoxic mechanism, PMR-116 has passed Phase I clinical trial in advanced solid tumours (CTRN12620001146987) with promising outcome and is entering a Phase II basket trial at the end of 2025.

14.50

Targeting the nucleoli as a strategy to treat ovarian cancer

Shalini Chelliah[1, 2], Ruofei Liu[1], Karla Cowley[3], Jiachen Xuan[3], Kaylene Simpson[3], Jian Kang[1], **Elaine Sanij**[1,2,3]

[1]DNA Damage and Cancer Therapy Laboratory, St Vincent's Institute of Medical Research, Fitzroy, Australia

[2]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia

[3]Research Department, Peter MacCallum Cancer Centre, Parkville, Australia

The nucleolus is the site for RNA polymerase I (Pol) transcription of ribosomal RNA genes and ribosome subunit assembly. The inhibitor of Pol I transcription CX-5461, has demonstrated therapeutic benefits in various preclinical cancer models and in Phase I clinical trials. Our work has shown that CX-5461 induces nucleolar stress, leading to cell cycle arrest and cell death in ovarian cancer cells. CX-5461 exhibits a unique sensitivity profile compared with chemotherapeutics. Thus, we propose that activating nucleolar stress represents a promising new therapeutic approach. To identify novel nucleolar stress pathways, we conducted an innovative arrayed whole-genome CRISPR-Cas9 screen to identify genes whose deletion causes changes in nucleolar morphology as an indicator of nucleolar stress. We identified and classified five distinct types of nucleolar stress phenotypes that are linked to the inhibition of specific biological processes, including ribosome biogenesis, DNA repair, cell cycle, and RNA metabolism. Our data highlight the tight coordination between these processes and nucleolar fidelity. Moreover, we have identified and characterized a new role for a protein phosphatase in the regulation of Pol I transcription. To complement this screen and identify drugs that target the nucleoli, we completed a high-throughput drug screen of 23,000 compounds utilizing the same screening approach, to identify compounds that trigger nucleolar stress and inhibit ovarian cancer cell growth. Cyclin dependent kinases (CDKs), topoisomerases, and other targets were identified as candidate hits that induce potent nucleolar stress. In summary, our work uncovered specific pathways/factors as mediators of nucleolar stress and potential cancer therapeutic targets.

15.05

The International Diamond Blackfan Anemia Syndrome Cancer Consortium: Preliminary Results

Adrianna Vlachos[1,2], Marije Bartels[3], Polyxeni Delaporta[4,5], Paola Quarello[6,7], Maryam Hussain[8], Eva

Atsidaftos[8,9], Katie L Mills[1], Dagmar Pospíšilová[10], Jana Volejníková[10], Alexander Puzik[11], Kruti Revagar[12,13], Deena Iskander[12,13], Thierry Leblanc[14], Jason E Farrar[15], Antonis Kattamis[4, 5], Jeffrey M Lipton[89,16]
[1]Cancer and Blood Disorders Institute, Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA
[2]Johns Hopkins University School of Medicine, Johns Hopkins University, Baltimore, MD, USA
[3]University Medical Centre Utrecht, Centre for Benign Hematology, Thrombosis and Hemostasis-Van Creveldkliniek, Utrecht, Netherlands
[4]Thalassemia Unit, First Department of Pediatrics, National and Kapodistrian University of Athens, Athens, Greece
[5]'Aghia Sophia' Children's Hospital, ERN-EuroBloodNet Center, Athens, Greece
[6]Department of Public Health and Pediatrics, University of Turin, Turin, Italy
[7]Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy, Regina Margherita Children's Hospital, Turin, Italy
[8]The Feinstein Institutes for Medical Research, Manhasset, NY, USA
[9]Hematology/Oncology and Stem Cell Transplantation, Cohen Children's Medical Center, New Hyde Park, NY, USA
[10]EuroBloodNet Center, Department of Pediatrics, Palacky University and University Hospital Olomouc, Olomouc, Czech Republic

Diamond Blackfan anemia syndrome (DBAS) is an inherited bone marrow failure syndrome characterized by red cell aplasia, congenital anomalies and predisposition to cancer. The International DBAS Cancer Consortium was established to quantify cases of cancer and myelodysplastic syndrome (MDS) and develop surveillance strategies. Individual country registries were queried. There were 2336 subjects with DBAS identified in Czech Republic, France, Germany, Greece, Italy, Netherlands, Poland, Spain, United Kingdom, and United States. There were 153 cases of cancer and/or MDS in 133 subjects: 118 cancer, 19 MDS, and 16 non-melanoma skin cancer (excluded from analysis). Diagnoses included colorectal carcinoma (n=25), osteosarcoma (21), breast cancer (14), lymphoma (10), acute myelogenous leukemia (9), MDS (19), among others. The median age at first non-skin cancer is 33.6 years; of MDS is 24.8 years. The genotypes follow the normal distribution seen in the DBAS population, with the exception of the absence of subjects with *RPS26* with cancer/MDS ($p<0.005$). Combining registry data provides a robust analysis of cancer/MDS in DBAS. The most common solid tumors are colorectal cancers and osteosarcoma. Colonoscopy is recommended beginning at age 20 years. The number of breast cancer cases has also increased; thus, breast cancer screening may need to be considered. There are international obstacles that must be overcome to accomplish full participation.

15.20

Ribosomal Subunits RPS19 and RPL5 Differentially Orchestrate Fetal Hematopoiesis

Lionel Blanc[1], Yuefeng Tang[1], Te Ling[2], Rashid Mehmood[2], Alexis Bertrand[1], Deena Iskander[3], Jeffrey Lipton[1], Narla Mohandas[4], Sebastien Durand[5], John Crispino[2]
[1]Pediatric Hematology/Oncology, Northwell, Manhasset, USA
[2]Hematology, St. Jude, Memphis, USA
[3]Pediatric Hematology/Oncology, Imperial, London, United Kingdom
[4]Red Cell Physiology, New York Blood Center, New York, USA
[5]Ribosome, Translation and Cancer, CRCL, Lyon, France

Precise control of ribosomal protein (RP) stoichiometry is critical for development, yet the impact of RP mutations remains poorly understood. Using Diamond-Blackfan anemia (DBA) as a model, we generated novel *in vivo* models of RP haploinsufficiency to define the roles of small and large RP

subunits in fetal hematopoiesis. *Rpl5* haploinsufficiency caused prenatal lethality due to a reduction in erythroid translation. Conversely, *Rps19* haploinsufficiency led to neonatal stem cell exhaustion and impaired erythroid progenitor expansion, resulting from combined translational and transcriptional defects. The stem cell factor RUNX1, upregulated in *RPS19*-haploinsufficient DBA patients, plays a critical role in these defects, and its deletion partially rescued hematopoiesis in *Rps19* mutant mice. Our findings reveal distinct, subunit-specific roles for RPs in fetal hematopoiesis and demonstrate that imbalanced RP stoichiometry disrupts developmental programs, providing key insights into DBA pathogenesis and underscoring the importance of balanced RP expression in stem cell or erythroid development and potentially other ribosomopathies.

Session #7 - Pre-rRNA Processing and Folding

16.00

RNA Shape-Shifting: DEAD-box ATPases Drive Structural Transitions to Assemble the Large Ribosomal Subunit

Victor E. Cruz[1], Xingyu Chen[2], Christine Weirich[1], Kai Stroh[3], Nagesh Peddada[1], Kamil Sekulski[1], Riccardo Pellarin[3], Florian Stengel[2], **Jan Erzberger**[1]
[1]Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, USA
[2]Department of Biology, University of Konstanz, Konstanz, Germany
[3]Laboratory of Biology and Modeling of the Cell, Ecole Normale Superieure de Lyon, Lyon, France

The ordered and efficient assembly of the large (60S) ribosomal subunit requires the concerted action of multiple NTPases. Among these, the molecular function of ATP-driven RNA remodeling by DEAD-box proteins has remained particularly elusive, with their individual substrates and roles in 60S maturation only now coming into focus. Here, we present insights into the activities of the essential and conserved DEAD-box ATPases Dbp10 (DDX54), Drs1 (DDX27), and Spb4 (DDX55), focusing on a common mechanistic function: catalyzing transitions between energetically comparable helical structures within topologically defined subdomains of pre-60S intermediates. In each case, local duplex unwinding by the DEAD-box protein triggers a broader rearrangement of rRNA secondary structure that drives a distinct, temporally regulated assembly step: Dbp10 initiates a structural switch essential for peptidyl transferase center (PTC) formation; Drs1 promotes the docking of rRNA domain III; and Spb4 stabilizes a high-energy intermediate to initiate domain IV assembly. Together, these activities demonstrate how DEAD-box ATPases reshape the rRNA landscape to promote productive 60S subunit maturation. This shared mechanistic theme supports mapping alternative, energetically comparable rRNA secondary structures within pre-60S intermediates as a predictive strategy for identifying additional DEAD-box protein substrates. Furthermore, our data support a model in which individual ATPase activities are temporally and spatially coordinated to enhance the fidelity and efficiency of ribosomal assembly.

16.25

Molecular interactions with nucleolar proteins and helicases chaperone pre-rRNA folding

Yunsheng Sun, **Sarah Woodson**
[1]T.C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, USA

Newly transcribed pre-ribosomal RNA may initially fold into structures that are incompetent for assembly, requiring chaperones to smooth the earliest steps of ribosome synthesis. We showed that the E. coli DEAD-box protein CsdA strongly accelerates ribosomal protein uS4 binding by facilitating proper folding of the nascent rRNA. CsdA unwinding became less frequent as additional ribosomal proteins joined the complex. We proposed that general chaperones create disassembly gradients that cull unstable RNPs while allowing native RNPs to continue assembly and maturation. We next asked whether proteins resident in the eukaryotic nucleolus facilitate rRNA folding and assembly. We directly compared RNA folding inside and outside Nop1 droplets, using single molecule fluorescence microscopy. The results show that Nop1 disrupts the tertiary structure of a ribozyme and an rRNA domain. However, this effect is identical inside and outside droplets and depends only on transient interactions between Nop1 and the RNA. We further show that Nop1 shields the rRNA from CsdA unwinding. In turn, non-specific Nop1 binding is displaced by specific assembly with ribosomal proteins. Our results show how the competition between helicases, nucleolar proteins and ribosomal protein shape the folding pathway of the nascent pre-rRNA.

16.40

RNA helicases in SSU processome formation and maturation

Valentin Mitterer

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria

During eukaryotic ribosome assembly, over 200 protein assembly factors, together with several snoRNAs, facilitate the stepwise incorporation of ribosomal proteins and the structural maturation of initially flexible, unstructured rRNA domains into their final conformations. Among these assembly factors, 19 RNA helicases facilitate key restructuring events by resolving immature RNA folds or displacing bound factors to enable downstream processing steps. However, their precise functions within their respective pre-ribosomal substrate particles remain largely unknown. To investigate the functions of RNA helicases, we employed tandem-affinity purifications to specifically isolate stalled ribosomal precursor particles following RNA helicase depletion or upon expression of dominant helicase mutants impaired in ATP binding or hydrolysis. Using a combination of genetic and biochemical approaches coupled with structural cryo-EM analyses of stalled pre-ribosomes, we have shed light on RNA helicase-driven maturation steps during early co-transcriptional assembly of the small ribosomal subunit (SSU). We identified distinct assembly stages during SSU processome maturation that require the activities of the DEAD-box helicase Dbp8 and the processive DEAH-box helicase Dhr2. While Dbp8 promotes maturation by integration of the 18S rRNA 5’ domain and associated assembly factors, Dhr2 facilitates the initial formation of the SSU processome emerging from the early 5’ ETS ribonucleoprotein complex. Furthermore, our findings indicate that the bulk of co-transcriptional assembly factors may only associate to form the SSU processome once transcription of the 18S rRNA is complete.

16.55

Early steps of eukaryotic ribosome assembly revealed by single-molecule microscopy

Margaret Rodgers

National Institutes of Health, Maryland, USA

Eukaryotic ribosome biogenesis begins with transcription of the 5' external transcribed spacer (5'ETS) of the 35S pre-ribosomal RNA (rRNA). During transcription, the pre-rRNA associates with dozens of assembly factors, ribosomal proteins, and small nucleolar RNPs (snoRNPs) to form pre-ribosome

intermediates that are processed and post-transcriptionally matured. The first subcomplex to be recruited is UtpA, which has been shown to assemble on rRNA fragments as small as 200 nucleotides *in vivo*. However, the mechanistic details of UtpA recruitment are not known. Using single-molecule colocalization microscopy, we have developed a real-time eukaryotic ribosome assembly assay to characterize the kinetic mechanisms driving early ribosome assembly. Using yeast whole cell extracts, we monitor colocalization of fluorescently-tagged UtpA complexes with immobilized pre-rRNAs to visualize the first step of assembly. UtpA associates rapidly and remarkably stably with fragments of the 5'ETS suggesting that assembly of UtpA is functionally irreversible. UtpA recruitment is specific for the 5'ETS as UtpA does not associate with an rRNA fragment containing only the 18S 5'domain. Fragments of the 5'ETS as short as 90 nucleotides can stably recruit UtpA, consistent with protein-RNA interactions anchoring the complex near the 5' end of the rRNA. However, UtpA recruitment becomes more efficient on longer 5'ETS fragments hinting at the influence of transcription directionality and the potential for cooperativity underlying early assembly. These results underscore the importance of a real-time mechanistic assembly assay for understanding how ribosome assembly is initiated and establish a foundation for studying later assembly steps.

17.20 (P50)

A UTP3-dependent nucleolar translocation pathway facilitates pre-rRNA 5'ETS processing

Jiayang Bao[1], Baochun Su[1], Zheyan Chen[1], Zhaoxiang Sun[2], Jinrong Peng[1], **Shuyi Zhao**[1]
[1]College of animal sciences, Zhejiang University, Hangzhou, China
[2]College of life sciences, Zhejiang University, Hangzhou, China

The ribosome small subunit (SSU) is assembled by the SSU processome which contains approximately 70 non-ribosomal protein factors. Whilst the biochemical mechanisms of the SSU processome in 18S rRNA processing and maturation have been extensively studied, how SSU processome components enter the nucleolus has yet to be systematically investigated. Here, in examining the nucleolar localization of 50 human SSU processome components, we found that UTP3, together with another 24 proteins, enter the nucleolus autonomously. For the remaining 25 proteins we found that UTP3/SAS10 assists the nucleolar localization of five proteins (MPP10, UTP25, EMG1 and the two UTP-B components UTP12 and UTP13), likely through its interaction with nuclear importing α. This 'ferrying' function of UTP3 was then confirmed as conserved in the zebrafish. We also found that knockdown of human UTP3 impairs cleavage at the A0-site while loss-of-function of either *utp3/sas10* or *utp13/tbl3* in zebrafish causes the accumulation of aberrantly processed 5'ETS products, which highlights the crucial role of UTP3 in mediating 5'ETS processing. Mechanistically, we found that UTP3 facilitates the degradation of processed 5'ETS by recruiting the RNA exosome component EXOCS10 to the nucleolus. These findings lay the groundwork for studying the mechanism of cytoplasm-to-nucleolus trafficking of SSU processome components and grants increased understanding of the multifaceted roles of UTP3 during pre-rRNA processing.

Wednesday 10th September

Session #8 - Non-eukaryotic ribosome assembly and advances in antibiotic development

9.00

Visualizing ribosomes in near native conditions through cryoEM and machine learning

Joey Davis
MIT, Massachusetts, USA

Structure determination through single particle cryogenic electron microscopy (cryoEM) has transformed our understanding of the structure of the ribosome as it is assembled, as it performs translation, and as it is repressed during times of cellular hibernation. Traditionally, such analyses use highly purified samples that are often trapped in desired states through genetic, pharmacological, or biochemical perturbations. However, such in vitro approaches can limit the full ensemble of states observed, including factors not previously known to bind to certain ribosomal states. Likewise, despite much progress in determining ribosomal structures at ever-increasing resolution in situ through sub-tomogram averaging and 2D-template matching, this work has focused on computationally isolating a small number of abundant states, often overlooking functionally important conformers. To overcome these limitations, we introduce an 'ex vivo' approach to sort and reconstruct near-atomic-resolution cryo-EM maps imaged directly from cell lysates. Specifically, we established lysis, vitrification, and image analysis methods that allowed us to determine dozens of unique states spanning ribosomal assembly, translation initiation, elongation, recycling, hibernation, and rescue from a single dataset, thereby demonstrating the ability of this approach to preserve interactions with a milieu of ribosomal factors. As a proof of principle, we applied this method to analyze ribosomal states in bacteria and Archaea, discovering new constellations of protein factors that modulate translational activity. Through these vignettes, we show that our approach is rapid, accessible, and broadly applicable for studying specific aspects of the ribosomal life cycle and comparing ribosomal landscapes under different treatments in near-native conditions.

9.25

Characterization of *cis*- and *trans*-acting elements required for ribosome synthesis in archaea

Ferreira-Cerca Group, **Sébastien Ferreira-Cerca**
Structural Biology of the Cell Laboratory | Laboratoire de Biologie Structurale de la Cellule (BIOC), UMR 7654—CNRS, École polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91128 Palaiseau, France

The ribosome is a universally conserved ribonucleoprotein complex responsible for the translation of mRNAs into proteins. Ribosome synthesis is a highly complex and tightly regulated task consisting of the stepwise hierarchical and coordinated assembly of the rRNA and r-proteins. During this process precursor (pre-)rRNAs get matured by endo- and exoribonucleases, properly folded, and chemically modified. In the past few decades, ribosome biogenesis has been extensively studied in Bacteria and Eukaryotes. In Archaea, on the other hand, little is known about this fundamental process.

Our team aims to characterize the specific and common principles of ribosome biogenesis in archaea. Accordingly, we study the role of key *cis*- and *trans*-acting elements involved in ribosome biogenesis in archaea. We will provide a summary of our efforts in characterizing this fundamental process in archaea and provide an updated view on the rRNA maturation pathway(s) and rRNA modifications in archaea as well as structural and functional insight into ribonucleases contributing to archaeal ribosome biogenesis.

Selected References:
Vayssières M, Jüttner M, Haas K, Ancelin A, Marchfelder A, Leulliot N, Ferreira-Cerca S and Blaud M.

RNase W, a conserved ribonuclease family with a novel active site. Nucleic Acids Research 2024. https://doi.org/10.1261/rna.079636.123.

Grünberger F, Jüttner M, Knüppel R, Ferreira-Cerca S and Dina Grohmann. Insights into rRNA processing and modification mapping in Archaea using Nanopore-based RNA sequencing. RNA 2023. doi: 10.1261/rna.079636.123.

Jüttner M & Ferreira-Cerca S. Looking through the lens of the ribosome biogenesis evolutionary history: possible implications for archaeal phylogeny and eukaryogenesis. Molecular Biology and Evolution 2022. https://doi.org/10.1093/molbev/msac054

9.40

Autonomous synthesis and assembly of early and late stage intermediates of the Escherichia coli 50S subunit in a minimal system

Noa Avidan[1], Shirley Shulman-Daube[1], Michael Levy[1], Yoshihiro Shimizu[2], Roy Bar-Ziv[1]
[1]Chemical and Biological Physics, Weizmann Institute of Science, Rehovot, Israel
[2]Center for Biosystems Dynamics research, RIKEN, Osaka, Japan

Cell-free autonomous biogenesis of ribosomes is a key step toward the realization of artificial self-replicating cells. Recently we demonstrated autonomous cell-free synthesis and assembly of the Escherichia coli small ribosomal subunit in the minimal PURE system, but the large subunit is far more challenging, due to the numerous components, the essential ribosomal RNA modifications and assembly factors, and the complexity of the assembly pathway. In this work, we used total internal reflection fluorescence microscopy (TIRF) to probe the dynamics of assembly of all the large subunit nascent components including 33 ribosomal proteins and 2 ribosomal RNAs coupled to their synthesis in a minimal recombinant cell-free gene expression system. A systematic screen of the binding of each of the ribosomal proteins to the 23S ribosomal RNA, enabled by surface localized reaction centers, revealed an assembly pattern indicative of formation of domains I, II, III and VI of the 23S rRNA, implying assembly of an early intermediate of the subunit. The addition of genes coding for the assembly factors EngA and ObgE facilitated association of late assembly r-proteins with the 23S rRNA, supporting formation of late-stage intermediates. Our approach lays a foundation for full ribosome synthesis in vitro, using a minimal expression system and simple genetic programming.

9.55

Role of the leader-trailer helix of pre-16S rRNA in biogenesis of the 30S subunit

Benjamin Warner, **Kurt Fredrick**
[1]Microbiology, Ohio State University, Columbus, USA

Ribosome biogenesis occurs co-transcriptionally and entails rRNA folding, r-protein binding, rRNA processing, and rRNA modification. In many bacteria, the 16S, 23S, and 5S rRNAs are co-transcribed, often with one or more tRNAs. Sequences flanking the rRNAs are complementary and form long helices known as leader-trailer helices. Using an orthogonal translation system, we have shown that the leader-trailer helix (hLT) of pre-16S rRNA is crucial for formation of active subunits. Truncation of either the 5’ or 3’ strand causes progressive loss of activity, with no detectable activity once the helix becomes shorter than 17 base pairs. Other mutations which abolish transcription antitermination or remove the leader helices (hA, hB) confer comparatively small effects on 30S biogenesis. To better understand hLT’s role, we tested whether an artificial protein-aptamer bridge could substitute for hLT

in *E. coli*. Constructs were made encoding one array of MS2-binding aptamers upstream of 16S rRNA and another array of PP7-binding aptamers downstream, and the corresponding RNA was expressed in the absence or presence of 2MS2-2PP7, a fused MS2 coat protein dimer linked to a fused PP7 coat protein dimer. We found that such engineered protein-aptamer bridges can facilitate subunit assembly. Surprisingly, the aptamer arrays themselves cause substantial rescue of translation activity. Activity is further increased by 2MS2-2PP7 or by depletion or loss of RNase E, RNase R, and RhlB. This work suggests that leader-trailer helices act to protect pre-rRNA from degradation and identifies those proteins involved in rRNA surveillance and turnover.

10.20

Functional coupling between ribosomal RNA transcription and early processing

Anastasiia Chaban, Nusrat Qureshi, Olivier Duss
[1]Molecular Systems Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

Efficient bacterial rRNA transcription is mediated by the rRNA transcription antitermination complex (rrnTAC; composed of six proteins NusA, NusG, NusB, NusE, S4, SuhB) assembling co-transcriptionally on the 5' rRNA leader and the RNA polymerase. However, the mechanism for efficient rrnTAC assembly as well as its potential role in mediating early co-transcriptional rRNA processing by RNase-III is not understood. Here, we use multi-color single-molecule fluorescence microscopy to investigate how transcription of rRNA, its co-transcriptional folding and processing are functionally coupled. First, we track in real-time the assembly of the complete rrnTAC onto an active transcription elongation complex and show that SuhB joins last thereby stabilizing the previously transient rrnTAC protein interactions with the transcription machinery. Next, by simultaneous and real-time tracking of rrnTAC assembly and co-transcriptional RNase-III processing, we demonstrate that the fully assembled rrnTAC facilitates RNase-III cleavage by co-transcriptional chaperoning the RNase-III substrate helix. This is the first direct experimental evidence of coupling between rRNA transcription and processing in bacterial ribosome assembly, which is mediated by long-range rRNA looping and raises the question if co-transcriptional RNA looping is a more general mechanism in co-transcriptional processes such as we previously showed for transcription-translation coupling (Qureshi & Duss, Nature, 2025).

Session #9 - Regulation of Mitoribosome Biogenesis and Non-Eukaryotic Ribosome Assembly

11.00

YsxC is a placeholder for ribosomal protein uL2 during 50S ribosomal subunit assembly

Amal Seffouh[1,2], Dominic Arpin[1,2], Kaustuv Basu[1,2], Joaquin Ortega[1,2]
[1]Anatomy and Cell Biology, McGill University, Montreal, Canada
[2]Centre de Recherche en Biologie Structurale, McGill University, Montreal, Canada

The maturation of the functional core of the 50S ribosomal subunit in *Bacillus subtilis*, a model organism for Gram-positive bacteria, is assisted by assembly factors that enhance the efficiency of the process. Two essential assembly factors, the GTPases RbgA and YphC, bind at or near the functional sites of the 50S subunit to promote the folding of key functional ribosomal RNA helices. This investigation focuses on a third ribosomal assembly GTPase, termed YsxC, whose

function remains unknown. We demonstrate that YsxC contributes to the maturation of the 50S subunit through a mechanism that is drastically different from those of RbgA and YphC. Using cryo-electron microscopy, we found that YsxC occupies the binding site of the core r-protein uL2 in the 44.5S large ribosome assembly intermediate. When the 44.5S intermediate has not been previously exposed to YsxC, uL2 binds unstably and wobbles at its binding sites. Accordingly, the temporary binding of YsxC conditions the binding site for uL2, and upon its release, uL2 can bind in the same conformation adopted in the mature 50S subunit. Overall, these results suggest that YsxC functions as a placeholder factor for the r-protein uL2. By this strategy, YsxC placeholder sterically prevents premature recruitment of uL2, premature formation of structures, avoids possible folding traps, and acts as a molecular clock that supervises the correct progression of pre-ribosomal particles into functional ribosomal subunits. Moreover, our high-resolution cryo-EM model provides new insights into the molecular mechanisms of GTP hydrolysis by YsxC.

11.15

A ribosome biogenesis associated RNA helicase impacts r-protein production

Gabriella Lopez-Perez[1], Mira May[1], Joseph Davis[1,2]
[1]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA
[2]Program in Computational and Systems Biology, Massachusetts Institute of Technology, Cambridge, MA, USA

Ribosome assembly is a highly coordinated process that involves the transcription, folding, and modification of ribosomal RNAs (rRNAs) and synthesis, folding, and association of ribosomal proteins (r-proteins), all occurring within ~ 2 minutes in bacteria cells. Assembly factors, such as DEAD-box RNA helicases, are thought to enhance speed and efficiency to the assembly process by facilitating rRNA folding and preventing the formation of non-native rRNA structures. Of the five DEAD-box RNA helicases encoded in *E. coli*, four (SrmB, DeaD, RhlE, and DbpA) have been linked to ribosome biogenesis. However, direct mechanistic evidence for how these helicases contribute individually or in concert to ribosome assembly remains limited. Here, we describe our development of a tunable genetic system to precisely regulate the individual or joint expression of DEAD-box helicases within a single *E. coli* strain, allowing us to dissect their contributions with minimal confounding variables. Combined with a high-throughput growth assay to screen for genetic interdependencies, this system builds on existing models for how these helicases jointly assist in ribosome assembly and enables the characterization of assembly intermediates that accumulate under specific depletion conditions. Critically, we identify a novel role for RhlE in modulating the levels of specific r-proteins, suggesting a broader regulatory function in ribosome biogenesis.

11.30

Mechanisms of human mitochondrial RNA biogenesis and mitoribosome synthesis

Hauke Hillen
Department of Cellular Biochemistry, University Medical Center Göttingen, Germany
Research Group Structure and Function of Molecular Machines, Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany

The mitochondrial ribosome is composed of ribosomal RNA (rRNA) encoded by the organellar genome and protein constituents that are encoded in the nucleus, synthesized in the cytosol and imported to the organelle. Mitochondrial ribosome biogenesis therefore requires the coordinated

expression of two genomes as well as a large number of dedicated maturation factors. However, the mechanisms underlying mitochondrial gene expression and mitoribosome assembly remain poorly understood at the molecular level. In contrast to nucleus-encoded rRNAs, human mitochondrial rRNAs are transcribed as part of polycistronic transcripts that additionally contain tRNAs, mRNAs and non-coding sequences. These primary transcripts are processed by dedicated molecular machineries, which liberates the individual RNAs for further maturation. Using a combination of structural biology and biochemistry, we have recently elucidated the mechanism of canonical pre-RNA processing in human mitochondria. Our data reveal how dedicated processing machineries enable the recognition of structurally degenerate mitochondrial tRNAs flanking rRNAs and mRNAs within the primary transcript and provide a mechanistic basis for the very first steps of mitochondrial ribosome and tRNA biogenesis. In addition, we have reported structural snapshots of several mitoribosome assembly intermediates, which provide insights into the mechanisms of late small and large subunit maturation in human mitochondria. Finally, our most recent data suggest a functional coupling between ribosome biogenesis and translation initiation in human mitochondria. In my talk, I will provide an overview of our work and highlight these recent advances in our understanding of mitochondrial RNA biogenesis and mitoribosome synthesis.

11.55

Integration of experimental data with model prediction and simulation reveals how Mettl15-Mettl17 modulates pre-mitoribosome

Alexey Amunts[1,2]

[1]Universität Münster, Munster, Germany

[2]Max Planck Institute for Molecular Physiology, Dortmund, Germany

We used an integrative approach combining cryo-EM data, molecular dynamics simulations, and computational modeling to uncover intermediate states and propose a sequential assembly mechanism of the mitoribosome orchestrated by the methyltransferases Mettl15 and Mettl17. Our analysis identified previously unassigned elements in the cryo-EM map of T. brucei mitoribosomal small subunit precursor, including homologs of assembly factors RbfA and Mettl15 tightly associated with Mettl17. Phylogenetic studies revealed the conservation of the Mettl15-Mettl17 heterodimer across eukaryotic groups. Using AlphaFold and molecular dynamics simulations, we modeled the human pre-mitoribosome and demonstrated the dynamic role of Mettl17 in recruiting Mettl15 and facilitating rRNA maturation through conformational rearrangements. Our results suggest that Mettl17 primarily acts as a structural organizer rather than an enzymatic methyltransferase. Finally, we combined our analysis with previously obtained structural insights into a coherent series of sequential assembly steps that lead to the catalytic mitoribosome. This work highlights how combining molecular dynamics with template-based modeling can reveal transient states, offering a powerful tool for studying complex macromolecular systems where experimental data are limited. This broadly applicable methodology can ultimately help reveal protein functions and more complete biogenesis pathways in other metabolic processes.

12.10

Mammalian mitochondrial ribosome assembly: insights from recent structural studies

Joanna Rorbach

Dept of Medical Biochemistry and Biophysics, Karolinska Institutet, 17177 Stockholm, Sweden

The correct assembly of mitochondrial ribosomes is critical for mitochondrial respiration and overall cellular function. Mutations that disrupt mitoribosome components or their biogenesis pathways are

linked to severe human mitochondrial diseases. While the mechanisms of ribosome assembly in bacteria and the eukaryotic cytosol are well characterized, the biogenesis of human mitoribosomes remains comparatively underexplored.
Here, we present high-resolution cryo-EM structures of late-stage assembly intermediates of the human mitochondrial large (mt-LSU) and small (mt-SSU) ribosomal subunits, captured in genetically engineered cell lines. Structural comparison of these intermediates with mature mitoribosomes reveals the sequential events underlying mitoribosome maturation. These insights clarify the mechanisms of known assembly factors and uncover previously unidentified factors involved in this process.
Notably, we identify unexpected roles for translation factors: elongation factor mtEF-Tu in mt-LSU assembly and initiation factors mtIF2 and mtIF3 in mt-SSU assembly. Our findings also demonstrate a tight coordination between mt-LSU and mt-SSU biogenesis, which prevents premature subunit joining prior to full maturation.
Collectively, these results illuminate the unique features of late-stage mitoribosome assembly in mammalian mitochondria and provide long-awaited structural insights into several key assembly factors.

Thursday 11th September

Session #10 - Specialized Ribosomes, Regulation, and Quality Control

9.00

Loss of different ribosomal proteins have distinct effects on antigen processing and presentation
William Faller
Netherlands Cancer Institute, Netherlands/ University of Bristol, Bristol, UK

Antigen processing and presentation (APP) is a key step in adaptive immune responses. It is a complex process during which proteins are broken down and processed, before the resulting peptides are loaded on to MHC molecules and transported to the cell surface, where they can be sampled by T-cells. In response to inflammatory cytokine exposure, the cell up-regulates APP, allowing the cell to become more visible to immune surveillance. Efficient APP is critical to health, and tumour cells are known to down-regulate it to avoid immune detection. Specialised ribosomes are known to play a key role in APP; however, it is not known whether different changes to the ribosome have different effects on APP.

We have shown that the loss of two different ribosomal proteins (P1 and uL14) both result in decreased T-cell recognition, but through distinct mechanisms. P1 is needed for the translation of key APP regulators and adaptors (including HLA), due to an increased ability to translate transmembrane domains. Ribosomes lacking uL14, on the other hand, translate a distinct population of proteases and peptidases, resulting in changes in the identity of peptides being presented. These peptides are predicted to have a shorter half-life, reducing APP. Thus, loss of P1 and uL14 both reduce APP and the ability of T-cells to recognise tumour cells, but they do this through distinct mechanisms.

In all, our work suggests that diverse ribosome populations may fine-tune APP on multiple levels and highlights the variability in ribosome function that is defined by the ribosomal proteins present.

9.25

Regulation of RNA Polymerase I activity and rRNA transcription by cell-type-specific transcription factor CEBPA

Charles Antony[1,2], Santosh Adhikari[2,4], Eleanor Sams[1,2], Victoria Feist[1,2], Jill Henrich[1,2], Mustafa Mir[2,3,4], **Vikram Paralkar[1,2,4]**

[1]Department of Medicine, University of Pennsylvania, Philadelphia, USA
[2]Epigenetics Institute, University of Pennsylvania, Philadelphia, USA
[3]Freeman Hrabowski Scholar, Howard Hughes Medical Institute, Philadelphia, USA
[4]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, USA

Transcription of rRNAs from rDNA repeats by Pol I is an energy-intensive process. Complex organ systems contain diverse cells that differ in size and proliferation rates. It remains unclear how rRNA transcription is tailored to meet the needs of each cell type. We used FISH-Flow to construct a detailed map of nascent and mature rRNA levels in normal and leukemic mouse hematopoiesis. Within normal hematopoiesis, rRNA levels varied 9-fold. Leukemic cells exhibited elevated rRNA compared to normal counterparts. We previously reported that the myeloid transcription factor (TF) CEBPA binds to a canonical motif on rDNA repeats, and that CEBPA degradation rapidly reduces Pol I occupancy and rRNA transcription. To dissect the steps of Pol I regulation by this cell-type-specific factor, we employed single molecule tracking. We engineered a mouse myeloid cell line with Halo sequence fused to Polr1a and Rrn3 loci, enabling live cell tracking of both molecules. We observed that Pol I and RRN3 exist in two populations: a slow-moving, rDNA-loaded pool (co-localizing with nucleolar marker UBTF), and a freely diffusing pool. 70% of Pol I molecules and 25% of RRN3 molecules are rDNA-loaded, supporting a model in which majority of Pol I is actively transcribing. Degradation of CEBPA (using FKBP degron) led to decrease in the fraction of rDNA-loaded Pol I and RRN3 within 2 hours. CEBPA loss also increased duration of rDNA-bound tracks for both, indicating delayed release of Pol I-RRN3 complex from promoters. Our work shows how a lineage-defining TF regulates Pol I activity in a cell-type-specific manner.

9.40

Translatome and translation dynamics analysis reveals mechanistic diversity of frequent ribosomal protein mutations in cancer, with profound changes for RPS15-mutations

Marino Caruso[1], Anaïs Astier[2], Stijn Vereecke[1], Paulo Espirito Santo[2], Coralie Capron[3], Francesca Rapino[3], Frédéric Catez[4], Julien Marcoux[5], Célia Plisson-Chastang[2], **Kim De Keersmaecker[1]**

[1]Department of Oncology, KU Leuven, Leuven, Belgium
[2]Centre de Biologie Integrative Toulouse, Université de Toulouse, CNRS, Toulouse, France
[3]GIGA institute, University of Liège, WELBIO, Liège, Belgium
[4]Cancer Research Center of Lyon, Inserm, CNRS, UCBL, Centre Léon Bérard, Lyon, France
[5]Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, UPS, Toulouse, France

Various somatic mutations in ribosomal proteins (RPs) have been described in cancer. With a genomewide translatome analysis (proteome, Ribo-seq and total mRNA-seq), we previously showed that RPL10-R98S (uL16-R98S) mutation drives hypertranslation of JAK-STAT, BCL2 and PSPH oncoproteins in T-cell leukemia. To verify whether other cancer-associated RP mutations act similarly, we generated an isogenic cell line library modeling the most recurrent RP mutations (Rpl5$^{+/-}$ (uL18$^{+/-}$), Rpl11$^{+/-}$ (uL5$^{+/-}$), Rpl22$^{+/-}$ (eL22$^{+/-}$), Rpl22$^{-/-}$, Rpl10-R98S, Rps15-P131S (uS19-P131S) and Rps15-H137Y). Genomewide translatome analysis of this library revealed little translational changes in the RP knock-outs, suggesting rather extra-ribosomal pro-oncogenic effects. The RP point mutations caused

extensive translational rewiring, with most significant translation efficiency (TE) changes for the Rps15 mutants. Further Cryo-EM and biochemical analyses revealed that Rps15 mutations alter the stability of the C-terminal Rps15 domain, affecting the translation elongation cycle dynamics of these mutant ribosomes, and deregulating accommodation of new aminoacylated tRNAs at the ribosomal A-site. Our Ribo-seq data indicate that this accommodation defect shows a specificity to certain tRNAs, as supported by translation reporter assays. Finally, the genes presenting significant TE changes in Rps15 mutants are enriched for transcriptional regulators, revealing an unappreciated cross-talk between translational and transcriptional regulation in these RP mutant cells.

9.55

Ribosomal Protein Paralogues of the eRpL22 family as Drivers of Developmental Changes in Spermatogenesis in *Drosophila*

Vassie Ware, Catherine Mageeney, Michael Kearse, and Oyedibu Oloyede
Department of Biological Sciences, Lehigh University, Bethlehem, PA, USA

The eukaryotic-specific RpL22 family (eRpL22) consists of paralogues eRpL22 and eRpL22-like, which in *Drosophila* are structurally distinct with an additional domain (similar to histone H1) at the N terminus, compared to other eukaryotic RpL22 paralogues. We have shown that expression of eRpL22 family paralogues in the male germline in the adult testis contributes to ribosome heterogeneity and functional differences by forming populations of specialized ribosomes containing eRpL22 or eRpL22-like with differential mRNA translation specificities. That eRpL22 and eRpL22-like are not functionally equivalent has also previously been demonstrated in RNAi-depletion/ conditional knock-out and overexpression experiments, showing a differential impact on sperm maturation and fertility depending on which paralogue is depleted or overexpressed. Here, we provide experimental updates on ribosomal and extraribosomal roles of eRpL22 paralogues at different developmental stages of spermatogenesis. We report that tissue-specific overexpression of eRpL22, but not eRpL22-like, causes cellular overproliferation in the apical tip of the testis within the stem cell niche. *In vivo* apical tip overgrowth is partially phenocopied when eRpL22 overexpression is confined to somatic cyst cells and accessory glands using cell type-specific GAL4 drivers. The mechanism underlying this phenomenon remains unknown, but overexpression phenotypes are abolished in the testis when truncated eRpL22 containing only the rRNA binding C-terminal end is overexpressed, suggesting a non-ribosomal role for the N-terminal domain of eRpL22 in processes affecting somatic cell proliferation and tissue overgrowth. Additionally, we present evidence supporting a late stage SUMOylation modification of eRpL22-like in mature sperm that may remove this paralogue from the ribosomal pool. Our previous work showed SUMOylation of a nuclear pool of eRpL22 in early-stage meiotic spermatocytes. Thus, mature spermatids may sequester both modified eRpL22 paralogues prior to fertilization. Overall, experiments are ongoing to determine changes in translation and sperm maturation outcomes when expression of specific paralogues is disrupted.

10.20

Compositionally distinct 5.8S-L rRNA ribosomes link differential mRNA translation to selective cell fitness in *S. cerevisiae*

Christian Trahan[1], Amal Seffouh[3], Ludivine Rachel[4], Justina Chu[2], Daniel Zenklusen[2], Malik Chaker-Margot[2], Denis Lafontaine[4], Joaquin Ortega[3], **Marlene Oeffinger[1,2,5]**
[1]RNPBiochemistry laboratory, IRCM, Montréal, Canada
[2]Département de biochimie & médecine moléculaire, Université de Montréal, Montréal, Canada

[3]Anatomy and Cell Physiology, McGill University, Montréal, Canada
[4]Faculté des Sciences, Université, Libre de Bruxelles, Bruxelles, Belgium
[5]Experimental Medicine, McGill University, Montréal, Canada

While heterogeneous ribosomes are largely considered at the level of ribosomal proteins and rRNA modifications, a so far less investigated source of ribosome heterogeneity are the long (L) and short (S) forms of mature 5.8S rRNA, whose 5'ends located in close vicinity to the peptide exit tunnel (PET) of translating cytoplasmic ribosomes. We studied the difference of the two 5.8S forms on translating ribosomes. In line with a functional heterogeneity of 5.8S-L- vs. -S-form ribosomes in translation of mRNA subsets, we observed increase translation of transcripts linked to membrane synthesis, including in the ER and mitochondria, and components of the nascent polypeptide-associated complex (NAC) in a *S. cerevisiae* strain with six-fold increased 5.8S-L-form ribosome levels (termed L^+), while translation of sensors linked to vacuole synthesis and meiosis were overall decreased. This was concomitant with higher L^+ cell fitness in response to cell wall, osmotic and antibiotic stress but decreased fitness under oxidative stress. Increased levels of Um2347 and increased occupancy of L^+ ribosomes at start and stop codons suggest a differential mode of translation, which is further supported by structural differences identified in L^+ ribosomes by cryo-EM, a significant enrichment of the NAC and ribosome-associated complex (RAC)–both linked to protein folding and sorting at the PET–with L^+ribosomes along with distinct changes in paralog stoichiometry of NAC–and RAC-linked ribosomal proteins around the PET, as well as localization of 5.8S-L ribosomes. Taken together, our data provides evidence of functional and compositional differences of 5.8S-Long-form containing ribosomes in *S.cerevisiae*.

Session #11 - New Developments in the Ribosome Epitranscriptome

11.00

Differential rRNA Methylation Orchestrates Ribosome Specialization to Fine-Tune Translation During the Cell Cycle

Sandra Blanco
[1]Cancer Research Centre, CSIC - University of Salamanca, Salamanca, Spain

Variations in ribosomal RNA (rRNA) modifications have been observed in both healthy and diseased tissues, raising questions about their role in translation regulation and potential contributions to disease. However, the dynamic regulation of these modifications and their impact on translation remain unresolved. Here, we identify differential cytosine-5-methylation (m5C) of rRNA as a significant source of ribosome heterogeneity in human cells. Using nascent proteomics, RNA bisulfite and ONT RNA direct sequencing, and molecular dynamics simulations, we demonstrate that rRNA m5C, mediated by the NSUN5 methyltransferase, undergoes dynamic changes during the cell cycle. High-resolution cryo-EM analysis reveals that the loss of m5C induces structural rearrangements at the subunit interface, altering codon usage to preferentially translate mRNAs enriched in optimal codons. This shift enhances the expression of early mitotic regulators and impacts the efficiency and accuracy of mRNA decoding during mitosis.Through biochemical, genetic, and functional analyses, we establish that rRNA m5C methylation dynamics are essential for cell cycle progression and tumor growth in vivo. Our findings demonstrate that differential rRNA m5C methylation generates specialized ribosomes with unique functional roles, providing a mechanism for fine-tuning translation, regulating codon usage, and modulating genome decoding during cell cycle progression.

11.25

Cytoplasmic uridylation monitors maturation of the human 18S rRNA 3' end

Maxime Aubert[1], Hélène Zuber[2], Claudia Gafko[3], Nathalie Montel-Lehry[1], Ulrike Kutay[3], Gagliardi Dominique[2], O'Donohue Marie-Françoise[1], **Gleizes Pierre-Emmanuel[1]**

[1]Centre de Biologie Intégrative, Université de Toulouse, CNRS, Toulouse, France

[2]Institut de Biologie Moléculaire des Plantes, Université de Strasbourg, CNRS, Strasbourg, France

[3]Department of Biology, Institute of Bochemistry, ETH Zürich, Zürich, Switzerland

In human cells, formation of the 18S rRNA 3’ end by endonuclease NOB1 is preceded by the 3’-5’ exonucleolytic removal of most of the ITS1 nucleotides remaining in the 18S-E precursor. This step begins in the nucleus with the action of the exoribonuclease PARN and proceeds in the cytoplasm, where the final steps of 40S subunit maturation occur. To gain insight into this process and how it coordinates with pre-40S particle maturation and nuclear export, we mapped 18S-E pre-rRNA processing at nucleotide resolution by 3'RACE-Seq. Our findings reveal that 3’ adenylation and uridylation are integral to 18S-E pre-rRNA trimming. Adenylation modifies the 3’ end at nearly every position during exonucleolytic trimming, whereas uridylation is largely restricted to nucleotides forming a large central stem-loop. Notably, 3’ uridylation occurs exclusively in the cytoplasm and is catalyzed by TUT4 and TUT7. Knocking down TUT4 and TUT7 delays the processing of the stem-loop and the release of pre-40S ribosome biogenesis factors from the particles. When pre-40S particle maturation is impaired in the cytoplasm, longer uridylated 3’ extensions form and the highly processive exoribonuclease DIS3L2 is recruited. Consistent with a role in quality control, RPS-haploinsufficient lymphoblastoid cells from patients with Diamond-Blackfan anemia syndrome exhibit increased 18S-E pre-rRNA uridylation rates. We conclude from this overview of 18S rRNA 3' end processing in human cells that cytoplasmic 3' uridylation plays a dual role: it facilitates the exonucleolytic processing of a strong secondary structure in the ITS1 under normal conditions, but promotes RNA decay when 40S ribosomal particle maturation is impaired.

11.40

Assigning functions to ribosomal 2’-O-methylations

Anders H. Lund

Biotech Research and Innovation Centre, University of Copenhagen, Denmark

2’-O-methylation (2’Ome) is the most abundant class of modifications found on ribosomal RNA. Using Ribo-meth-seq, we have mapped the pattern of 2’Ome across more than 250 different cell and tissue types, including cancers, stem cell differentiation models, and neurons. We find that about 75% of the approximately 114 2’Ome sites are highly methylated in the ribosome population across all samples analyzed, whereas the remaining around 25% displayed sub-stoichiometric methylation with levels differing between cell and tissue types and between normal and disease tissues. Using classical gain/loss-of-function studies, we demonstrated the roles of a few 2’Ome sites.

In this presentation, data will be presented on 1) ribosome modifications in colorectal cancer and an analysis on how a particular 2’Ome site affects cancer-relevant translation programs, and 2) a single rRNA sequencing approach to determine which 2’Ome appear on the same rRNA molecule in a first attempt to classify ribosome subtypes.

12.05

A key erythroid transcriptional switch coincides with an EpoR/Stat5-driven spike in ribosome biogenesis and protein synthesis

Divya Nair[1], Daniel Hidalgo[1], Ashley Winward[1], Logan Lalonde[1], Colin Sieff[2], Vikram Paralkar[3], **Merav Socolovsky[1]**

[1]MCCB, UMASS Chan Medical School, Worcester, MA, USA

[2]Division of Hematology/Oncology, Boston Children's Hospital and Dana-Farber Cancer Institute, Boston, MA, USA

[3]Department of Medicine, Hematology-Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

Transcriptional switches lead to altered cell fate, but the manner by which this process is coordinated is poorly understood. We study an S-phase dependent transcriptional switch that controls the transition of erythroid progenitors (CFU-e) from a self-renewal stage into a maturational phase (erythroid terminal differentiation, ETD). The CFU-e/ETD switch takes place during an unusually short cell cycle and S phase. Surprisingly, in spite of the shortened cycle, cells maintain their size, suggesting an unusually fast growth in cell biomass. Using $EpoR^{-/-}$ and $Stat5^{-/-}$ fetal livers (FL), we found that EpoR/Stat5 signaling induce a spike in the rates of ribosome biogenesis (Ribi, ~2.5 fold increase), protein synthesis (PS, ~3-5 fold) and growth in cell size (~3 fold) during the narrow developmental window of the CFU-e/ETD switch, which subsided as cells progressed beyond the switch into terminal differentiation. This pattern was supported by multiple lines of evidence, including quantitative rRNA 47S transcription rate using FISH-FLOW, imaging flow cytometry to measure nucleolar size, and a novel approach to polysome profiling using size-exclusion chromatography. Single-cell RNA-sequencing of $EpoR^{-/-}$ and $Stat5^{-/-}$ FLs suggests that EpoR/Stat5 signaling is essential for expression of multiple genes involved in ribosomal RNA (rRNA) transcription and processing and for translation initiation genes. The pattern we describe of Stat5-dependent, brief but intense augmentation in the rates of RiBi, PS and the cell-cycle during a transcriptional switch differs from previously-described patterns of RiBi and PS regulation in stem and progenitor cells and may recur in other instances of rapid transcriptional switches.

12.20

Nucleolar integration and molecular mechanism of ribosomal RNA modification by liquid-liquid phase separation

Koceila Meznad[1], Manisha Deogharia[1], Ludivine Wacheul[2], Christiane Zorbas[2], Denis L.J. Lafontaine[2]**, U. Thomas Meier[1]**

[1]Cell Biology, Albert Einstein College of Medicine, New York, USA

[2]RNA Molecular Biology, Université Libre de Bruxelles, Gosselies, Belgium

Ribosome synthesis depends on cotranscriptional modification of roughly 200 nucleotides in nascent rRNA. This process takes place in the dense fibrillar component (DFC) of the nucleolus and achieves nearly 100% efficiency across ~10 million rRNAs per cell, despite lacking canonical proofreading. Our findings reveal that this accuracy arises because H/ACA and C/D snoRNPs are concentrated around nascent rRNA forming a liquid–liquid phase–separated condensate, the DFC. This enrichment relies on unique intrinsically disordered regions (IDRs) in snoRNP core proteins, composed of lysine–rich nuclear localization signals (NLSs) separated by stretches dotted with glutamic acid residues. We identify similar NLS–IDRs in additional DFC proteins, including the 350–amino–acid C–terminal domain (CTD) of the RNA polymerase I–associated factor PAF49, and in RNA helicase cofactors PINX1, GPATCH4, and UTP23. The NLS–binding chaperone Nopp140—whose own IDR comprises ten

alternatingly charged repeats—binds multiple NLS–IDRs and, in an NLS–dependent manner, coalesces these components into a liquid phase condensate using a “stickers and spacers” interaction model. Glycine–phenylalanine–rich IDRs within snoRNP core proteins and transient snoRNA–rRNA base pairing further boost local component concentration. Together, these weak, multivalent interactions enable snoRNPs to efficiently sample every nucleotide of nascent rRNA for modification and establish the DFC. We further present data showing how dyskeratosis congenita disrupts snoRNA levels and rRNA modification.

Session #12A - Hot Topics and Technological Innovations in Ribosome Assembly

14.00
Advancing Ribosome Research through Direct RNA Sequencing with Oxford Nanopore Technologies
Mark Bruce
Oxford Nanopore

Understanding ribosome biogenesis demands detailed insights into RNA processing, chemical modifications, and structural transitions throughout maturation. Oxford Nanopore Technologies (ONT) offers a unique long-read sequencing platform capable of directly analysing full-length native RNAs, including ribosomal RNAs, without the need for amplification or reverse transcription.

In this talk, we will highlight how recent developments in ONT’s direct RNA sequencing, including improved basecalling, expanded RNA modification detection, and enhanced read length, enable novel investigations into rRNA processing, heterogeneity, and epitranscriptomic regulation. We will present data demonstrating the ability to directly sequence native rRNAs and detection of key modifications such as **pseudouridine** and **2’-O-methylation**.

By integrating real-time analysis with modification-aware basecalling, ONT’s platform provides a powerful toolkit for dissecting ribosome assembly pathways and uncovering previously inaccessible regulatory layers—opening new avenues for both fundamental research and therapeutic discovery

14.25
Investigate nucleolar pre-ribosome assembly in situ
Xiaohan Zhao[1], Herman Fung[2], Yuki Hayashi[3], Sara Cuylen-Häring[3], Julia Mahamid[1,3], Christoph Müller[1]
[1]Molecular and Systematic biology, EMBL, Heidelberg, Germany
[2]Cell and Developmental Biology, UMich, Michigan, USA
[3]Cell biology and biophysics, EMBL, Heidelberg, Germany

Effective ribosome biogenesis is essential for cell growth, proliferation, and differentiation, and makes this process a drug target of great interest. Eukaryotic ribosome biogenesis happens in the nucleolus, a phase-separated condensate. The rDNA transcription occurs at the interface of the fibrillar center (FC) and the dense fibrillar component (DFC), the initial transcripts are then processed and modified, and assembled into pre-ribosomes in the granular component (GC). The integrity of nucleolar structure is closely tied to active RNA polymerase I (Pol I) transcription. When transcription is inhibited, the nucleoli become spherical and FC and DFC migrate to the periphery of the GC, forming distinct structures known as ‘nucleolar caps’. What drives this condensation process and how this nucleoli deformation affects pre-ribosome assembly is still unknown. Our interest is to

investigate the pre-ribosome assembly in GC and how it changes after nucleolar cap formation. Using cryo-ET and on-lamella CLEM, we visualized the nucleolar molecular landscape in situ and found that the nucleolar reorganization induced by the transcription inhibitor Actinomycin D (ActD) changed the distribution of pre-60S ribosomes. Specifically, the absence of Rixosome binding states provides a mechanistic explanation for the stalling of 32S rRNA processing. More notably, we observed a marked increase in the abundance of late pre-60S ribosomes, suggesting a defect in the transport of pre-60S ribosomes from the nucleolus to the nucleoplasm. In addition, we identified an extra density near the 28S rRNA in ActD-treated samples, which could also play a role in the accumulation of late pre-60S ribosomes.

14.40

Visualization of translation reorganization upon persistent ribosome collision stress in mammalian cells

Juliette Fedry
MRC LMB, Cambridge, UK

Aberrantly slow ribosomes incur collisions, a sentinel of stress that triggers quality control, signaling, and translation attenuation.
Although each collision response has been studied in isolation, the net consequences of their collective actions in reshaping translation in cells is poorly understood. Here, we apply cryoelectron tomography to visualize the translation machinery in mammalian cells during persistent collision stress. We find that polysomes are compressed, with up to 30% of ribosomes in helical polysomes or collided disomes, some of which are bound to the stress effector GCN1. The native collision interface extends beyond the in vitro-characterized 40S and includes the L1 stalk and eEF2, possibly contributing to translocation inhibition. The accumulation of unresolved tRNA-bound 80S and 60S and aberrant 40S configurations identifies potentially limiting steps in collision responses. Our work provides a global view of the translation machinery in response to persistent collisions and a framework for quantitative analysis of translation dynamics in situ.

15.05

Towards autonomous cell-free biogenesis of the central dogma of biology

Shirley Daube[1], Noa Avidan[1], Yuval Oren[1], Matthaeus Schwarz-Schilling[1], Ilan Cohen[1], Michael Levy[1], Yoshihiro Shimizu[2], Roy Bar-Ziv[1]
[1]Chemical and Biological Physics, Weizmann Institute, Rehovot, Israel
[2]Laboratory for Cell-Free Protein Synthesis, RIKEN Center for Biosystems Dynamics Research, Suita, Osaka, Japan

Establishing a cell-free autnomous minimal system that can support self regeneration of the entire protein translation machinery is a major goal, opening numerous possibilities, from the deciphering of fundemental principles of complex biological systems, to the screening of novel antibiotics targetting ribosome assembly, and the engineering of ribosomes and translation factors with expanded functions. Dozens of ribosomal proteins, ribosome assembly factors, translation factors and amino acyl tRNA synthetases should be simultanously self-synthesized in a functional form, challanged by the limited capacity of the minimal cell-free system and by the ability to demonstrate active participation of the nascent proteins in their own synthesis. We developed a surface methodology that facilitates simultanous cell-free synthesis of dozens of proteins in miniaturized silicon compartments, providing favored conditions for efficient synthesis coupled to assembly.

Genes coding for the proteins of interest are immobilized on the surface at high density as DNA brushes, serving as a focal point for the gene expression machinery and the subsequent accumulation of nascent protein products. Genetically encoded peptide tags facilitate capture of nascent proteins on the surrounding surface for sensitive detection, spatially resolved from the machinery that created them. Using sensitive fluorescence imaging and multiplexed screening of gene combinations we have already made progress, demonstrating the assembly pathway of the E. coli and S. Aureus small ribosomal subunits; the E. coli large ribosomal subunit; and the biogenesis of E. coli's thirty translation factors, paving the way for a collective self-regenerated artificial cell as a simple model for complex cellular systems.

15.20

Mechanistic Insights into Non-AUG Codon Stringency

Ximena Zottig[1,2], Chun-Ying Huang[2], Nikolaus Grigorieff[1,2], Andrei Korostelev[2]
[1]RNA Therapeutics Institute, Howard Hughes Medical Institute, Worcester, USA
[2]RNA Therapeutics Institute, University of Massachusetts Chan Medical School, Worcester, USA

Non-AUG translation initiation expands the mammalian proteome by generating alternative proteoforms from the same transcript. The balance between AUG and non-AUG initiation is context-dependent and critical for normal cellular function, with imbalances linked to diseases such as cancer and neurodegeneration. Although regulation by specific initiation factors has been proposed, the molecular mechanisms governing non-AUG initiation remain poorly understood, largely due to the difficulty of visualizing translation in cellular environments. In this work, we present a mechanism of non-AUG regulation by directly visualizing a non-AUG regulator protein (nARP) in action within cellular lysate. Using cryo-EM and 2D Template Matching (2DTM), we resolved 48S pre-initiation complexes at near-atomic resolution (2.6–3.0 Å). The structures reveal that nARP stabilizes the scanning conformation of the initiation complex, leading to bypass of non-AUG codons and promoting start codon selection specifically at AUG sites. We show nARP's molecular interactions with tRNA, eIF2, and eIF1A, in the 48S complex, along with details of the scanning and arresting conformations, including effects on codon recognition at the P site, all within cellular-like environments. We also quantify codon stringency and show that nARP alters the translation of c-myc isoforms derived from an upstream non-AUG open reading frame. This shift in isoform usage underscores the need for precise regulation of non-AUG initiation, particularly given nARP's emerging role as an oncogene Together, we present near-atomic resolution structures of 48S initiation complexes formed in a cellular context and provide a molecular framework for understanding how non-AUG initiation is regulated in a native-like context.

Session #12B Hot Topics and Technological Innovations in Ribosome Assembly

16.00

Rebecca Thompson

Thermofisher

16.30

Ribosomal protein paralogues in germline development

Katarina Grobicki[1], **Felipe Karam Teixeira[1,2]**
[1] University of Cambridge, Department of Genetics, United Kingdom
[2] University of Cambridge, Department of Physiology, Development and Neuroscience, United Kingdom

Many ribosomal protein genes have been duplicated during evolution, leading to co-existing paralogous genes within species, the implications of which are only starting to be determined. In *Drosophila melanogaster*, one paralogous ribosomal protein gene is usually expressed ubiquitously and is essential for translation; mutations in these genes are homozygous lethal and heterozygotes usually display the *minute* phenotype. Less is known about their paralogues, however several show tissue-specificity and many have enriched expression in the germline. Following up on results obtained through a genome-wide RNAi screen, we performed systematic CRISPR mutagenesis of ribosomal protein paralogue genes. Our results demonstrate the vast majority of ribosomal protein paralogues expressed in a tissue-specific manner are not required for viability or fertility, while identifying one essential for female fertility, RpS5b. Gene swap experiments revealed that RpS5b is functionally interchangeable with its canonical paralogue, suggesting that the retention of both paralogues is due to Duplication-Degeneration-Complementation rather than neofuntionalisation. Nonetheless, by studying the phenotype induced by loss of RpS5b, we established that ribosome biogenesis stress in germ cells is sufficient to trigger activation of the Tor pathway, leading to remodelling of germline metabolism. This germline stress response is transduced to the neighbouring somatic epithelium, leading to overgrowth, disorganisation, incomplete Notch activation, and non-autonomous activation of Tor kinase. In conjunction, alterations halt oogenesis and causes complete female sterility. Altogether, our analyses provide evidence against functionally-specialised ribosomes based on ribosomal protein paralogues, while uncovering an essential inter-tissue mechanism coordinating the control of growth, metabolism and development.

Poster Abstracts

Poster Number: P01

Regulation of pre-rRNA processing by the exoribonuclease XRN2

Savannah Seely, Jacob Gordon, Robin Stanley
Molecular and Cellular Biology Branch, National Institute of Environmental Health Sciences, Durham, USA

Across all domains of life, protein synthesis is carried out by ribosomes, made up of 60S and 40S subunits. In mammals, ribosome biogenesis is a hierarchical process that begins in the nucleolus and undergoes regulated steps of RNA folding, RNA processing and integration of ribosomal proteins. Regulation of ribosome biogenesis is critical to the cell, as hyperactivation of ribosome synthesis is a hallmark of cancer initiation and progression. Mutations in ribosomal proteins and assembly factors can also lead to ribosomopathies, which are developmental disorders characterized by dysregulation of ribosome synthesis. A critical aspect of ribosome assembly is processing of the external and internal transcribed sequences from the pre-rRNA. These spacer regions are removed by ribonucleases to form the mature rRNAs. Removal of the ITS2 (internal transcribed spacer 2) is initiated by endonucleolytic cleavage followed by 5' exonucleolytic decay by XRN2 and 3' exonucleolytic decay by the nuclear exosome. XRN2 is the cells major nuclear 5'-3' exoribonuclease, degrading numerous nuclear RNA targets, interacting with a myriad of associating factors in the nucleus and nucleolus. A diverse profile of substrates, apparent lack of target sequence specificity, and the enigmatic relationship between XRN2 and its associating factors has rendered its mechanism elusive. We are employing a complement of biochemical assays and structural biology, to investigate how XRN2 regulates ITS2 processing.

Poster Number: P02

Targeting RPA194 unmasks mutant p53 for ubiquitination and suppresses pancreatic tumor growth

Mudassier Ahmad, Sahir Alvi, Haider Ahsan, Alfia Bilal, Dae Kim, Murali Yallapu, Subhash Chauhan, **Bilal Hafeez**
Medicine and Oncology, School of Medicine, UTRGV, McAllen TX, USA

Human pancreatic cancer (PanCa) is one of the most aggressive malignancies, with very limited therapeutic options that typically involve surgery, chemotherapy and targeted therapy. Thus, developing new treatment strategies is an urgent required for PanCa treatment. In this study, we explored the therapeutic potential of BMH-21, a pharmacological inhibitor of RNA polymerase I (Pol I), against PanCa by uncovering a novel molecular mechanism involving interaction of RPA194 mediated ubiquitination of mutant p53 without affecting wild-type p53. Our key findings include i) BMH-21 selectively induces apoptosis and cell growth inhibition of PanCa cells with no effect on normal human pancreatic ductal epithelial cells (HPDEC), ii) degrades RPA194 protein, iii) inhibits recruitment of both RPA194 and RPA135 on rDNA to suppress pre-rRNA synthesis, iv) RPA194 physically interacts with p53 and BMH-21-induced degradation of RPA194 selectively exposes truncated and mutated p53 for ubiquitination with no effect on wild-type p53 in PanCa cells and v) BMH-21 treatment significantly reduces the growth of orthotopic xenograft pancreatic tumors in athymic nude mice with no observe toxicity. Altogether, these findings suggest that BMH-21 is a promising, non-toxic therapeutic agent for PanCa patients with aberrant ribosome biogenesis and mutant p53, offering a potential new avenue for targeted treatment.

Poster Number: P03

Genome-wide CRISPR screen identifies regulators of POLR1A turnover

Sheetanshu Saproo, Jaime A Espinoza, Dimitris C Kanellis, Jiri Bartek, Mikael S Lindström
[1]Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [2]SciLifeLab, Stockholm, Sweden

RNA polymerase I (Pol I) transcribes ribosomal DNA to precursor ribosomal RNA which is further matured prior to its integration in the functional ribosome. The subunits of Pol I enzyme along with the Pol I-specific transcription initiation factors are frequently dysregulated in cancer, favouring increased protein synthesis and tumor growth. Targeting Pol I is thus a therapeutic strategy and has primed the development of small-molecules such as BMH-21 that disrupts Pol I transcription through degradation of its catalytic subunit, POLR1A. To identify regulators of POLR1A protein levels, we conducted a genome-wide CRISPR screen. We transduced colon carcinoma RKO cells expressing Cas9 protein with the Brunello CRISPR library (Doench et al, 2016) targeting 20,000 genes with 4 sgRNAs per gene. The transduced cells were treated with BMH-21 or DMSO vehicle, fixed and FACS-sorted based on intracellular POLR1A protein levels. Our screen revealed multiple putative pathways influencing POLR1A turnover. We confirmed the hit TAF1C as a critical factor and additional hits included Pol I-specific SL1 subunits TBP, TAF1A, TAF1B, and TAF1D as required for BMH-21 to degrade POLR1A. The initiation of Pol I transcription requires recognition and binding of rDNA core promoter by SL1 complex, composed of TBP and TBP-associated factors TAF1A-D. Our findings provide new insights into Pol I regulation highlighting numerous enzymes involved in post translational modifications as well as metabolic pathways. Furthermore, our data identified various oncogenes previously not known to have impact on POLR1A protein levels, providing novel insights into the oncogene-mediated regulation of Pol I transcription.

Poster Number: P04

Investigating the biogenesis of snoRNA-retaining transcripts

Federico Zacchini[1], Giulia Venturi[2], Guglielmo Rambaldelli[2], Sidra Asghar[2], Chiara Barozzi[2], Lorenzo Montanaro[1,2]
[1]Departmental Program in Laboratory Medicine, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, [2]Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum - University of Bologna, Bologna, Italy

Small nucleolar RNA-retaining transcripts (snoRTs) are mRNA isoforms that retain introns containing snoRNA sequences. These abundant and heterogeneous transcripts exist as full-length unprocessed transcripts and shorter processed variants. Although snoRTs have been observed under diverse experimental conditions, their biogenesis remains poorly understood. To address this, we first explored the interaction between H/ACA box snoRTs and the pseudouridine synthase dyskerin (DKC1) using RNA immunoprecipitation, revealing that several snoRTs, especially the shorter forms, bind DKC1. To investigate whether this interaction is mediated by the retained snoRNA sequence, we analyzed the EIF4A1 snoRT, which contains the SNORA67 sequence, in SNORA67 knockout cells (KO). Loss of SNORA67 significantly reduced binding to DKC1, suggesting that the snoRNA sequence is crucial for this interaction. Moreover, the SNORA67 KO selectively decreased the levels of the shorter processed variants, further supporting its role in snoRT maturation. Given that snoRTs are cytoplasmic mRNAs, we then investigated their involvement in translation. In fact, polysome profiling showed that full-length snoRT transcripts associate with polysomes, indicating active translation, while the shorter variant co-sediment mainly with free 40S ribosomal subunits. Because retained introns often harbor premature stop codons, we explored whether nonsense-mediated decay (NMD)

or other RNA decay mechanisms contribute to short variant generation. Our data suggest that translation is required for snoRT processing, but the precise role of NMD or alternative decay pathways remains unclear.
Overall, our findings suggest that different pathways are involved in snoRTs biogenesis and processing, warranting further investigation into the underlying molecular mechanisms.

Poster Number: P05

RPL8 amplification reveals a novel vulnerability in High-Grade Serous Ovarian Cancer

Kamil Filipek[1, 2], Daniela Pollutri[1, 2], Lorenza Bacci[1, 2], Marianna Penzo[1, 2]
[1]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy, [2]Center for Applied Biomedical Research (CRBA), University of Bologna, Bologna, Italy

Ovarian cancer (OC) is one of the most lethal gynecological malignancies, with high-grade serous OC (HGSOC) representing the most prevalent and aggressive subtype. HGSOC is characterized by extensive genomic instability and frequent TP53 mutations, while alterations in ribosome biogenesis are increasingly recognized as contributors to tumor progression. Through analysis of a public HGSOC dataset, we identified frequent amplification (30%) of Ribosomal Protein L8 (RPL8/uL2), a component of the large ribosomal subunit essential for peptidyl transferase activity and ribosome assembly. RPL8 expression levels correlated with gene copy number alterations. To investigate its functional relevance, we developed HGSOC cellular models with modulated RPL8 expression. Altered RPL8 levels significantly impacted proliferation, invasion, clonogenic potential, and response to chemotherapeutic agents. These phenotypic changes were accompanied by disruptions in ribosome biogenesis, although global protein synthesis remained largely unaffected under basal conditions. Notably, under stress conditions, RPL8 overexpression affected cell adhesion and suppressed global translation through increased phosphorylation of eIF2α, suggesting a potential role in the integrated stress response (ISR). These results point to an extra-ribosomal function of RPL8 in modulating cellular adaptation to stress. Taken together, our findings propose that RPL8 amplification may support HGSOC development by influencing both ribosome-dependent and independent processes. RPL8 may thus represent a novel vulnerability in HGSOC and a candidate biomarker for patient stratification in the context of precision medicine.

Poster Number: P06

Structural disruption of EFL1 communication networks underlie Shwachman-Diamond Syndrome pathogenesis

Abril Gijsbers[1], Jonathan Zuñiga Hernandez[2], Rohit Jain[3], Martin Gonzalez Andrade[1], Erik Farquhar[3], Mark Chance[3], **Nuria Sanchez Puig[2]**
[1]Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Ciudad de Mexico, Mexico, [2]Instituto de Quimica, Universidad Nacional Autonoma de Mexico, Ciudad de Mexico, Mexico, [3]Centre for Synchrotron Biosciences, Case Western Reserve University, Cleveland, Ohio, USA

Shwachman-Diamond Syndrome (SDS) is a rare genetic disorder associated with mutations in the ribosomal maturation factors EFL1 and SBDS, which impair eIF6 release and disrupt ribosome assembly. To investigate the structural and functional consequences of SDS- associated mutations, we focused on the yeast-equivalent pathogenic variant Efl1 R0186Q. Using hydroxyl radical footprinting (XFP), molecular dynamics simulations (MDS) and functional assays, we examined how this mutation affects protein conformational dynamics. Despite only subtle changes in global stability and fold, XFP revealed that the mutation – located in domain IV - induces widespread alterations in

solvent accessibility, extending even to domain I located 80 Å away. This suggests a breakdown in intramolecular communication and a loss of coordinated domain motions. MDS further confirmed reduced flexibility and a rigidified mutant ensemble. Strikingly, a second-site compensatory mutation (P151L) within a b-sheet connecting the GTP-binding pocket to domain IV rescued yeast growth defects and restored nuclear localisation of Tif6 (yeast eIF6), indicating that this second mutation reestablishes disrupted allosteric communication.

Our findings reveal a critical long-range structural pathway in EFL1 that links the nucleotide-binding status to eIF6 release. Disruption of this network underlies SDS pathology, while its targeted modulation offers a potential strategy to restore mutant function. This study provides mechanistic insights into EFL1-linked SDS and highlights conformational rewiring as a potential therapeutic avenue.

Poster Number: P07

Exploration of Ribosome Structure-function relationships by systematic introduction of Pseudouridines and 2'O-methyl groups at novel ribonucleotides

Vinithra Iyer, Joshua Bugis, Rotem Morag, Schraga Schwartz
Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Ribosomes are essential molecular machines that mediate mRNA translation. Their rRNAs, which form the structural and functional core, contain diverse evolutionarily conserved modifications that contribute to ribosome biogenesis, pre-rRNA processing, and translation. Pseudouridylation and 2'-O-methylation are the most abundant rRNA modifications, introduced via small nucleolar RNAs (snoRNAs) that guide the modification machinery to specific target sites. However, only a subset of rRNA sites are modified, and the functional significance of individual modifications remains unclear. Our study aims to explore why only certain target sites undergo modification and to systematically dissect the properties conferred by modified rRNA sites. To achieve this, we introduced novel pseudouridines employing a pool of 6000 H/ACA snoRNAs targeting all available uridines in yeast rRNA. To assess their impact, we performed an expedited in vitro evolution experiment, where yeast cells, each expressing a different snoRNA, competed in a pooled culture. We observed widespread fitness changes, suggesting gain- and loss-of-function phenotypes. We are currently validating top candidates to examine their effects on yeast growth, bulk translation, and translation fidelity using biochemical assays, NGS, and structural analyses. This study will provide insights into the constraints guiding snoRNA evolution and into how rRNA modifications influence ribosome structure and function.

Poster Number: P08

Ribosomal RNA biogenesis factors as therapeutic targets in myeloid leukaemia

Kim-Phuong To [1], Bertina Dragunaite[1], Quang-Anh Hoang[1], Pedro Casado-Izquierdo[1], Emilie Allard[1], Tommy Shield[1], Keith Woodley[1], Alex Schurer[2], Michael Kharas[2], **Diu TT Nguyen[1]**, Mandy Chan[2], Thomas Rohwetter[2], Anthony Velleca[2], Ly Vu[2, 3], Anna Rio-Machin[1], Pedro Cuttilas [1], Paolo Gallipoli[1], Ben Towler[3], Faraz Mardakheh[1, 4]
[1]Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, [2]Pharmacology programme, Memorial Sloan Kettering Cancer Centre, New York, USA, [3]Terry Fox Laboratory, University of British Columbia, Vancouver, Canada, [4]Biochemistry, University of Oxford, Oxford, United Kingdom, [5]Biochemistry and Biomedicine, University of Sussex, Sussex, United Kingdom

Ribosome biogenesis is often deregulated in cancer, making it an attractive therapeutic target. Our proteomics analysis of poorest risk acute myeloid leukaemia (AML) patients revealed majority of rRNA biogenesis factors are overexpressed compared to healthy counterparts, with a higher extent in undifferentiated vs differentiated AML subtypes. Among these factors, MYB-Binding Protein 1A (MYBBP1A) was a top hit in our previous in vivo drop-out screen for genes essential for AML stem cell survival. However, MYBBP1A's role and molecular function in AML is unclear.

Here, we found MYBBP1A depletion significantly inhibited cell proliferation, induced apoptosis, and increased myeloid differentiation in multiple human and mouse AML cell lines and primary AML patient cells, and delayed leukaemogenesis in vivo. Furthermore, MYBBP1A knockdown in normal blood stem cells decreased 50% clonogenicity, with no effects on apoptosis, suggesting a therapeutic index.

Transcriptomics analysis revealed that MYBBP1A depletion downregulated mTORC1 signalling and unfolded protein response pathways, whereas innate immune response programmes are upregulated. Mechanistically, our IF and co-immunoprecipitation analysis showed MYBBP1A localised in nucleolus and interacted with multiple ribosomal proteins and ribosome biogenesis factors. MYBBP1A loss impaired fibrillarin (FBL) recruitment into nucleolus. Nascent RNA captures revealed that premature rRNA synthesis and processing was modulated by MYBBP1A knockdown. Interestingly, proteomics analyses identified little change in the proteome but downregulation of the whole MCM complex. Concordantly, we found G1/S cell cycle arrest and increased DNA damage in MYBBP1A-deficient cells. Overall, we demonstrated that MYBBP1A controls leukaemia cell survival via regulating rRNA biogenesis, presenting a novel therapeutic target in AML.

Poster Number: P09

Dysregulation of the RNA Acetylome and Cytidine Acetyltransferase Drives Aberrant Ribosome Biogenesis in Human Liver Cancer

Mengjie Wu[1,2], Chun-Ming Wong[1,2]

[1]Li Ka Shing Faculty of Medicine, Department of Pathology, The University of Hong Kong, Hong Kong SAR, China, [2]State Key Lab of Liver Research, The University of Hong Kong, Hong Kong SAR, China

Ribosome epitranscriptomics represents an emerging frontier in gene expression regulation, offering new insights into how chemical modifications on RNA fine-tune ribosomal behaviours and modulate the translatome. This layer of regulation holds significant promise for the development of innovative cancer therapies. Beyond the well-characterized transcriptional regulation mediated by ε-acetylation of histone tails, the functional implications of RNA cytidine N4-acetylation (ac4C) in regulating the flow of genetic information from mRNA to protein remain largely unexplored.

In this study, we employed single-nucleotide-resolution RNA N4-acetylome profiling, optimized ribosome footprinting (Opti-Ribo-seq), and atomic-resolution cryo-electron microscopy (cryo-EM) resources to systematically dissect the role of the NAT10-mediated RNA acetylation axis in liver cancer.

Our findings reveal that dysregulation of NAT10 and its associated RNA acetylation landscape profoundly alters ribosome dynamics and translational output—globally, preferentially, and transcript-specifically—thereby contributing to hepatocarcinogenesis.

Crucially, we developed a novel pharmacological strategy to modulate ribosome RNA acetylation events, effectively reprogramming the ribosome acetylome. This intervention significantly

suppressed liver tumor growth in vivo, highlighting a promising therapeutic avenue centered on the ribosome epitranscriptome.

Poster Number: P10

Early Transcriptional Responses to Pol I Inhibitors. Lessons to learn

Xiyuan Qi[1], Nadine Hein[2], Ross Hannan[2], **Konstantin Panov[1, 3]**

[1]School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom, [2]John Curtin School of Medical Research, Australian National University, Canberra, Australia, [3]Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, United Kingdom

Ribosome biogenesis is a major determinant of cell growth and proliferation, with RNA Polymerase I (Pol I)-mediated transcription of ribosomal RNA (rRNA) representing a key regulatory step. rRNA transcription occurs within the nucleolus, a membrane-less nuclear compartment that plays a critical role in chromatin organization. Pol I activity is essential for nucleolar formation and integrity, and its hyperactivation in cancer (caused by MYC activation and/or loss of tumour suppressors) may induce chromatin changes that support malignant phenotypes. These alterations make cancer cells “addicted” to elevated Pol I transcription.

This has made Pol I an attractive target for cancer therapy, leading to the development of selective inhibitors such as first-in-class CX-5461, BMH-21, and the second-generation compound PMR-116. These compounds are known to induce nucleolar stress pathways and, in some cases, activate DNA damage response, causing cell cycle arrest and cell death.

However, surprisingly little is known about the immediate transcriptional consequences of Pol I inhibition. We therefore performed time-resolved transcriptome profiling at early timepoints (1, 4, and 12 hours) following treatment with physiologically relevant concentrations of CX-5461, BMH-21, and PMR-116. We observed substantial early transcriptional changes independent of classical stress pathways. Gene ontology analysis revealed shared changes in chromatin organization and nuclear structure pathways across all three inhibitors. We propose that rapid nucleolar morphological changes induced by Pol I inhibition trigger transcriptional reprogramming that precedes classical stress responses. This hypothesis and the associated transcriptional changes induced by Pol I inhibitors will be discussed in detail.

Poster Number: P11

The effect of cellular signalling and nutrient availability on pre-rRNA processing in human cells

Isabella R Lawrence[1, 2], Susan J Baserga[2], Claudia Schneider[1]

[1]Biosciences, Newcastle University, Newcastle Upon Tyne, United Kingdom, [2]Molecular Biophysics and Biochemistry, Yale University, New Haven, USA

Ribosome biogenesis is a major consumer of cellular energy and therefore tightly regulated in response to external and internal growth stimuli. Processing of the pre-ribosomal (r)RNA internal transcribed spacer (ITS1) can occur through two distinct pathways: the proximal (cleavage at site 2a/E) and distal (cleavage at site 2) pathways, separating the small (SSU) and large (LSU) subunit rRNA precursors. Prior studies have suggested that ITS1 pathway choice and stalling is a key regulatory step in modulating the ribosome biogenesis response to nutrient signalling in yeast and humans (Kos-Braun 2017, Iadevaia 2012, Pan 2022). However, the mechanisms that link these pre-rRNA processing choices to nutrient signalling remain unclear in human cells.

We used an array of approaches to simulate nutrient stress, including treatment with rapamycin (mTORC1 inhibitor) or TBB (CK2 inhibitor) and growth medium lacking glucose or L-glutamine. We observed that these treatments cause accumulation of distal pathway pre-rRNAs and inhibit both SSU and LSU processing in HEK293 cells. Surprisingly, when we performed a refeed following glucose depletion, we detected a switch to the proximal pathway. We are currently investigating whether NKRF, previously identified as a thermosensor during heat stress (Coccia 2017), is also a key player in nutrient-dependent regulation of ITS1 processing. Finally, we are investigating whether later stage pre-rRNAs are degraded during the nutrient stress response as previously reported in yeast (Kos-Braun 2017). Determining how nutrient signalling in the cytoplasm affects pre-rRNA processing in the nucleolus will expand our understanding of how ribosome biogenesis and growth control are intertwined.

Poster Number: P12

Abstract can be found in Speaker Abstracts, Monday 8th September at 17.10

Poster Number: P13

Decoding the role of the non-essential yeast ribosomal protein eL22 during cytoplasmic maturation of 60S ribosomal subunits

Sara Martín-Villanueva[1, 2], José Fernández-Fernández[1, 2], Carla Verónica Galmozzi[1, 2], Cristina Arjona-Torres[1], Taylor N. Ayers[3], John L. Woolford[3], Jesús de la Cruz[1, 2]

[1]Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain, [2]Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Seville, Spain, [3]Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, USA

Ribosome biogenesis is an essential cellular process involving the coordinated processing of ribosomal RNA precursors and assembly of ribosomal proteins (r-proteins). In Saccharomyces cerevisiae, this complex pathway involves around 300 trans-acting factors and 79 r-proteins. Although many r-proteins have well-defined roles, the function of some non-essential proteins remains unclear. This study focuses on the r-protein of the large subunit eL22. It has been reported that eL22 plays an important but still undefined role not only during biogenesis of large r-subunits, but also during splicing, replicative lifespan, and meiosis; notably, human eL22 is the second r-protein more dysregulated during cancer.

This study provides novel insights into the specific contribution of eL22 during ribosome synthesis. Absence of eL22 causes mild growth defects, especially at low temperatures, and results in a shortage of 60S subunits and a mild nuclear export defect of pre-60S particles. Moreover, eL22 is essential for efficient processing of 27S pre-rRNAs. Most importantly, our data suggest that proper assembly of eL22 is required for the function of several ribosome assembly factors involved in late cytoplasmic maturation of the 60S r-subunit.

Poster Number: P14

Oxaliplatin causes RNA damage by multiple mechanisms

Zornitsa Kotopanova[1], Michael Braun[2], Mark Saunders[2], John Knight[1]

[1]Division of Cancer Sciences, University of Manchester, Manchester, United Kingdom, [2]The Christie Hospital, Manchester, United Kingdom

The three clinically approved platinum compounds are used to treat different tumour types. For example, oxaliplatin is used to treat colorectal cancer (CRC), a tumour type where cisplatin and

carboplatin bring no benefit. This suggests an underlying molecular difference in how these compounds work. Emerging evidence for oxaliplatin has implicated RNA, rather than DNA, as its major target. Here we aim to understand the molecular effects of oxaliplatin on RNA in the HCT116 cell line.

Using a qPCR method based on primer extension analysis we analyse platinum adducts present in specific highly abundant RNAs, of which 18S rRNA exhibits dose- and time-dependent oxaliplatin damage. By contrast, significant 18S rRNA damage is not observed for cisplatin- or carboplatin-treated cells. In parallel we have developed and applied AquIRE (Aqueous Identification of RNA Elements) to demonstrate oxaliplatin adduct formation on total RNA. Finally, using AquIRE we have identified a previously unknown RNA damage modality for oxaliplatin, whereby the drug induces RNA-protein crosslinks. This type of damage is more broadly explored by mass spectrometry revealing enrichment of cysteine-type endopeptidase inhibitors and proteins involved in the ribosome biogenesis pathway in RNA samples extracted from oxaliplatin-treated cells, which is further validated by AquIRE and qPCR.

Poster Number: P15

Remodelling of Rea1 linker domain drives the removal of assembly factors from pre-ribosomal particles

Johan Busselez[1,2], Geraldine Koenig[1,2], Carine Dominique[3], Torben Klos[1,2], **Deepika Velayudhan[1,2]**, Piotr Sosnowski[1,2], Nils Marechal[1,2], Corinne Crucifix[1,2], Anthony Henras[3], Helgo Schmidt[1,2], Benjamin Albert[3], Yves Henry[3]

[1]Integrated Structural Biology Department, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France, [2]Université de Strasbourg, Illkirch, France, [3]Molecular, Cellular and Developmental Biology Unit (MCD), Centre de Biologie Intégrative (CBI), CNRS, Université de Toulouse, Toulouse, France

The ribosome maturation factor Rea1 (or Midasin) catalyses the removal of assembly factors from large ribosomal subunit precursors and promotes their export from the nucleus to the cytosol. Rea1 consists of nearly 5000 amino acid residues and belongs to the AAA+ protein family. It consists of a ring of six AAA+ domains from which the ≈1700 amino acid residue linker emerges that is subdivided into stem, middle and top domains. A flexible and unstructured D/E rich region connects the linker top to a MIDAS (metal ion dependent adhesion site) domain, which is able to bind the assembly factor substrates. Despite its key importance for ribosome maturation, the mechanism driving assembly factor removal by Rea1 is still poorly understood. Here we demonstrate that the Rea1 linker is essential for assembly factor removal. It rotates and swings towards the AAA+ ring following a complex remodelling scheme involving nucleotide independent as well as nucleotide dependent steps. ATP hydrolysis is required to engage the linker with the AAA+ ring and ultimately with the AAA+ ring docked MIDAS domain. The interaction between the linker top and the MIDAS domain allows direct force transmission for assembly factor removal.

Poster Number: P16

Nucleolar NOC1/CEBPZ protein controls rRNA Maturation and Genome Integrity: A Novel Nucleolar Surveillance Link with MYC and p53 controlling Apoptosis

Shivani Bajaj[1], Valeria Manara[1], Andrea Vutera Cuda[1], Marianna Penzo[2,3], Guglielmo Rambaldelli[2,3], Paola Bellosta[1,4]

[1]CIBIO, University of Trento, Trento, Italy, [2]DIMEC , University of Bologna, Bologna, Italy, [3]IRCCS-

Policlinico Sant'Orsola, University of Bologna, Bologna, Italy, [4]Medicine, New York University Grossman School of Medicine, New York, USA

Ribosome biogenesis is critical for cellular homeostasis, and its disruption is increasingly linked to cancer. Among nucleolar proteins involved, NOC1, a C/EBP transcription factor, regulates rRNA processing and 60S subunit maturation via heterodimerization with NOC2 and NOC3. Beyond this canonical role, we propose that NOC1 also modulates RNA-DNA hybrids (R-loops), whose dysregulation promotes genomic instability.

Using Drosophila, we show that NOC1 downregulation impairs pre-rRNA processing, triggering nucleolar stress, DNA damage, and a unique nucleolar surveillance response marked by MYC and p53 activation. This response is mediated by Xrp1, a stress-induced C/EBP factor that promotes MYC and p53 expression, in a delicate balance that controls apoptosis. We further demonstrate that defective expression of NOC1 leads to R-loop accumulation, linking ribosome stress to transcription-associated DNA lesions in the nucleolus.

We show that this mechanism is conserved for human CEBPZ, highlighting a novel conserved stress-response axis linking ribosome function and disruption to genome protection. Moreover, human homologs, CEBPZ, NOC2L, and NOC3L, are differentially expressed across tumors, and their expression correlates with genes fundamental in the regulation of ribosomal stress and genomic instability. Our data highlight a novel potential dual role of these factors in rRNA processing and R-loop resolution, suggesting their context-dependent function as tumor suppressors or as regulators of genome instability.

Poster Number: P17

Sequencing translationally-active tRNAs under stress: An insight into ribosomal associated tRNAs

Hasan Yilmaz[1,*], Mie Monti[2,*], Alessia Del Piano[1], Michele Arnoldi[1], Isabelle Bonomo[1], Laia Llovera[2], Eva Maria Novoa[2,3,4], Massimiliano Clamer[1]
[1]IMMAGINA Biotechnology, Pergine Valsugana (TN), Italy, [2]Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, [3]Universitat Pompeu Fabra (UPF), Barcelona, Spain, [4]ICREA, Barcelona, Spain

Living organisms can adapt to environmental changes by regulating gene expression at multiple levels, including protein synthesis. During translation, tRNA molecules deliver specific amino acids to the ribosome in a manner coordinated with the sequence encoded in the mRNA. However, the precise role of tRNAs- and their modifications- in modulating translation remains poorly understood due to lack of methods to map ribosome selected tRNAs. Traditional analyses of tRNA dynamics often focus on total cellular tRNAs, which can mask subtle, stress-induced changes. To address this challenge, we developed a method to isolate ribosome-associated tRNAs from actively translating ribosomes and applied direct nanopore sequencing to compare these functionally active tRNAs with the total cellular pool in immortalized human cells. This approach combines antibody- and tag-free pull-down of active ribosomes with multiplexed sequencing, enabling comprehensive and comparative measurements of tRNA abundance, charging, and modifications in both the total and ribosome-embedded RNA pools.

Poster Number: P18

***RPS26*-haploinsufficient patient-derived iPSCs exhibit impaired processing of ribosomal RNA and upregulate ribosome biogenesis factors**

Teun Slijkerman[1, 3], Marien van der Stel[1, 2], Marie-Françoise O'Donohue[4], Pierre-Emmanuel Gleizes[4], Marieke von Lindern[1], Sjaak Philipsen[2], Chantal Clark[1, 3], Marije Bartels[3], Emile van den Akker[1]
[1]Landsteiner laboratory, Sanquin Research, Amsterdam, The Netherlands, [2]Department of Cell Biology, Erasmus MC, Rotterdam, The Netherlands, [3]Centre for Benign Hematology, Thrombosis and Hemostasis, University Medical Centre Utrecht, Utrecht, The Netherlands, [4]Molecular, Cellular and Developmental biology department, University of Toulouse, Toulouse, France

Diamond-Blackfan Anemia Syndrome (DBAS) is characterized by hypoplastic anemia, congenital malformations and cancer predisposition, and is predominantly caused by ribosomal protein gene mutations, including *RPS26* in ~10% of patients. Pathogenic mechanisms underlying *RPS26*-DBAS are largely unknown. To address this, we established induced pluripotent stem cell (iPSC) models derived from patients with *RPS26* mutations to study defects in ribosome biogenesis and hematopoiesis.

We generated iPSC lines from a patient with *RPS26*-DBAS from the Dutch registry. Erythroblasts were reprogrammed using the Cytotune-Sendai-IPS2.0 kit. Using CRISPR/Cas9-mediated homology-directed repair, we corrected the *RPS26* mutation in the patient iPSC lines. We compared control, mutant and corrected lines through differentiation into embryoid bodies (EB), hematopoietic organoids, and hematopoietic progenitors. All lines were characterized by a multi-omics approach.

Whole genome sequencing confirmed successful correction of the *RPS26* mutation. Mutant lines exhibited disaggregation of iPSC-derived EBs and failed hematopoietic organoid formation, while corrected lines showed restored EB integrity and hematopoietic development. Pre-ribosomal-RNA profiling revealed accumulation of 26S and 18S-E pre-ribosomal RNA in mutant lines, consistent with *RPS26* haploinsufficiency, which was fully reverted in corrected lines. Proteomic analysis demonstrated increased expression of ribosomal biogenesis factors, including PNO1, in mutant lines. Increased expression was not determined with RNA sequencing, suggesting a distinct protein translation signature in *RPS26*-mutant cells, compared to corrected and control cells. Our data demonstrate that patient-iPSC lines represent valid models to study DBAS-disease mechanisms. Molecular characterization of our *RPS26*-DBAS lines elucidated key proteins involved in ribosomal processing that contribute to a unique *RPS26*-associated ribosomopathy.

Poster Number: P19

Contribution of an alternative 16S rRNA helix to biogenesis of the 30S subunit of the ribosome

Benjamin Warner[1, 2], Kurt Fredrick[1, 2]
[1]Department of Microbiology, The Ohio State University, Columbus, USA, [2]Center for RNA Biology, The Ohio State University, Columbus, USA

30S subunits become inactive upon exposure to low Mg^{2+} concentration, due to a reversible conformational change that entails nucleotides (nt) in the neck helix (h28) and 3' tail of 16S rRNA. This active-to-inactive transition involves partial unwinding of h28 and re-pairing of nt 921-923 with nt 1532-1534, which requires flipping of the 3' tail by ~180 degrees. A growing body of evidence suggests that immature 30S particles adopt the inactive conformation in the cell, and transition to the active state occurs at a late stage of maturation. Here, we target nucleotides that form the alternative helix (hALT) of the inactive state. Using an orthogonal ribosome system, we find that disruption of hALT decreases translation activity in the cell modestly, by ~2-fold, without compromising ribosome fidelity. Ribosomes carrying substitutions at positions 1532-1533 support

growth of *E. coli* strain Δ7 prrn (which carries a single rRNA operon), albeit at rates 10-20% slower than wild-type ribosomes. These mutant Δ7 prrn strains accumulate free 30S particles and precursor 17S rRNA, indicative of 30S biogenesis defects. Biochemical analysis of purified 30S subunits harboring the tandem mutation U1532A / C1533G suggests that hALT stabilizes the inactive state by 1.2 kcal/mol with little-to-no impact on the active state or the transition state of conversion.

Poster Number: P20

Stoichiometric changes in rRNA modification during stem cell differentiation revealed by parallel rRNA and snoRNA sequencing

Tessa Chan, Ivana Barbaric, Emma Thomson
School of Biosciences, The University of Sheffield, Sheffield, United Kingdom

Ribosomal RNA (rRNA) undergoes extensive modification, primarily pseudouridylation (pseU) and 2'-*O*-methylation, mediated by specific H/ACA and C/D box snoRNAs, respectively. Changes in the stoichiometry of rRNA modifications can lead to ribosome heterogeneity and the potential for regulating cellular processes.

We investigate how rRNA modification stoichiometry varies during embryonic stem cell differentiation from pluripotency to the three primary germ layers. Using the modification basecalling capability of Nanopore direct RNA sequencing, we quantified pseU levels across all known sites and observed distinct stoichiometric changes in pseU patterns between pluripotent and differentiated cells. In parallel, next-generation sequencing of small RNAs profiled snoRNA expression, revealing differential expression of snoRNAs involved in mediating both pseU and ribose methylation. Interestingly, several rRNA pseU residues with altered stoichiometry during differentiation correlated with differential expression patterns of the corresponding snoRNA.

Our integrated direct RNA and snoRNA sequencing approach, provides a comprehensive profile of rRNA pseU dynamics during early embryonic cell fate decisions, highlighting a potential regulatory mechanism for ribosome heterogeneity in development.

Poster Number: P21

Estimating rDNA copy number using nanopore sequencing

Zaka Yuen[1,2,3], Ross Hannan[4], Eduardo Eyras[1,2,3], Nadine Hein[4]
[1]EMBL Australia Partner Laboratory Network, Australian National University, Canberra, Australia, [2]The Shine-Dalgarno Centre for RNA Innovation, John Curtin School of Medical Research, Australian National University, Canberra, Australia, [3]The Centre for Computational Biomedical Sciences, John Curtin School of Medical Research, Australian National University, Canberra, Australia, [4]Division of Genome Sciences and Cancer, John Curtin School of Medical Research, Australian National University, Canberra, Australia

The ribosomal RNA genes (rDNA) are encoded in tandem arrays on the five acrocentric chromosomes. These highly repetitive and GC-rich regions show variation in copy number (CN), which has been linked to disease, ageing, external stress, and environmental exposure. However, accurate quantification of rDNA CN remains challenging. Conventional qPCR methods provide only relative estimates, and short-read sequencing lacks the resolution to span full rDNA units.

We developed a computational pipeline that uses nanopore sequencing to estimate absolute rDNA CN at single-molecule resolution. Nanopore sequencing offers a powerful alternative to other sequencing methods by producing reads long enough to span the entire or even multiple units,

making it possible to assess CN variation. To ensure accurate alignment and improve specificity, our pipeline aligns reads to a modified reference genome containing the standard assembly and multiple copies of an rDNA sequence, while masking rDNA-like regions and retaining only primary alignments. We also include two strategies for calculating rDNA CN - mean and modal coverage, where both are normalised against a panel of autosomal single-copy genes to allow internal validation and improve reproducibility across experiments.

We validated our method using nanopore whole-genome data from a reference human sample, recovering the expected rDNA CN with consistent estimates across biological replicates. We also tested our method on simulated datasets with known rDNA CN and accurately recovered CN. We further benchmark it using mouse samples with a known reduction of rDNA CN.

Our pipeline provides a robust framework for quantifying rDNA CN and lays the groundwork for evaluating its potential as a biomarker in diseases where rDNA instability may play a role.

Poster Number: P22

18S rRNA G1412 residue is critical for maintaining ribosome biogenesis and translation initiation fidelity

A Akhil Kumar[1,2], Charles Antony A[1,2], Pankaj V Alone[1,2]
[1]School of Biological Sciences, National Institute of Science Education and Research, Bhubaneswar, India, [2]Homi Bhabha National Institute, Training School, Mubai, India

Ribosome biogenesis is a highly regulated process essential for accurate translation. While several factors contribute to the maturation of the small ribosomal subunit, the contribution of specific 18S rRNA residues to both biogenesis and translational regulation remains less explored. Here, we identify an 18S rRNA ^{G1412A} mutation in helix 40 of 18S rRNA that disrupts 40S subunit biogenesis and impairs translational control in *Saccharomyces cerevisiae.* The 18S rRNA ^{G1412A} mutation showed reduced 40S ribosomal subunit levels and accumulation of the 20S pre-rRNA precursor, suggesting a late-stage defect in 40S maturation. These biogenesis defects coincide with aberrant translation initiation, like increased initiation at near-cognate UUG codons, and repress GCN4 translation under amino acid starvation (gcn$^-$ phenotype). 18S rRNA^{G1412A} mutation also shows enhanced reinitiation efficiency, linking subunit maturation defects to altered post-initiation dynamics. However, the 18S rRNA^{G1412C} mutation exacerbates the growth and the 40S ribosomal biogenesis defects, highlighting the importance of this residue. Our findings highlight a critical role for 18S rRNA residue G1412 in regulating 40S ribosomal subunit maturation and regulation of translation initiation, providing new insights into the relation between ribosome assembly and function.

Poster Number: P23

Exploring the Functional Impact of Ribosome Biogenesis in AML Progression and Treatment

Sophia Miliara[1], Xiangfu Zhong[1], Styliani Papadaki[2], Dapnhe Devesa[1], Ann-Kristin Östlund Farrants[3], Sören Lehmann[4,5,6], Jiri Bartek[2,7], **Dimitris C. Kanellis[2]**, Andreas Lennartsson[1]
[1]Department of Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden, [2]Department of Medical Biochemistry and Biophysics, Science for Life Laboratory, Karolinska Institute, Solna, Sweden, [3]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [4]Department of Medicine , Center for Hematology and Regenerative Medicine, Karolinska Institute, Huddinge, Sweden, [5]Department of Medical Sciences,Hematology Unit, Uppsala University, Uppsala, Sweden, [6]Hematology Centre, Karolinska University Hospital, Stockholm, Sweden, [7]Danish Cancer Institute, Danish Cancer Society, Copenhagen, Denmark

Acute Myeloid Leukemia (AML) remains a highly challenging malignancy, characterised by poor prognosis and limited effective treatment options. Ribosome biogenesis (RiBi) has gained attention for its role in cancer development and therapy resistance. Through RNA-Seq analysis, we defined a 28-gene RiBi signature with strong prognostic and predictive significance in AML. Elevated RiBi scores were linked to inferior survival outcomes and impaired differentiation. Utilising chemical screening data, we identified drug classes with selective efficacy across AML subtypes stratified by RiBi activity. Notably, primary AML cells with elevated RiBi activity demonstrated heightened sensitivity to the DNA intercalator Actinomycin D. Actinomycin D triggered ribosomal stress and activated the tumour suppressor p53. Additionally, RiBi inhibition alleviated the differentiation block, facilitating the maturation of leukemic progenitor cells. These findings highlight RiBi as a promising dual-action therapeutic target in AML, capable of simultaneously triggering apoptosis and promoting cellular differentiation. By exploiting this vulnerability, RiBi-targeted strategies could significantly enhance personalised treatment approaches and improve outcomes in this aggressive disease.

Poster Number: P24

Dissecting the Dose-Dependent Anticancer Properties of Actinomycin D

Styliani Papadaki[1], Athina Eleftheraki[1], Mikael S Lindström[1], Jiri Bartek[1,2], Dimitris C Kanellis[1]
[1]SciLifeLab, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [2]Danish Cancer Society Research Center, Copenhagen, Denmark

Actinomycin D (ActD) was the first antibiotic used in cancer therapy, currently used as a first-line chemotherapeutic in various cancer types such as Wilms Tumor and Ewing's Sarcoma at concentrations with ambiguous mechanisms of action. ActD is known to inhibit transcription by intercalating into GC-rich DNA regions, thereby stabilizing topoisomerase I-DNA complexes that prevent RNA polymerase-mediated elongation. It blocks all three RNA polymerases in a dose-dependent manner, inhibiting RNA polymerase I at low concentrations and gradually blocking RNA polymerases II and III at higher doses. In this study, we investigated the effects of low versus high concentrations of ActD, trying to characterize its multiple mechanisms of action and broaden its anti-cancer potential. We showed that low ActD dose activates p53 through the impaired ribosome biogenesis checkpoint, leading to G1 arrest while high ActD dose activates the DNA damage response arresting cells in G2 phase. We provide evidence that ActD dosage could be an essential factor to consider in cancer treatment particularly when taking into account the patient ribosome biogenesis status. Our data highlight the multifaceted roles a single chemical inhibitor can exert and emphasize the importance of dose optimization in patient treatment for minimizing off-target effects.

Poster Number: P25

rRNA Operon Redundancy as a Bacterial Genome Stability Insurance Policy

Ivan Matic
Institut Cochin, Université Paris Cité - Inserm - CNRS, Paris, France

The ability to modulate translational capacity—largely determined by ribosome abundance—confers robustness in fluctuating environments. As translation is the most energetically demanding cellular process, bacteria tightly regulate ribosome production in response to nutrient availability, primarily by controlling ribosomal RNA (rRNA) synthesis. Most bacterial genomes contain multiple rRNA operons, exceeding the need for maximal growth in stable conditions. We show that this redundancy prevents RNA polymerase saturation of individual operons during rapid adaptation. Such saturation

can hinder chromosome replication and cause cell death, making rRNA operon multiplicity a critical safeguard for maintaining genome stability under stress.

Poster Number: P26

***Drosophila melanogaster* as a model to study the ribosomopathy ANE syndrome**

Isabella R Lawrence, Susan J Baserga
Molecular Biophysics and Biochemistry, Yale University, New Haven, USA

Alopecia, neurological defects and endocrinopathy (ANE) syndrome is a rare ribosomopathy caused by mutations in the large subunit (LSU/60S) ribosome biogenesis factor gene *RBM28*. To date, three disease-causing *RBM28* variants have been identified; p.(Leu351Pro) (Nousebeck et al 2008; McCann et al 2016), and two biallelic splicing variants, an in-frame deletion of exon 5 (E5) and deletion of exon 8 (E8) which leads to a premature stop codon (Bryant et al 2021). We confirmed that these variants result in growth and LSU pre-rRNA processing defects in yeast (Bryant et al 2021). Like many ribosomopathies, ANE syndrome targets specific tissues and organs, most notably the brain, despite ribosome biogenesis being essential for all cells and RBM28 being an essential protein.

Here, we aim to determine the role of RBM28 in brain development, structure and function, and ribosome biogenesis, in *Drosophila melanogaster,* a powerful and well characterised model organism, for the first time. Using the GAL4-UAS system we are currently generating flies with tissue specific or ubiquitously expressed RNAi targeting CG4806 (the *Drosophila* ortholog of RBM28). We are also generating CRISPR flies expressing ANE syndrome variants to determine the effects of each variant. This work will generate valuable *in vivo* models to better define the development and underlying mechanisms behind this disease.

Poster Number: P27

SURF2 is a key player in nucleolar stress responses, representing a new therapeutic target for a range of diseases

Manon Bongers[1], Paulo Espirito Santo[1], Lorena Maili[2], Dana Rinaldi[1], Paul Trainor[2], Virginie Marcel[3], Célia Plisson-chastang[1], Simon Lebaron[1]
[1]MCD, Centre de Biologie Intégrative, Université de Toulouse, CNRS, Toulouse, France, [2]Stowers Institute for Medical Research, University of Kansas Medical Center, Kansas City, USA, [3]Centre de Recherche en Cancérologie de Lyon, Inserm U1052, CNRS UMR5286, Université de Lyon, Lyon, France

Ribosome synthesis is a highly energy-consuming process involving over 200 factors. Disruptions due to stresses like nutrient deprivation, UV/drug exposure, or genetic mutations can alter this process, leading to the accumulation of unassembled 5S RNP particles in the nucleoplasm. These free 5S RNPs can inhibit MDM2, stabilizing p53 and causing cell cycle arrest, both in a p53 dependant and independent manner which is crucial in responding to nuclear stresses. Finding new inhibitor of ribosome synthesis to promote nucleolar stress response is an important research area in cancer therapies. Furthermore, in ribosomopathies, the activation of p53 by free 5S RNPs significantly contributes to the disease's etiology. While studying free 5S regulation, we recently identified SURF2 as a new interaction partner of free 5S RNP (Tagnères et al, Nat. Comm. 2024). We were able to show that SURF2 competes with MDM2 for 5S RNP binding, and its depletion promotes cell cycle arrest and apoptosis under stress, while its overexpression protects cells from nucleolar stresses and prevents p53 activation upon drug exposure.
During this presentation we will discuss our recent data on SURF2 characterization, supporting it as a

potential therapeutic target for ribosomopathies, including Diamond-Blackfan anemia (DBA), and as an anti-cancer treatment, especially for adrenocortical carcinomas.

Poster Number: P28

Characterising the biochemical, structural and functional differences between eL22 paralogs in *D. melanogaster*

Bulat Fatkhullin[1,2], Karl Norris[1,3], Tayah Hopes[1,3], Antonio Calabrese[1,2], Julie Aspden[1,2,3], Juan Fontana[4]
[1]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom, [2]Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom, [3]LeedsOmics, University of Leeds, Leeds, United Kingdom, [4]Instituto Biofisika, CSIC-UPV/EHU, Leioa, Spain

Many eukaryotic ribosomal proteins possess paralogs that exhibit tissue-specific expression and are ribosome-incorporated. In *D. melanogaster*, eL22-containing ribosomes have been found in germ and somatic cells, and its knockdown is embryonically lethal. In contrast, eL22-like-containing ribosomes are enriched in the testis germ cells. The tissue specificity of the eL22-like paralog suggests it has a different function to eL22. eL22 paralogs are localised on the exterior of the ribosome near the peptide exit tunnel. Notably, the flexible N-terminal tails of eL22/eL22-like, which extend from the ribosome, are highly different (32% amino acid sequence identity, while C-domains share 60%) and were not resolved on previous cryo-EM maps of ribosomes from various *D. melanogaster* tissues.

We hypothesize that the N-terminal tails of eL22 paralogs contribute to ribosome specialisation, i.e. the translation of distinct mRNA pools by eL22 and eL22-like containing ribosomes. To discover if the N-terminal tails of eL22/eL22-like paralogs interact with different protein partners, they were recombinantly expressed and used for pull-downs from the cytoplasm of both S2 cells and whole flies. This revealed unique potential partners for each paralog. Extensive cryo-EM data analysis around eL22-like region revealed that 42% of the testis-specific pulled-down ribosomes do not contain an eL22 paralog protein. Additionally, the N-terminal tail of eL22 protein contacts the expansion segment ES27L of 28S rRNA in the ribosomes from testis, providing a potential specialisation mechanism. Together, this work will shed light on the fundamental mechanisms of specialised ribosomes and has the potential to improve our understanding of ribosomopathies.

Poster Number: P29

A comprehensive, multi-omics and structure-function study of RPS15-mutant 'onco-ribosomes' involved in Chronic Lymphocytic Leukemia

Anaïs Astier[1], Marino Caruso[2], Stijn Vereecke[2], Paulo Espirito Santo[1], Dana Rinaldi[1], Simon Lebaron[1], Frédéric Catez[3], Julien Marcoux[4], Kim De Keersmaecker[2], Célia Plisson-Chastang[1]
[1]Université de Toulouse, Centre de Biologie Integrative, Toulouse, France, [2]Department of Oncology, Laboratory for disease mechanisms in cancer, Leuven,Belgium, [3]Centre Léon Bérard, Cancer Research, Center of Lyon, Lyon, France, [4]Université de Toulouse, Institut de Pharmacologie et de Biologie Structurale, Toulouse, France

Ribosomes modulate mRNA translation, playing a central role in gene expression. They have long been thought to harbor strictly identical composition and production capacities for each cell of an organism. However, the heterogeneity of ribosomes has been highlighted by the discovery of mutations in numerous ribosomal proteins (RP) associated with different cancer types.

Our study focuses on RPS15 (uS19), a protein belonging to the small ribosomal subunit, whose point

mutations in the C-terminus domain are found in up to 20% patients suffering from Chronic Lymphoid Leukemia. This region structurally extends into the active site of ribosomes, in close proximity with the ribosomal A- and P-sites tRNAs. Thus, mutations carrying by this domain might directly affect the translation mechanics, and consequently alter the translatome of cells displaying them. To characterize their molecular and cellular effect, we generated an 'onco-ribosome' cell line library, ie CRISPR-Cas9 engineered isogenic lymphoid Ba/F3 cells expressing homozygous mutant ribosomal proteins, among which RPS15 P131S and H137 point mutations. Genomewide translatome analysis of the onco-ribosome library revealed a profound translational rewiring in RPS15-mutant cells. Using cryo-electron microscopy, we performed a comparison of the 3D structures of both WT and onco-ribosomes, which showed significant differences in the dynamics of their translation elongation cycle, notably with a A-site tRNA accommodation defect in onco-ribosomes. This has led us to assess whether RPS15 mutant ribosomes exhibit a specific bias for decoding specific codons, which will allow to identify a novel mechanism by which oncoribosomes dysregulate translation and promote oncogenesis.

Poster Number: P30

Mutation Matters: RPL5 Defects Impair Ribosome Function in Diamond-Blackfan anemia

Federica Marchesini[1], Veronica Pepe[1], Christiane Zorbas[2], Eliza Sack[2], Denis L.J. Lafontaine[2], Marianna Penzo[1]

[1]Department of Medical and Surgical Sciences, University of Bologna, 40138 Bologna, Italy, [2]RNA Molecular Biology, Fonds de la Recherche Scientifique (F.R.S./FNRS), Université libre de Bruxelles (ULB), B-6041 Gosselies, Belgium

Diamond-Blackfan anemia (DBA) is a rare congenital bone marrow failure syndrome frequently caused by heterozygous mutations in ribosomal protein genes, including RPL5/uL18, a critical component of large subunit biogenesis. While defects in ribosome production and nucleolar stress pathways are established contributors to DBA pathogenesis, the mutation-specific impact on mature ribosome function remains poorly understood.

To investigate these effects, we analyzed a panel of RPL5/uL18 mutations identified in DBA using patient-derived lymphoblastoid cell lines (LCLs). Total ribosome quantification revealed a consistent reduction in ribosome number per cell across all mutant lines compared to healthy controls.

To probe intrinsic ribosome function, we employed a cell-free translation assay developed in our lab, based on ribosome-free rabbit reticulocyte lysate reconstituted with ribosomes from patient-derived LCLs and in vitro–transcribed reporter mRNAs. All mutant ribosomes showed reduced translational efficiency, including under CrPV-IRES–driven conditions that bypass canonical Cap-driven initiation, indicating intrinsic defects in ribosomal function.

Fidelity assays revealed aberrant decoding behaviour—such as increased stop codon readthrough and amino acid misincorporation—in several mutants, particularly those predicted to cause early truncations. These defects varied in severity, supporting the presence of mutation-specific effects on distinct aspects of translation.

Our findings highlight that RPL5/uL18 mutations can disrupt ribosome activity through diverse mechanisms, resulting in variable penetrance of translational defects. This work underscores the importance of monitoring both efficiency and fidelity of translation in ribosomopathies, and offers mechanistic insight into how ribosome dysfunction contributes to inherited bone marrow failure. Such knowledge may inform mutation-tailored therapeutic strategies in DBA and related ribosome biogenesis dysfunction disorders.

Poster Number: P31

Transcriptional adaptation promotes leukemia cell resistance to protein synthesis inhibition

Daniel Sjövall, Carolina Guibentif, **Pekka Jaako**
Sahlgrenska Center for Cancer Research, University of Gothenburg, Gothenburg, Sweden

Manipulation of the protein synthesis machinery using the mRNA translation elongation inhibitor homoharringtonine (HHT) has emerged as a promising therapeutic strategy for hematologic malignancies, including acute myeloid leukemia (AML). However, a limited understanding of the mechanisms that underlie leukemia cell resistance to HHT hamper the rational development of optimized treatment approaches.

Here, we demonstrate that a five-day HHT treatment significantly extends survival in a mouse model of MLL-rearranged AML by eradicating the majority of leukemia cells in the bone marrow. Flow cytometry analysis reveals that resistance to HHT is associated with an aberrant accumulation of lineage marker negative leukemia cells in the bone marrow. Single-cell RNA sequencing indicates that these cells represent a novel transcriptional state marked by upregulation of Myc and ribosome biogenesis genes and downregulation of key myeloid transcription factors, including members of the C/EBP family. Our ongoing work aims to determine whether this adaptive transcriptional state confers increased sensitivity to ribosome biogenesis inhibitors. Importantly, parallel studies in healthy mice show that HHT does not reduce hematopoietic stem cell numbers or impair their ability to reconstitute lethally-irradiated recipient mice in competitive transplantation assays, despite depleting c-Kit+ progenitor cells. Taken together, our findings support HHT as an efficient and relatively safe treatment strategy in AML and provide novel insights into how leukemia cells adapt to protein synthesis inhibition, highlighting potential avenues for combination therapy.

Poster Number: P32

Uncovering the role of small nucleolar RNA-retaining transcripts in tumor biology

Giulia Venturi[1], Guglielmo Rambaldelli[1], Sidra Asghar[1], Federico Zacchini[2], Lorenzo Montanaro[1, 2]
[1]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy, [2]Departmental Program in Laboratory Medicine, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Small nucleolar RNAs (snoRNAs) are non-coding RNAs whose genes are hosted within the introns of protein-coding and non-coding genes. However, a significant fraction of these exists as retained introns of specific transcripts—referred to as snoRNA-retaining transcripts (snoRTs). The biological and pathological roles of snoRTs remain largely unexplored.

By reanalyzing publicly available Oxford Nanopore Technology data, we found that snoRTs are widely represented in both human normal tissues and cancer-derived cell lines, appearing in a full-length (FL) form and as shorter variants for which we mapped the truncation sites. To validate these data, we developed a droplet digital PCR (ddPCR) assay to quantify selected snoRTs and their corresponding snoRNAs. Our findings indicate that a consistent portion of snoRNA sequences detected in cells, cancer tissues, and liquid biopsy samples are derived from snoRTs.

Moreover, in a previous study we demonstrated that one particular snoRT was associated with estrogen receptor status in breast cancer tumors and with nuclear hormone receptor pathway in a breast cancer cell line. We thus investigated the impact of selected snoRTs knockdown in cell lines derived from hormone dependent tumors such as breast and prostate. Our results in these models show that snoRT knockdown affects global protein synthesis, growth rate, and metastatic potential. In addition, a transcript-based re-analysis of publicly available NGS data from prostate cancer patients was performed to detect new FL-snoRTs in comparison with normal prostate tissue

and in association with pathological data. Collectively, our findings reveal previously unrecognized molecular players potentially involved in cancer development and progression.

Poster Number: P33

U14 small nucleolar RNA-protein complexes and early assembly factors orchestrate the integrity of pre-rRNA folding

Yi-Lan Chen, Sarah Woodson
Thomas C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, USA

Certain small nucleolar RNA-protein complexes (snoRNPs) guide pre-rRNA folding and assembly. Although the timing of snoRNP joining and release is crucial for pre-rRNA folding, these transient interactions are challenging to detect. U14 snoRNP, an early 40S assembly factor, targets two sites in the 18S rRNA (h6 and h14) that connect the two halves of the 18S 5′ domain. To study how U14 snoRNP recognizes its targets, which are separated by ~380 nt, we directly visualized the binding of AF555-labeled *C. thermophilum* U14 snoRNP to Cy5-labeled refolded pre-rRNA using single-molecule fluorescence microscopy. Stable mid-FRET and low-FRET complexes were assigned to base pairing with h14 or h6, respectively. When naked rRNA was used as a target, U14 snoRNP randomly bound to upstream (h6) and downstream (h14) sites. When co-assembly factors Bud22 and Efg1 were added, U14 snoRNP rarely bound h6 alone and instead preferentially bound the downstream h14 site first, consistent with crosslinking data showing that Bud22 interacts with h14. We next tested if U14 snoRNP can associate with the rRNA during transcription. Interestingly, we found that most U14 snoRNP molecules were only able to bind after both target sites were transcribed. Experiments are in progress to decipher how other assembly factors and helicases affect U14 snoRNP recruitment and how U14 snoRNP facilitates the folding of the pre-rRNA. This study aims to show how the recruitment of early factors is orchestrated and how they help maintain the integrity of ribosome assembly.

Poster Number: P34

A novel human induced Pluripotent Stem Cells-based model of Diamond-Blackfan Anemia to explore translational regulation in erythropoiesis throughout life

Deborah Donzel[1], Anne N. Mihindukulasuriya P.[1], Senthil Bhoopalan[2], Johan Flygare[3], Andrea Ditadi[1]
[1]San Raffaele Telethon Institute for Gene Therapy, IRCCS San Raffaele Scientific Institute, Milano, Italy, [2]Department of Bone Marrow Transplantation and Cellular Therapy, St. Jude Children's Research Hospital, Memphis, TN, USA, [3]Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Lund, Sweden

Diamond-Blackfan Anemia (DBA) is a ribosomopathy caused primarily by mutations in ribosomal proteins (RPs), leading to severe defects in red blood cell (RBC) production. RBCs are fundamental to life and are therefore constantly produced across successive developmental waves from embryogenesis through adulthood. Notably, DBA manifests only postnatally with a severe anemia that is lethal if not treated. This raises the intriguing hypothesis that distinct waves of erythropoiesis differ in their sensitivity to RP haploinsufficiency, potentially due to differences in translation regulation or ribosome composition. However, existing models limit our ability to test this hypothesis, as they fail to fully replicate this specific disease phenotype.
To address this, we developed a human induced pluripotent stem cell (iPSC)-based model that enables selective derivation of erythroid progenitors from either embryonic/fetal or postnatal developmental stages, via stage-specific modulation of WNT and Nodal-Activin signalling. These

progenitors were differentiated into RBCs displaying stage-appropriate globin expression patterns. Using iPSCs haploinsufficient for RPS19, the most frequently mutated gene in DBA, we found that embryonic and fetal RBCs develop normally, while postnatal-like RBCs exhibit the differentiation block observed in patients. This defect can be rescued with small-molecule modulators targeting specific cellular stress pathways. This iPSC-based model provides an innovative human-specific platform to investigate translational regulation across distinct erythropoietic waves in health and disease, and to develop targeted therapies for ribosomopathies.

Poster Number: P35

The N-terminal intrinscially disordered region of the assembly factor Nop2 is necessary for nucleolar stages of 60S subunit assembly

Stefanie Hedayati, Geqi Li, **John Woolford**
Biological Sciences, Carnegie Mellon University, Pittsburgh, USA

About 200 assembly factors (AFs) are required for the maturation of both the large and small ribosomal subunit in yeast. More than half of these AFs contain intrinsically disordered regions (IDRs), whose presence is conserved across other eukaryotes. IDRs have emerged as important participants in numerous cellular processes, such as participating in macromolecular interactions or sensing cellular physiology, yet their roles in ribosome biogenesis have been investigated for only a handful of AFs**.** Although cryo-EM structures of co-transcriptional to cytoplasmic pre-ribosome intermediates have provided structural information about the composition and conformation of assembly factors, ribosomal proteins and compacted rRNA, the IDRs in AFs are **not** visualized because they are conformationally heterogeneous. Therefore, we have an incomplete understanding of the role of IDRs, and thus assembly factors, in ribosome assembly. To investigate the roles of these IDRs in ribosome biogenesis, we deleted the non-visible IDRs in select AFs and assessed growth, polysome profiles and pre-ribosome composition. We found that even partial deletion of the 196-amino acid long N-terminal IDR of the 60S subunit assembly factor Nop2 leads to a dominant-negative slow-growth phenotype, reduced levels of 60S subunits, and a block in ribosome assembly around the NE2 state, as evidenced by decreased amounts of Nog2, Bud20 and Arx1 present in pre-ribosomes. We speculate that the Nop2 IDR may be involved in L1 stalk rotation, and plan to further investigate this hypothesis using cryo-EM of *nop2* mutant particles.

Poster Number: P36

Quantitative approaches to measure ribosome abundance in Diamond-Blackfan anemia-associated haploinsufficiency

Kate Schole[1-3], Rachel Green[1, 4]
[1]Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Biochemistry, Cellular & Molecular Biology Graduate Program, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [3]Medical Scientist Training Program, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, [4]Howard Hughes Medical Institute, Chevy Chase, MD, USA

Given their essential role in protein synthesis, ribosome and mRNA concentrations are finely tuned to optimize protein output according to cell need. In Diamond-Blackfan anemia (DBA), a rare genetic disease caused by mutations in ribosomal proteins, ribosome abundance decreases and thus this carefully coordinated process is perturbed. While it is generally understood that ribosomal protein mutations lead to decreased ribosome abundance and protein synthesis, it is challenging to

quantitively measure such changes. To this end, we have developed several biochemical and sequencing-based approaches to quantify ribosome abundance in the context of DBA. To obtain concentration measurements, we have begun to employ confocal microscopy to measure cell volume changes following siRNA-mediated knockdown of ribosomal proteins. In comparing knockdowns of commonly mutated ribosomal proteins in DBA, we have also found differences in imbalances between the large and small subunit of the ribosome. Through careful quantitation of ribosome abundance and cell size across common DBA disease alleles we hope to reveal how changes in ribosome concentration affect translation dynamics that contribute to pathogenesis.

Poster Number: P37

Liver Microenvironment and Metastatic Potential Drive Translational Remodeling of Ribosome Biogenesis and RNA Processing in Colorectal Cancer

Barış KELEŞ [1,2,3], Alana Panzenhagen [4], Peyda Korhan [3,5], Yasemin Öztemur Islakoğlu[2], Safiye Neşe Atabey [2,3,5]

[1]Department of Biomedicine and Health Technologies, İzmir International Bİomedicine and Genome Institute, İzmir, Turkey, [2]İzmir Bİomedicine and Genome Center, İzmir, Turkey, [3]Galen Research Center,İzmir Tınaztepe University ,İzmir, Turkey, [4]Department of Oncology-Pathology, Science for Life Laboratories, Karolinska Institute, Stockholm, Sweden, [5]Department of Medical Biology, Faculty of Medicine, İzmir Tınaztepe University, İzmir, Turkey

Colorectal cancer (CRC) to liver metastasis involves coordinated changes in both transcript abundance and translation of ribosome–related genes. In our RNA–seq data, comparing primary versus liver–metastatic cell lines, we observed upregulation of canonical ribosome biogenesis transcripts (e.g., UBF1, NOP58). Conversely, co–culture of CRC cells with LX–2 cells to model liver microenvironment effects resulted in downregulation of same ribosomal genes at the transcript level. KEGG mapping of these transcriptome fold–changes onto the “ribosome” pathway (hsa03010) confirmed that metastatic–high CRC cells exhibited higher expression of many ribosomal proteins and rRNA processing factors, whereas liver micro–environment stimulation suppressed those nodes. This transcriptome–based KEGG overlay highlights how metastatic cues drive ribosome assembly while liver microenvironment signals the opposite. To determine whether transcript–level patterns extend to translation, we integrated polysome profiling results from GSE164492 dataset. Translationally upregulated genes are enriched in rRNA processing and ribosome biogenesis, indicating metastasis–driven ribosome assembly. Translationally downregulated genes are enriched in RNA splicing, mRNA surveillance, and RNA stability, showing de–prioritization of general RNA processing. These results show that metastatic CRC cells reprogram translation to boost ribosome biogenesis, complementing our transcriptome–based KEGG overlay. Together, this highlights how liver–metastatic cues reshape both transcript abundance and ribosome engagement of RNA–related pathways.

Poster Number: P38

Pre-Ribo Mega-SEC enables separation of nucleolar pre-ribosomal particles in human cells

Harunori Yoshikawa[1, 2], Angus Lamond[2]

[1]Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan, [2]Molecular Cell and Developmental Biology, School of Life Sciences, University of Dundee, Dundee, Scotland, United Kingdom

Ribosome assembly, which comprises the processing and folding of the transcribed pre-rRNA and the assembly of the ribosomal proteins on the pre-rRNA, has been extensively characterised in budding yeast with the composition and structure of many assembly intermediates having been determined. In comparison, analysis of ribosome assembly in humans lags behind, in part due to the lack of effective method to isolate human early nucleolar pre-ribosomal particles.

We have developed an efficient approach for the systematic characterization of cellular protein complexes using uHPLC-based Size Exclusion Chromatography (SEC), and have shown that very large cellular complexes, including ribosomes and polysomes, can be separated within 15 minutes with high reproducibility by this SEC-uHPLC method, which we term, “Ribo Mega-SEC”.

We show here that we can apply Ribo Mega-SEC to effectively separate human pre-ribosomal particles from nucleolar extracts (“Pre-Ribo Mega-SEC”). We have used Pre-Ribo Mega-SEC in conjunction with mass spectrometry-based quantitative proteomics to characterise human nucleolar pre-ribosomal particles.

Poster Number: P39

Pre-ribosomal WDR74 module coordinates the early and late pre-rRNA processing stages for the NVL2-mediated regulation of 60S ribosome biogenesis

Yuya Hirooka[1, 2], Sotaro Miyao[2], Keiichi Izumikawa[2], Masami Nagahama[2]
[1]Insitute for Advanced Bioscieces, Keio University, Tsuruoka, Japan, [2]Graduate School of Pharmaceutical Sciences, Meiji Pharmaceutical University, Kiyose, Japan

WD repeat domain 74 (WDR74) is a nucleolar protein involved in early stages of pre-60S ribosomal subunit maturation in humans. At later stages, WDR74 is dissociates with MTR4-exosome complex via ATP hydrolysis by the nuclear AAA-ATPase NVL2. We previously showed that ATPase-defective NVL2 causes aberrant WDR74 accumulation on the MTR4–exosome complex at the nucleolar periphery and in the nucleoplasm, leading to abnormal cleavage at the internal transcribed spacer 1 (ITS1) of pre-rRNA. However, the molecular mechanism underlying this regulation remained unclear. In this study, co-immunoprecipitation and mass spectrometry analysis revealed that WDR74 forms a conserved pre-ribosomal subcomplex, composed of WDR74, RPF1, MAK16, and RRP1, which we term “WDR74 module”. Each component of the WDR74 module was mutually essential for the interaction of other members with MTR4 and all components were essential for the accurate pre-rRNA cleavage during 60S biogenesis. Disruption of any component or NVL2 ATPase activity impaired the release of WDR74 from MTR4–exosome, preventing MTR4 from recruiting PICT1, an adaptor required for 3’-end processing of 5.8S rRNA. We suggest that the WDR74 module coordinates a transition in MTR4-binding partners, linking early ITS1 cleavage with later steps of 5.8S rRNA maturation in an ATP-dependent manner.

Poster Number: P40

Starvation and meiosis dependent modulation of yeast ribosome

Mathieu Catala, Jonathan Wilson, Sherif Abou Elela
Microbiologie et Infectiologie, Université de Sherbrooke, Sherbrooke, Canada

Ribosomes play a crucial role in protein synthesis across all organisms. Traditionally, ribosomes have been considered uniform entities regulated in a coordinated manner to optimize output and minimize waste. However, recent research suggests that ribosomal protein genes are not uniformly regulated and often possess duplicated copies with specific functions. Meiosis in yeast is triggered by

starvation and induces a change in ploidy followed by encapsulation of the gametes in spores. Cells entering meiosis undergo major gene expression changes to reprogram their mode of division and ribosome composition alteration might contribute to the translation regulation of meiotic genes. In this study, we demonstrate that the abundance of nutrients can modify the composition of yeast ribosomes, allowing them to adapt translation patterns in response to amino acid scarcity. Starvation conditions were found to inhibit the expression of the major copies of ribosomal protein genes, resulting in a ribosome population that primarily expresses minor copies of these genes. This modulation slows down translation, preventing defects caused by amino acid scarcity and avoiding abortive translation. The transition from nutrient-rich to starvation ribosomes is facilitated by alterations in paralog splicing efficiency and translation. Entry into meiosis dictates further changes to sustain cell differentiation. These findings highlight that cellular responses to starvation and meiosis involve not only a decrease in ribosome production but also changes in translation patterns in response to amino acid availability and cell division program.

Poster Number: P41

New insights into the role of ribosome biogenesis in cellular senescence and tumor initiation

Kexin Zhu[1], Emilie Dejonckere[1], Jean-Michel Flaman[1], Juliette Royer[2], Laury Baillon[2], Joanna Czarnecka-Herok [1], Jean-Jacques Diaz[2], Frédéric Catez[2], David Bernard[1], **Nadine Martin[1]**
[1]Cellular senescence, cancer and aging team, Cancer Research Center of Lyon, Inserm U1052 / CNRS UMR 5286 / UCBL / CLB, Lyon, France, [2]Ribosome, translation and cancer team, Cancer Research Center of Lyon, Inserm U1052 / CNRS UMR 5286 / UCBL / CLB, Lyon, France

Cellular senescence is a stable cell proliferation arrest which can be induced by many stresses and which plays a crucial role in many physio-pathological contexts including cancer and aging. In addition to the cell cycle arrest, senescent cells display a particular secretome named senescence-associated secretory phenotype (SASP) which impacts their microenvironment. In response to pro-tumoral signals, cellular senescence is induced and acts as a critical anti-tumoral barrier that needs to be bypassed for tumor development. It is therefore essential to understand the regulatory mechanisms controlling cellular senescence. Here we will present our latest results expanding our knowledge on the role of ribosome biogenesis in cellular senescence and tumor initiation.

Poster Number: P42

The RNA helicase Dhr2 promotes early assembly steps during small subunit processome maturation

Nika Mikulic Vernik[1], Matthias Thoms[2], Sanem Ayaz Kök[1], Natalia Kunowska[3], Ulrich Stelzl[3], Roland Beckmann[2], Valentin Mitterer[1]
[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria, [2]Gene Center, LMU Munich, Munich, Germany, [3]Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria

Eukaryotic ribosome biogenesis starts with the co-transcriptional formation of the SSU processome, a huge macromolecular complex that promotes pre-rRNA folding coupled to RNA cleavage and processing steps. These early maturation steps are driven by a variety of essential assembly factors, including several RNA helicases.

We investigated the role of the essential but poorly characterized DEAH-box helicase Dhr2 during SSU processome maturation. Using tandem-affinity purifications upon auxin-induced degradation of endogenous Dhr2, we isolated stalled pre-ribosomal maturation intermediates that we further analyzed by mass spectrometry, RNA sequencing, and cryo-electron microscopy. This unveiled that in

the absence of Dhr2, ribosome biogenesis is arrested at an early stage, in which initial 5'ETS RNP complexes are formed but formation of the complete SSU processome is impaired.

Functional analysis showed that Dhr2's N-terminus is non-essential, while C-terminal truncations are lethal and disrupt pre-ribosome binding. The Dhr2 C-domain furthermore mediates a strong Yeast two-hybrid interaction with the N-terminal α-helix of assembly factor Nop19. Subsequent ATPase assays showed that Nop19 stimulates the activity of Dhr2 and *in vitro* maturation assays with purified Utp6 particles demonstrated that recombinant Dhr2 and Nop19 together initiate SSU processome restructuring, which is potentially exhibited by the associated exosome.

Our data unveil an essential role of Dhr2 in maturation of the 5' ETS RNP during early SSU processome assembly, and identify the small protein Nop19 as a specific cofactor for the helicase. Ongoing investigations aim to further elucidate Dhr2´s specific function and restructuring target on the nascent SSU processome.

Poster Number: P43

FUS modulates the level of rRNA modifications and ribosome activity in health and disease

Dorota Raczynska[1], Anna Bieluszewska[1], Patrycja Swiergiel[1], Kishor Gawade[1], Patrycja Plewka[1], Virginie Marchand[2], Yuri Motorine[2], Hannes Glass[3], Anreas Hermann[3]
[1]Department of Gene Expression, Adam Mickiewicz University, Poznan, Poland, [2]CNRS, University of Lorraine, Nancy, France, [3]University Medical Center Rostock, University of Rostock, Rostock, Germany

FUS is a multifunctional protein involved in many aspects of RNA metabolism. Recently, we have showed that FUS depletion results in a change of expression of numerous small nucleolar RNAs snoRNAs that guide posttranscriptional modifications at specific positions in rRNAs[1]. Using RiboMeth-seq and HydraPsiSeq for profiling site-specific 2'-O-methylation and pseudouridylation of rRNAs, respectively, we demonstrated considerably higher modification at several sites in HEK293T and SH-SY5Y cells with FUS knockout (FUS KO) compared to wild-type (WT) cells. Interestingly, the rRNA modification pattern partially correlated with the abundance of corresponding guide snoRNAs. Furthermore, similar direction of changes in rRNA modification and snoRNA levels were observed in SH-SY5Y cells with the FUS mutation (R495X) related to the early-onset disease phenotype of amyotrophic lateral sclerosis (ALS).

Next, we used the isogenic line of iPSCs derived from ALS-FUS patient (with P525L mutation) further differentiated into neuronal progenitor cells (NPCs) and motoneurons (MNs). Using RiboMeth-seq and HydraPsiSeq we analyzed rRNA modification profiles and snoRNA levels in mutant NPCs and MNs compared to WT cells. Additionally, we tested the translation activity of ribosomes in mutant NPCs compared to WT NPCs by Ribo-seq. Our findings suggest a role for FUS in modulating the modification patterns of rRNAs and contributing to ribosome heterogeneity that may constitute a finetuning mechanism for translation efficiency/fidelity. This in turn may represent a new translation-related mechanism that underlies ASL-FUS disease progression.

1. Gawade, K., et al. *Sci Rep*, FUS regulates a subset of snoRNA expression and modulates the level of rRNA modifications, 2023. 13(1):2974.

Poster Number: P44

Investigating 40S ribosome maturation in human cells reveals a recruitment platform for processing enzymes

Désirée Marchand[1], Andreas Schäffler[1], Siyi Chen[1], Diederik Lamen Trip[2], Yaren Karakoç[1], Pedro Beltrao[2], Stefanie Jonas[1]

[1]IMBB, ETHZ, Zurich, Switzerland, [2]IMSB, ETHZ, Zurich, Switzerland

In eukaryotes the life of a ribosome starts in the nucleolus, where the ribosomal (r)RNA is transcribed and co-transcriptionally assembled into the first pre-ribosomal particles. These precursors are then further matured on their path through the nucleoplasm into the cytoplasm. Human ribosome biogenesis has been so far studied through siRNA screens and many precursors were structurally resolved, however an interactome based approach comparing different stages is missing. Here, we investigated the biogenesis of the human small ribosomal subunit (40S) and engineered several tagged human cell lines of known trans-acting factors involved in different stages of ribosome biogenesis. Using affinity purification of these assembly factors from different nucleolar, nuclear and cytoplasmic extracts and their subsequent analysis by mass spectrometry, we obtained their interactomes across cellular compartments. These interactomes reflect the protein compositions of different ribosome assembly intermediates, revealing additional factors and new interaction patterns. For example, we identify that the trans-acting factor RRP12 serves as a docking platform for flexible attachment of rRNA processing enzymes. Using functional and biochemical analysis, we find that it directly recruits the exonuclease PARN which trims the 3' end of the 18S-E rRNA and the methyltransferase RRP8 to pre-40S subunits. We define the molecular basis of their interaction and demonstrate that RRP8 is not only involved in 60S but also 40S biogenesis. Additionally, we show that PARN-RRP12 binding is necessary both for nucleolar PARN localization and for successful 18S-E trimming.

Poster Number: P45

Dissecting the assembly line of the SSU processome

Tina Lence[1], Magdalena Gerhalter[1], Michael Prattes[1], Gertrude Zisser[1], Isabella Klein[1], Juliane Merl-Pham[2], Lorenz Emanuel Grundmann[3], Irina Grishkovskaya[3], David Hasselbach[3], Stefanie M. Hauck[2], Helmut Bergler[1]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria, [2]Metabolomics and Proteomics Core, Helmholtz Center Munich, Munich, Germany, [3]Research Institute of Molecular Pathology, Vienna BioCenter, Vienna, Austria

Ribosomes are ribonucleoprotein machineries responsible for the translation of mRNA to proteins in all living cells. They contain two subunits that are composed of ribosomal RNA (rRNA) and ribosomal proteins (RBS). The small subunit (SSU; 40S) decodes messenger RNA (mRNA), whereas the large subunit (LSU; 60S) forms the peptide bond. Ribosome biogenesis is one of the most energy consuming processes in a cell. It requires the contribution of over 200 transiently interacting assembly factors and multiple snoRNAs that facilitate pre-rRNA folding and ribosomal protein association. The pathway starts with the co-transcriptional assembly of the 90S/SSU-processome where the four domains of the 18S rRNA (the 5', central, 3' major, and 3' minor domains) are initially compacted and the first 15 ribosomal proteins are stepwise incorporated. However, the exact assembly line of the very early steps of SSU-processome maturation is currently unknown.

We recently applied a pulse-chase SILAC-qMS strategy to gain deeper insights into these early steps of SSU-processome assembly. This identified "seed" ribosomal proteins that initially bind to the rRNA domains (our unpublished results).

Here we investigate which role assembly factors play in the incorporation of these seed ribosomal proteins and how they cooperate to compact individual domains and in turn facilitate incorporation of later binding ribosomal proteins. We address this by using cryo-EM, qMS, genetic means and newly developed in vitro binding models.

Poster Number: P46
Deciphering the role of catalytic activity of uS11 assembly chaperones
Bruno Bonnettaz[1], Anna Smirnova[2], Yelda Abou Khalil[2], Emilie Zélie[1], Lila Delbos[1], Memgmeg Zhang[2], Wangqing Liu[2], Magali Blaud[1], **Nicolas Leulliot[1]**, Alexandre Smirnov[2]
[1]Cibles Thérapeutiques et Conception de Médicaments (CiTCoM), Université Paris Cité, Paris, France, [2]Génétique Moléculaire, Génomique, Microbiologie (GMGM), Université de Strasbourg, Strasbourg, France

Two universally conserved proteins have been identified as essential for the assembly of the uS11 ribosomal protein on the ribosome: YbeY in prokaryotes and Fap7 in eukaryotes and archaea. The proteins do not share any sequence or structural similarities. Both protein have homologies to two different classes of enzymes. Ybey is a putative single strand-specific metallo-endoribonuclease while Fap7 is an atypical kinase with an unknown substrates. Although the catalytic activity of YbeY and Fap7 are essential for ribosome synthesis, its role has remained enigmatic.

Here, using biochemical, enzymology and genetic approaches, structural studies of proteins by X-ray crystallography and Cryo-EM of ribosomes isolated from mutant strains, we show that these two independently evolved highly conserved enzymes catalyse the installation of an isoaspartate residue in the C-terminal tail of uS11. In bacteria and their derivatives, YbeY converts an invariant asparagine into isoaspartate through a mechanism partially emulated on that of zinc-dependent proteinases. Similarly, Fap7 isomerises an aspartate in the same position of uS11 using a totally different, ATP-dependent, activation mechanism. We show that, in either case, the isoaspartylation is functionally critical: this strategically positioned modification radically changes the geometry of the peptide chain of uS11 and enables its correct incorporation into the platform of the ribosomal small subunit, which is required for efficient translation.

Poster Number: P47
Withdrawn

Poster Number: P48
Withdrawn

Poster Number: P49
Deciphering the RPS26 mutation involvement in DBA through a mouse model
Alexis Bertrand, Riaz Rio, Julien Papoin, Lionel Blanc
Pediatric hematology oncology, Feinstein Institute for Medical Research, Manhasset, USA

Diamond Blackfan Anemia (DBA) is a rare inherited bone marrow failure syndrome caused by mutations in ribosomal protein genes. It is characterized by severe hypo-proliferative anemia, congenital abnormalities, and a predisposition to cancer. Among the various ribosomal genes

implicated in DBA, mutations in RPS26 stand out, as patients with these mutations do not exhibit an increased risk of cancer.

To investigate this unique feature, we have developed a Cre-dependent RPS26 mutant mouse model of DBA. Using a Vav-Cre background, we are currently studying erythropoiesis and ribosome biogenesis to understand the effects of RPS26 haploinsufficiency in hematopoietic cells. These results will be directly compared to data from existing RPS19 and RPL5 DBA mouse models previously generated in our laboratory.

Preliminary observations show that Vav-Cre RPS26 newborn mice display erythropoietic defects similar to those seen in the RPS19 model, although notable differences exist. These comparative analyses aim to determine whether each ribosomal protein gene mutation leads to distinct pathophysiological features.

To further explore the relationship between ribosomal protein defects and cancer predisposition, we will also introduce a Tp53 knockout into the model to assess the potential for leukemogenesis.

The latest findings from this novel and uniquely engineered RPS26 haploinsufficient DBA mouse model will be presented.

Poster Number: P50
Abstract can be found in Speaker Abstracts, Tuesday 9th September at 17.20

Poster Number: P51
RMB34 Function in 60S Ribosomal Subunit Assembly is Regulated by the MAPK Signaling Pathway
Florine Roses[1], Jessie Bourdeaux[1], Emilie L. Cerezo[1,2], Yves Henry[1], **Anthony K. Henras[1]** and Yves Romeo[1]
[1] Molecular, Cellular and Developmental biology Unit (MCD), Centre de Biologie Integrative (CBI), Université de Toulouse, CNRS, UPS, 31062 Toulouse, France.
[2] Present address: Lengefeld Lab, Institute of Biotechnology, Helsinki Institute of Life Science (HiLIFE), University of Helsinki.

Ribosome assembly starts with ribosomal RNA (rRNA) synthesis by RNA polymerases I and III, their packaging into precursor particles (pre-ribosomes) and their modification, processing and association with ribosomal proteins (RPs) to generate the mature ribosomal subunits. This process requires scores of assembly factors (AFs) that transiently interact with pre-ribosomes to fulfill specific functions in the maturation process. Signal transduction cascades tightly regulate this process to adapt ribosome biogenesis to cell growth requirements. Among these, the MAPK pathway regulates transcription by RNA polymerases and translation of RPs. Our recent data suggest that the MAPK pathway also regulates the co- and post-transcriptional stages of ribosome synthesis, to coordinate the synthesis of the primary transcripts and the whole maturation process. We identified RBM34 as a nucleolar substrate of RSK kinases, the downstream effectors of the MAPK pathway. We showed that RBM34 is required for efficient production of the 60S ribosomal subunit in human cells and can substitute for Nop12 in yeast ribosome synthesis. Both in yeast and human cells Nop12/RBM34 knockout affects global translation and cell proliferation. We further showed that in human cells, RBM34 is phosphorylated by RSK at a specific serine residue (S14), which is required for optimal production of the large ribosomal subunit, highlighting the importance of this phosphorylation event in ribosome synthesis. Our ongoing work will lead to a comprehensive view of the impact of the MAPK signaling pathways on the co- and post-transcriptional stages of ribosome biogenesis in human cells.

Keywords: ribosome biogenesis, RBM34, MAPK signaling, RSK kinases, Translation

Poster Number: P52

Characterizing the intracellular cellular organization of ribosome biogenesis and function in archaea by RNA-FISH and super-resolution microscopy

Tom Garnier[1,2], Maximilian Liebl[3], Michael Jüttner[3], Daniela Strauß[1,3], Nicolas David[2], Roxane Lestini[2] and Sébastien Ferreira-Cerca [1,3]

[1]Structural Biology of the Cell Laboratory | Laboratoire de Biologie Structurale de la Cellule (BIOC), UMR 7654—CNRS, École polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91128 Palaiseau, France

[2]Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS UMR7645-INSERM U1182, École polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91128 Palaiseau, France

[3]Regensburg Center for Biochemistry, Biochemistry III – Institute for Biochemistry, Genetics and Microbiology, University of Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany

Archaea consists of a very diverse group of poorly understood microorganisms, commonly known for their capacity to thrive in diverse and sometimes extreme environments. Interestingly, recent phylogenetic analysis suggests the emergence of the eukaryotic lineage from within the archaeal domain.

Eukaryotic ribosome biogenesis is characterized by a high degree of functional compartmentalization, from the nucleolus to cytoplasm. Moreover, the translation process is separated from the stored genetic information. In contrast, the intracellular organization of these fundamental processes in archaea remains largely unexplored.

Using wide-field fluorescence microscopy and super-resolution techniques (dSTORM) we aim to explore the putative diversity and dynamics of intracellular organization of ribosome biogenesis and the translation process in archaea.

Poster Number: P53

New Insights into nuclear import and nucleolar localization of yeast RNA exosome subunits

Valdir Gomes Neto[1,2], Leidy Paola P. Cepeda[1], Bruno R.S. Queiroz1[1], Sylvain Cantaloube[3], Isabelle Leger-Silvestre[2], Thomas Mangeat[3], Benjamin Albert[2], Olivier Gadal[2], **Carla C. Oliveira**[1]

[1]Department of Biochemistry, Institute of Chemistry, University of São Paulo, SP, Brazil;

[2]MCD (CBI), Université de Toulouse, CNRS, UPS, 31000, Toulouse, France. 3Centre de Biologie

[3]Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31000, Toulouse, France

The RNA exosome is a multiprotein complex essential for RNA maturation and degradation. In budding yeast, a nine-subunit protein complex (Exo9) associated with Rrp44 forms Exo10 in the cytoplasm and, in complex with Rrp6, Exo11 in the nucleus. Depending on its subcellular localization, the exosome interacts with different cofactors and RNA substrates. In the cytoplasm, Exo10 associates with the SKI complex via Ski7, while in the nucleus, Exo11 interacts with the TRAMP complex. Within the nucleolus, the exosome participates in ribosomal RNA (rRNA) processing, facilitated by Mtr4-dependent adaptors Utp18 and Nop53. In this manuscript, we have performed a comprehensive study that addresses the targeting mechanism and precise subcellular localization of all members of the Exo11 complex. We observed a high concentration of all Exo11 subunits in the nucleolus and identified the importins Srp1 (α) and Kap95 (β) as responsible for the nuclear import of Exo9 subunits. Notably, Exo9 subunits localization was not significantly disrupted in the

simultaneous absence of NLS-containing subunits Rrp6 and Rrp44, suggesting redundant nuclear import pathways for Exo9. Additionally, we show evidence that Ski7 may play a role in the Exo9 retention in the cytoplasm. To explore the exosome sub-nucleolar localization, we compared Rrp43 with nuclear exosome cofactors and show that it is enriched in the same nucleolar region as Mtr4 and Nop53. In conclusion, our findings provide a detailed characterization of Exo11 distribution, highlight the primary nuclear import mechanisms for Exo9, and reveal the specific localization of the exosome within the granular component (GC) of the yeast nucleolus, suggesting a spatial regulation of the RNA processing pathway.

Sponsors

We are grateful to our sponsors for their support of this meeting

Exhibitor Information

Stand 1
Thermofisher

Thermo Fisher Scientific is proud of our Mission: To enable our customers to make the world healthier, cleaner and safer. Through our electron microscopy solutions and expertise, we help customers accelerate innovation and enhance productivity across the life sciences. We offer a broad range of products covering all imaging scales from near-atomic resolution molecular data to tissue and whole cell ultrastructure. By revealing molecular detail at biologically relevant resolutions, we enable scientific discoveries and breakthrough drug discovery.

Stand 2
Immagina Biotechnology

Immagina mission is to advance RNA and ribosome biology by enabling scientists to study translation in action. We offer novel technologies—from high-throughput ribosome profiling, ribosome isolation and native-tRNA sequencing to cfRNA and RNA-mods detection—that provide new insights protein synthesis for our academic and pharma communities. We leverage this knowledge to co-create transformative treatments for diseases with unmet clinical needs.

Stand 3
Shanghai Tanon Life Science Co., Ltd

Shanghai Tanon Life Sciences Co., Ltd. was established in 1994 and is a manufacturer specializing in WB experimental instruments. Shanghai Tanon Life Sciences Co., Ltd. was established in 1994 and is a manufacturer specializing in WB experimental instruments. Our customers are mainly in China and the United States. Our customers have mentioned using our products in nearly 10,000 papers they have published, 20 of which are in Cell, Nature and Science.

Attendee List

List correct as at 28.08.25, please note you will only be included in the attendee list below if you ticked on the registration form that you would like to be included

Full Name	Company	Job Title	Registration contact email
Kohei Abe	Ludwig-Maximilians-Universität München	PhD Student	abe@genzentrum.lmu.de
Pablo Aguirre Azorín	CiMUS / University of Santiago de Compostela	PhD Researcher	pablo.aguirre.azorin@usc.es
Alexey Amunts	Munster University	Group Leader	alexey.amunts@gmail.com
Shajahan Anver	University College London	Research Associate	s.anver@ucl.ac.uk
Michele Arnoldi	Immagina Biotechnology	Tech Support and Product Specialist	michele.arnoldi@immaginabiotech.com
Julie Aspden	University of Leeds	PI	J.Aspden@leeds.ac.uk
Anaïs Astier	Centre de Biologie Intégrative	PhD Student	anais.astier@univ-tlse3.fr
Noa Avidan	Weizmann Institute of Science	Post-Doc	noa.stern@weizmann.ac.il
Taylor Ayers	Carnegie Mellon University	PhD Student	tayers@andrew.cmu.edu
Shivani Bajaj	Department CIBIO - University of Trento	PhD Student	shivani.bajaj@unitn.it
Roland Beckmann	Munich University LMU	Professor for Biochemistry	beckmann@genzentrum.lmu.de
Paola Bellosta	University of Trento	Associate Professor	paola.bellosta@unitn.it
Helmut Bergler	University Graz	Ao Univ. Prof. Dr.	helmut.bergler@uni-graz.at
Alexis Bertrand		Post Doc Trainee	abertrand2@northwell.edu
Lionel Blanc	Feinstein Institute for Medical Research	Professor	lblanc@northwell.edu
Sandra Blanco	CSIC	Tenured Scientist	sandra.blanco@usal.es
Manon Bongers	CBI Toulouse	PhD Student	manon.bongers@univ-tlse3.fr
Anthony Bougas	University of Edinburgh	Postdoctoral Research Associate	abougas@ed.ac.uk
Ahmed Boukerrou	CIMR - Haematology	Research Assistant	azb22@cam.ac.uk
Michael Buszczak	UT Southwestern Medical Center	Professor	Michael.Buszczak@UTSouthwestern.edu
Eliezer Calo	Massachusetts Institute of Technology	Associate Professor	calo@mit.edu
Mathieu Catala	Université de Sherbrooke	Research Agent	mathieu.catala@usherbrooke.ca
Tessa Chan	University of Sheffield	PhD Student	wcvchan1@sheffield.ac.uk

Ling-Ling Chen	Shanghai Institute of Biochemistry and Cell Biology	Principal Investigator	linglingchen@sibcb.ac.cn
Yi-Lan Chen	Johns Hopkins University	Post-Doc	ychen548@jh.edu
Shi-An Chen	Altos Labs Inc	Scientist	schen@altoslabs.com
Justina Chu	Université de Montréal	PhD Candidate	justina.chu@umontreal.ca
Chantal Clark	Sanquin Research / University Medical Center Utrecht	Post-Doc	chantalclark@me.com
Seth Corey	Cleveland Clinic	Professor and Staff	coreylab@mac.com
Shirley Daube	Weizmann Institute	Senior Staff Scientist	shirley.daube@weizmann.ac.il
Joey Davis	MIT	Associate Professor of Biology	jhdavis@mit.edu
Jef De Brabander	UT Southwestern Medical Center	Professor	Jef.DeBrabander@UTSouthwestern.edu
Kim De Keersmaecker	KU Leuven	Full Professor	kim.dekeersmaecker@kuleuven.be
Jesus De La Cruz	Institute of Biomedicine of Seville/University of Seville	Professor	jdlcd@us.es
Deborah Donzel	Fondazione Telethon ETS C.F. 04879781005	Post-Doc	donzel.deborah@hsr.it
Mercedes Dosil	University of Salamanca	Full Professor	mdosil@usal.es
Jan Ezberger	UT Southwestern Medical Center	Associate Professor	Jan.Erzberger@UTSouthwestern.edu
Alexandre Faille	University of Cambridge	Postdoctoral Scientist	RAF58@cam.ac.uk
William Faller	Netherlands Cancer Institute	Group Leader	w.faller@nki.nl
Bulat Fatkhullin	University of Leeds	Postdoctoral Researcher	b.fatkhullin@leeds.ac.uk
Juliette Fedry	MRC LMB	Programme Leader Track	jfedry@mrc-lmb.cam.ac.uk
Sébastien Ferreira-Cerca	Ecole polytechnique - UMR7654 (BIOC)	Principal Investigator	sebastien.ferreira-cerca@polytechnique.edu
Aroa Ferrer Pinós	CiMUS - Universidade de Santiago de Compostela	PhD Student	aroa.ferrer@rai.usc.es
Kamil Filipek	University of Bologna	Postdoctoral Fellow	kamil.filipek@unibo.it
Kurt Fredrick	Ohio State University	Professor	fredrick.5@osu.edu
Olivier Gadal	CBI Toulouse	PI	olivier.gadal@univ-tlse3.fr
Carla Veronica Galmozzi	Universidad de Sevilla	Post-Doc	cgalmozzi@us.es
Jianan Gao	Zhejiang University	PhD Student	12317020@zju.edu.cn
Tom Garnier	Ecole polytechnique - UMR7654 (BIOC)	PhD Student	tom.garnier@polytechnique.edu

Alexander Geiger	University of Zurich	Post-Doc	alexandergregor.geiger@uzh.ch
Amee George	The Australian National University	Associate Professor in Pharmacogenomic Technologies	amee.george@anu.edu.au
Abril Gijsbers Alejandre	National Autonomous University of Mexico	Assistant Professor	abril@bq.unam.mx
Pierre-Emmanuel Gleizes	University of Toulouse	Professor	pierre-emmanuel.gleizes@univ-tlse3.fr
Jacob Gordon	National Institute of Environmental Health Sciences	Postdoctoral Fellow	jacob.gordon@nih.gov
Anastasia Goryanin	CIMR	Researcher	ag2544@cam.ac.uk
Mantas Gudelis	University of Cambridge	PhD Student	mpg62@cam.ac.uk
Holly Guo	UT Southwestern Medical Center	Graduate Student	holly.guo@utsouthwestern.edu
Bilal Hafeez	UTRGV Biomedical Research Facility	Assistant Professor	bilal.hafeez@utrgv.edu
Ross Hannan	Australian National University	Academic Lead, Health and Medicine	ross.hannan@anu.edu.au
Anthony Henras	CBI Toulouse	CNRS Research Director	anthony.henras@univ-tlse3.fr
Yves Henry	CBI/CNRS/Toulouse University	Group Leader	yves.henry@univ-tlse3.fr
Hauke Hillen	University Medical Center Göttingen	Professor	hauke.hillen@med.uni-goettingen.de
Yuya Hirooka	Keio University	Postdoctoral Researcher	hirooka@keio.jp
Wenjing Huang	University of Glasgow	PhD student	2470980h@student.gla.ac.uk
Sebastian Iben	University of Ulm	Principal Investigator	sebastian.iben@uni-ulm.de
Deena Iskander	Imperial	Consultant	d.iskander@imperial.ac.uk
Vinithra Iyer	Weizmann Institute of Science	Post Doctoral Fellow	vinithra.iyer@weizmann.ac.il
Pekka Jaako	University of Gothenburg	Researcher	pekka.jaako@gu.se
Jianhang Jia	University of Kentucky	Professor	jianhang.jia@uky.edu
Dimitris Kanellis	Karolinska Institutet	Researcher	dimitris.kanellis@ki.se
Felipe Karam Teixeira	University of Cambridge	Assistant Professor	fk319@cam.ac.uk
Katrin Karbstein	Vanderbilt University	Professor	katrin.karbstein@vanderbilt.edu
Vasileios Kargas	Cambridge Institute for Medical Research	Post-Doc	vk335@cam.ac.uk
Barış Keleş	İzmir Biomedicine and Genome Institute	M.Sc. Student	bariskeles04@gmail.com
Anas Khawaja	Karolinska Institutet	Research Specialist	anas.khawaja@ki.se

Marianthi Kiparaki	Biomedical Sciences Research Centre, "Alexander Fleming"	Junior Group Leader	kiparaki@fleming.gr
Sebastian Klinge	The Rockefeller University	Professor	klinge@rockefeller.edu
John Knight	University of Manchester	Lecturer in Cancer Biology	john.knight@manchester.ac.uk
Takehiko Kobayashi	University of Tokyo	Professor	tako2015@iqb.u-tokyo.ac.jp
Zornitsa Kotopanova	University of Manchester	PhD Student	zornitsa.stoichkova@postgrad.manchester.ac.uk
A Akhil Kumar	National Institute of Science Education and Research	PhD Scholar	aakhil.kumar@niser.ac.in
Denis Lafontaine	Université libre de Bruxelles	Professor	denis.lafontaine@ulb.be
Dorthe Helena Larsen	Danish Cancer Society	Group Leader	dhl@cancer.dk
Isabella Lawrence	Yale University	Postdoctoral Associate	isabella.lawrence@yale.edu
Simon Lebaron	CBI Toulouse	Researcher	simon.lebaron@univ-tlse3.fr
Sharonda LeBlanc	NC State University	Assistant Professor	sharjohn1@gmail.com
Tina Lence	University of Graz / Institute of Molecular Biosciences	Post-Doc	tina.lence@uni-graz.at
Nicolas Leulliot	Université Paris Cité - Site PRG	Professor	nicolas.leulliot@u-paris.fr
Jeffrey Lipton	Feinstein Institutes for Medical Research, Zucker School of Medicine at Hofstra/Northwell	Professor	jlipton@northwell.edu
Yajuan Liu	University of Kentucky	Senior Research Associate	yajuan.liu@uky.edu
Gabriella Lopez Perez	Massachusetts Institute of Technology	Graduate Student	gslopez@mit.edu
Anders Lund	Biotech Research and Innovation Centre, University of Copenhagen	Director	anders.lund@bric.ku.dk
Désirée Marchand	ETH	Post-Doc	desiree.marchand@mol.biol.ethz.ch
Federica Marchesini	University of Bologna	PhD Student	federica.marchesini9@unibo.it
Faraz Mardakheh	University of Oxford	Associate Professor	faraz.mardakheh@bioch.ox.ac.uk
Nadine Martin	Cancer Research Center of Lyon	INSERM Research Scientist	nadine.martin@lyon.unicancer.fr
Sara Martín-Villanueva	University of Seville	Post-Doc	smartin9@us.es
Ivan Matic	Institut Cochin - Inserm U1016	Research Director	ivan.matic@inserm.fr
Tom Meier	Albert Einstein College of Medicine	Professor	tom.meier@einsteinmed.edu
Chengwei Meng	Shanghai Tanon life Science Co., LTD.	Sales Director	Jack@biotanon.com

Davide Merulla	Immagina Biotechnology	Inside Sales & Business Development Specialist	davide.merulla@immaginabiotech.com
Michal Minczuk	University of Cambridge	Professor	mam201@cam.ac.uk
Valentin Mitterer	Institute of Molecular Biosciences	Principal Investigator	valentin.mitterer@uni-graz.at
Lorenzo Montanaro	Bologna University	Professor	lorenzo.montanaro@unibo.it
Frédéric Murisier	Unil	PhD Student	frederic.murisier@unil.ch
Diu Nguyen	Barts Cancer Institute	Group Leader	t.d.nguyen@qmul.ac.uk
Henrik Nielsen	Department of Cellular and Molecular Medicine, University of Copenhagen	Professor	hamra@sund.ku.dk
Deepak Nijhawan	University of Texas Southwestern Medical Center	Professor	deepak.nijhawan@utsouthwestern.edu
Marlene Oeffinger	Montreal Clinical Research Institute	Associate Professor	oeffinm@gmail.com
Carla Oliveira	University of Sao Paulo	Professor	ccoliv@iq.usp.br
Joaquin Ortega	McGill University	Professor	joaquin.ortega@mcgill.ca
Manuel Palacios Perez	CIEMAT/CIBERER/IIS-FJD, UAM		Manuel.Palacios@externos.ciemat.es
Konstantin Panov	Queen's University Belfast	Senior Lecturer in Cell Signalling	k.panov@qub.ac.uk
Styliani Papadaki	Karolinska Institutet	Postdoctoral Researcher	styliani.papadaki@ki.se
Julien Papoin	Northwell	Associate Investigator	jpapoin@northwell.edu
Vikram Paralkar	University of Pennsylvania	Assistant Professor of Medicine	Vikram.Paralkar@pennmedicine.upenn.edu
Reejuana Parveen	University of Cambridge	PhD Student	rp670@cam.ac.uk
Marianna Penzo	University of Bologna	Associate Professor	marianna.penzo@unibo.it
Brigitte Pertschy	University of Graz	Research Group Leader	brigitte.pertschy@uni-graz.at
Célia Plisson-Chastang	CBI Toulouse	Principal Investigator	celia.plisson-chastang@univ-tlse3.fr
Sofia Quinodoz	Princeton University	Postdoctoral Fellow	quinodoz@princeton.edu
Dorota Raczynska	Adam Mickiewicz University in Poznan	Associate Professor	doracz@amu.edu.pl
Vardhman Rakyan	QMUL	Professor	v.rakyan@qmul.ac.uk
Venki Ramakrishnan	MRC Laboratory of Molecular Biology	Staff	ramak@mrc-lmb.cam.ac.uk
Pedro Rebelo-Guiomar	University of Cambridge	PostDoc / Research Associate	pfrg2@cam.ac.uk
Margaret Rodgers	National Institutes of Health	Investigator	margaret.rodgers@nih.gov
Joanna Rorbach	Karolinska Institutet	Associate Professor	joanna.rorbach@ki.se
Florine Roses	CBI Toulouse	PhD Student	florine.roses@univ-tlse3.fr

Michael Ruoss	University of Zurich	PhD Student	mruoss@imm.uzh.ch
Nuria Sánchez Puig	UNAM	Principal Investigator	nuriasp@unam.mx
Elaine Sanij	St Vincent's Institute for Medical Research	Laboratory Head	esanij@svi.edu.au
Sheetanshu Saproo	Karolinska Institutet	Postdoctoral Fellow	sheetanshu.saproo@ki.se
Helgo Schmidt	CERBM GIE	Team Leader	schmidth@igbmc.fr
Kate Schole	Johns Hopkins School of Medicine	MD-PhD student	kschole1@jh.edu
Savannah Seely	National Institute of Environmental Health Sciences	Post Doctoral Fellow	seelysm@nih.gov
Lin Shan	Shanghai Institute of Biochemistry and Cell Biology	Postdoctoral Fellow	shanlin2017@sibcb.ac.cn
Dhriti Singh	Umeå Plant Science Centre	Post-Doc	dhriti.singh@umu.se
Sameer Singh	Charité-Universitätsmedizin Berlin	Group Leader	sameer-kumar.singh@charite.de
Maria Siomos	European Research Council	Scientific Officer	maria.siomos@ec.europa.eu
Teun Slijkerman	University Medical Center Utrecht / Sanquin Research	PhD candidate	t.slijkerman@sanquin.nl
Merav Socolovsky	UMASS Chan Medical School	Professor	merav.socolovsky@umassmed.edu
Robin Stanley	National Institute of Environmental Health Sciences/National Institutes of Health	Senior Investigator	robin.stanley@nih.gov
Baochun Su	Zhejiang University	PhD Candidate	12117014@zju.edu.cn
Sophie Tagnères	Department of Molecular Biology	Post-Doc	sophie.tagneres@med.uni-goettingen.de
Shengjiang Tan	Univeristy of Cambridge	Senior Research Associate	st299@cam.ac.uk
Rebecca Thompson	Thermo Fisher Scientific	Director Business Development	rebecca.thompson@thermofisher.com
Matthias Thoms	Gene Center and Department of Biochemistry	Staff Scientist	thoms@genzentrum.lmu.de
Herbert Tschochner	University of Regensburg	Professor	h.tschochner@mac.com
Marien van der Stel	ErasmusMC	PhD Student	h.vanderstel@erasmusmc.nl
Deepika Velayudhan	Institut de génétique et de biologie moléculaire et cellulaire	PhD Student	velayudd@igbmc.fr
Giulia Venturi	University of Bologna	Post-Doc	giulia.venturi13@unibo.it
Adrianna Vlachos	Johns Hopkins All Children's Hospital	Chief of Hematology	avlacho1@jhmi.edu
Tobias von Arx	University of Zuerich	Scientific Employee	tobias.vonarx2@uzh.ch
Vassie Ware	Lehigh University	Professor of Molecular Biology	vcw0@lehigh.edu

Benjamin Warner	The Ohio State University	Postdoctoral Scholar	warner.474@osu.edu
Alan Warren	CIMR - Cambridge	Professor of Haematology	ajw1000@cam.ac.uk
Martin Weissenborn	University Halle	Professor	martin.weissenborn@chemie.uni-halle.de
John Woolford	Carnegie Mellon University	Professor	jw17@andrew.cmu.edu
Mengjie Wu	University of Hong Kong	PhD Student	meng2020@connect.hku.hk
Yongpan Xu	Zhejiang University	PhD	12307014@zju.edu.cn
Harunori Yoshikawa	Keio University	Project Lecturer	hyoshikawa@keio.jp
Hongyan Yu	Zhejiang University	PhD	3500549315@qq.com
Ulrika Yuan	Department of Biochemistry	Post-Doc	cy293@cam.ac.uk
Zaka Wing Sze Yuen	Australian National University	Post-Doc	zaka.yuen@anu.edu.au
Federico Zacchini	IRCCS Azienda Ospedaliero-Universitaria di Bologna	Health Researcher	federico.zacchini@aosp.bo.it
Xin Zhang	Westlake University	Professor	zhangxin@westlake.edu.cn
Shuyi Zhao	Zhejiang University	Research Fellow	syzhao@zju.edu.cn
Xiaohan Zhao	EMBL	Graduate Student	xzhao@embl.de
Haozhe Zhu	Zhejiang University	PhD Candidate	12217012@zju.edu.cn
Ximena Zottig	UMass Chan Medical School	Post-Doc	ximena.zottig@umassmed.edu

10 ways to engage with the Biochemical Society

We've been at the forefront of advancing molecular bioscience for over 100 years, facilitating the sharing of knowledge and expertise, and supporting molecular bioscientists across all career stages. Join our community and get involved in an exciting range of activities to match your interests!

Become a member
Join our diverse community from across the world and expand your molecular biosciences network.

Attend an upcoming event
Present your work, network with experts or propose a topic with our extensive range of scientific conferences and training. Members get discounted rates.

Apply for a grant or bursary
Secure funding to participate in meetings, develop new skills, undertake research and enhance your scientific events and outreach projects.

Publish in one of our journals
Featuring world-leading research and reviews, share your expertise to drive forward molecular bioscience research.

Write for The Biochemist
Share news and views on current scientific topics, careers, policy, outreach and more in our community magazine.

Participate in a webinar
Discuss your latest insights, educate participants on new technologies or enhance your skills by attending or presenting in our free webinar series.

Explore our resources
Discover useful advice and practical support to help you transition through a variety of careers, studies, and opportunities.

Nominate a colleague
Celebrate excellence across the molecular biosciences by nominating an outstanding individual or team for a Biochemical Society Award.

Join a committee
Get involved with one of our committees, panels, or boards to influence activities in EDI, grants, publications, scientific meetings, education and more.

Become an advocate
Promote our activities through your networks and help us support your community.

Follow us

Visit our website to find out more
biochemistry.org