OddPols 2025

Stowers Institute

Kansas City, Missouri, USA
May 29 - June 1, 2025

#OddPols25

Program

Thursday, May 29	**Stowers Institute for Medical Research** **1000 E 50th St, Kansas City, MO 64110**
3.00pm – 5.00pm	**Check-in, hang posters.** **Free parking is available in the Stowers Institute parking garage.**
5.00pm – 7.00pm	**Structure and Function of OddPols**
	Session Chair: Paul Trainor
	Keynote – Dr Christoph Mueller, EMBL "Structural biology of RNA polymerase I and III transcription" (25 + 5)
	Herbert Tschochner, University of Regensburg, "Why RNA polymerase I can make Christmas trees" (15+5)
	Andreas Schmidbauer, University of Regensburg, "Single-molecule FRET experiments with fluorescently labeled human RNA polymerase I reveals dynamics of the A49 subunit" (15+5)
	Xiaochan Zhao, EMBL, "Structural reorganization of the nucleolus and change in ribosome assembly upon Pol I inhibition with Actinomycin D" (15+5)
7.00pm - 9.00pm	Welcome Reception and Posters (odd)
Friday, May 30	**Stowers Institute for Medical Research**
9.00am - 10.30am	**Prokaryotic, Virus and Plant RNA Polymerases**
	Session Chair: Jennifer Gerton
	Keynote – Dr Craig Pikaard, Indiana University (25 + 5)
	Ramya Enganti, Indiana University, "Evidence for inside-out upregulation of nucleolus organizers in Arabidopsis" (15+5)
	Clemens Grimm, University of Wuerzburg, "Host tRNA(Gln) acts as an assembly chaperone for the poxviral early transcription machinery" (15+5)
	Akihito Fukudome, Indiana University, "Rules of RNA polymerase V transcription elongation" (15+5)
10.30am - 11.00am	Morning Break, All-conference picture on the stairs
11.00am - 12.30pm	Session Chair: Ritwick Sawarkar
	Kyleigh Castillo, University of Alabama, "Transcription sabotage: nucleotide analog drugs vs. Mtb RNAP" (15+5)

Alexandra Harte, Colorado State University,
"Tight coupling of transcription and translation regulates archaeal gene expression" (15+5)

Stefan Jungwirth, University of Wurzburg,
"A unique mechanism of promoter recognition enables poxvirus intermediate transcription" (15+5)

Feng Wang, Indiana University,
"Evidence for TFIIB and TFIIH-like activities in the RNA polymerase V transcription factor DRD1" (15+5)

12.30pm - 1.30pm Lunch

1.30pm - 3.00pm **Mechanisms Regulating Transcription and Translation**

Session Chair: Olivier Gadal

Paul Pease, LUMICKS,
"A single-molecule platform for dissecting the molecular mechanisms of transcription" (15+5)

Fred van Leeuwen, Netherlands Cancer Institute,
"Recruitment of Fpt1 to tRNA genes in budding yeast independently requires the RNA polymerase III general transcription factors TFIIIB and TFIIIC" (15+5)

Rajendra KC, University of Illinois Urbana-Champaign,
"Genomic metamap of RNA polymerase I, II, and III reveals RNA polymerase III occupancy and transcription at protein coding genes promoter" (15+5)

Ewan Ramsey, Human Technopole,
"Structural insights into distinct mechanisms of RNA polymerase II and III recruitment to snRNA promoters" (15+5)

3.00pm - 3.30pm Afternoon Break, Speaker picture on the stage

3.30pm - 5.00pm Session Chair: Jessica Finlay-Shultz

Kevin Van Bortle, University of Illinois Urbana-Champaign,
"An expanding universe of RNA polymerase III transcription, regulation, and function" (15+5)

Ruiying Cheng, University of Illinois Urbana-Champaign,
"The NuRD chromatin remodeling complex regulates tRNA genes through direct interactions with RNA polymerase III" (15+5)

Sahil Shah, University of California, Berkeley,
"RNA polymerase III transcription-associated polyadenylation promotes the accumulation of noncoding retrotransposons during herpesviral infection" (15+5)

Brandon Fagen, Stowers Institute,
"5S rRNA regulatory changes in fission yeast are dynamic and rapid" (15+5)

5.00pm - 7.00pm Reception and Posters

7.00pm - 9.00pm Dinner

May 31 Saturday **Stowers Institute for Medical Research**

9.00am - 10.30am **Genome Organization, Nucleolar Structure**

Session Chair: Elif Sarinay Cenik

Keynote – Dr Susan Baserga, Yale University (25 + 5)

Kamena Kostova, Stowers Institute,
"Ribosome heterogeneity in zebrafish" (15+5)

Paxton Kostos, Stowers Institute,
"Genetic and epigenetic features of rRNA gene arrays impact rRNA pools and chromosome organization" (15+5)

Anastasia McKinlay, Indiana University,
"Higher-order chromatin organizations of highly repetitive NOR, 5S gene, and centromeric loci in Arabidopsis" (15+5)

10.30am-11.00am	Morning Break
11.00am-12.30pm	Session Chair: Kevin Van Bortle
	Anaswara Sugathan, Stockholm University, "Dysregulated ribosomal transcription due to reduced levels of WSTF in the chromatin remodelling complex B_WICH induces the integrative stress response" (15+5)
	Mareike Polenkowski, Stockholm University, "IGS38s ncRNA from human rDNA intergenic spacer regulates rDNA chromatin organization and 45S rRNA transcription" (15+5)
	Olivier Gadal, University of Toulouse, "Ribosomal RNA synthesis by RNA polymerase I is subject to premature termination of transcription" (15+5)
	Maria Jose Blanco Salazar, Stowers Institute, "Identifying factors involved in the codon optimality mechanism in human cells" (15+5)
12.30pm - 1.30pm	Lunch
1.30pm - 3.00pm	**Odd Pols in Development and Genetic Disorders**
	Session Chair: Bruce Knutson
	Elif Sarinay Cenik, University of Texas at Austin, "A ribosome-biogenesis-independent role of RNA pol I and nucleolar integrity in the maintenance of proper germline H3K4 remodeling and oogenesis" (15+5)
	Rita Ferreira, Australian National University, "In vivo functions of UBF1 and UBF2 isoforms" (15+5)
	Lorena Maili, Stowers Institute, "Ribosome biogenesis is essential for neural crest cell differentiation and craniofacial cartilage and bone development" (15+5)
	Jonathan Chung, SUNY Upstate Medical University, "Awakening mutant p53 with the RNA Polymerase 1 inhibitor BMH-21" (15+5)
	Afternoon Break, Speaker picture on the stage
3.00pm - 3.30pm	Session Chair: Herbert Tschochner
3.30pm - 5.00pm	**Ritwick Sawarkar**, University of Cambridge, "A spatiotemporal expression atlas of mouse tRNA genes" (15+5)
	Kristin Watt, University of Colorado, "RNA polymerase III subunit Polr3a is required for craniofacial cartilage and bone development" (15+5)
	Nazif Alic, University College London, "Loss of pol III repressor Maf1 in neurons promotes longevity by preventing the age-related decline in 5S rRNA and translation" (15+5)
	Jessica Finlay-Schultz, University of Colorado, "Maf1 cooperates with progesterone receptor to repress RNA polymerase III transcription of tRNAs" (15+5)
	Take down posters
5.00pm	Dinner on own
June 1 Sunday	**Nelson Atkins Museum of Art,** **4525 Oak St, Kansas City, MO 64111** **Parking Fee: $14/per vehicle**
	Odd Pols in Cancer and Diseases
10.00am-12.00pm	Session Chair: Kristin Watt
	Keynote – Dr Ian Willis, Albert Einstein College of Medicine, "Mouse models of Polr3-related disease reveal neurodevelopmental and neurodegenerative phenotypes" (25 + 5)

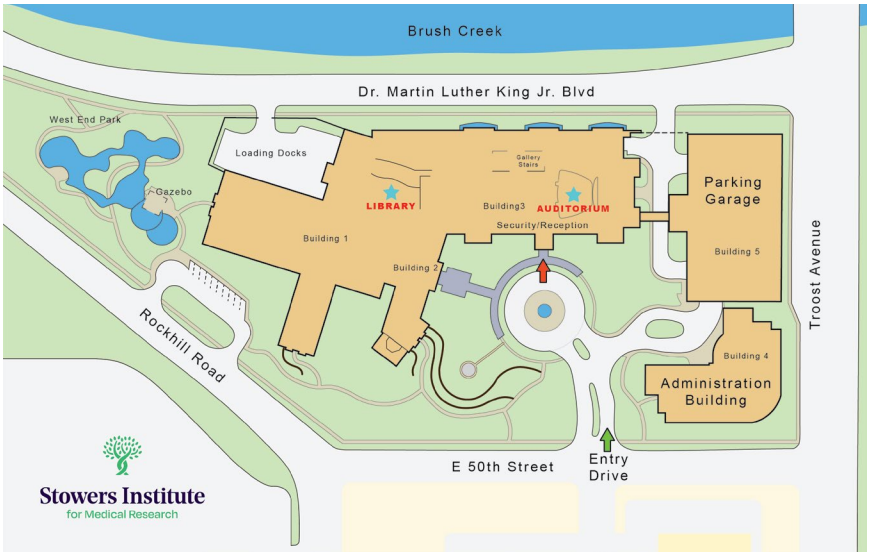
	Shalini Sundramurthi Chelliah, St. Vincent's Institute of Medical Research, "Targeting the nucleoli as a strategy to treat ovarian cancer" (15+5)
	Lalita Shevde-Samant, University of Alabama, "Targeting ribosomal RNA biogenesis mitigates hypoxia-induced tumor progression and functionally reprograms tumor-associated macrophages in breast cancer" (15+5)
	Konstantin Panov, Queen's University Belfast, "Expanding horizon: Novel Pol I inhibitor PMR-116 demonstrates versatile anti-tumour activity across a broad spectrum of high MYC malignancies" (15+5)
12.30pm - 1.30pm	Lunch + Museum Free Time
1.30pm - 3.00pm	**New and Emerging Ideas in Odd Pols Biology and Function**
	Session Chair: Rita Ferreira
	Sheetanshu Saproo, Karolinska Institutet, "Genome-wide CRISPR screen identifies regulators of POLR1A turnover" (15+5)
	Catarina Mendes Felgueira, University of Alabama, "Unlocking the power of long read sequencing to study regulation of rRNA synthesis" (15+5)
	Michay Diez, Stowers Institute, "Evolution of ribosome heterogeneity in teleosts" (15+5)
	Wenjun Fan, Johns Hopkins University, "Inactivation of mTOR is a strategy for tumor resistance to ribosome biogenesis inhibition" (15+5)
Closing Remarks	Paul Trainor and Jennifer Gerton
3.00pm-3.30pm	Afternoon Tea
3.30pm-5.00pm	Museum Free Time
6.30pm-9.30pm	Conference Banquet Dinner – catch the shuttle from your hotel 5:45 pm Intercontinental, 6 pm Courtyard Marriott Jack Stack BBQ Restaurant, Freight House, 101 W 22nd St. #300 Return shuttles will depart the restaurant at approximately 9.30pm

Speaker Instructions

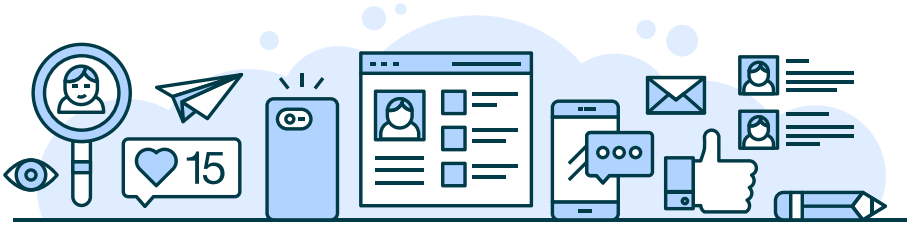
On Thursday, Friday and Saturday, speakers **must upload their presentations at the podium** prior to their scheduled sessions.

Speakers **must get equipped with a microphone at the podium** 15 minutes prior to their scheduled sessions.

Campus Map



SRC Social Media Guidelines



The Conference organizers **encourage the use of social media to share information and network with other attendees.**

We remind you to **remain courteous and respectful in your comments and posts.**

Avoid sharing speaker or poster presentation content that's not your own, copyrighted or trademarked, or material protected by other intellectual property rights.

Follow and tag **@stowersinstitute** on Instagram

Follow and tag **Stowers Institute for Medical Research** on LinkedIn

Follow and tag **@ScienceStowers** on X (Twitter)

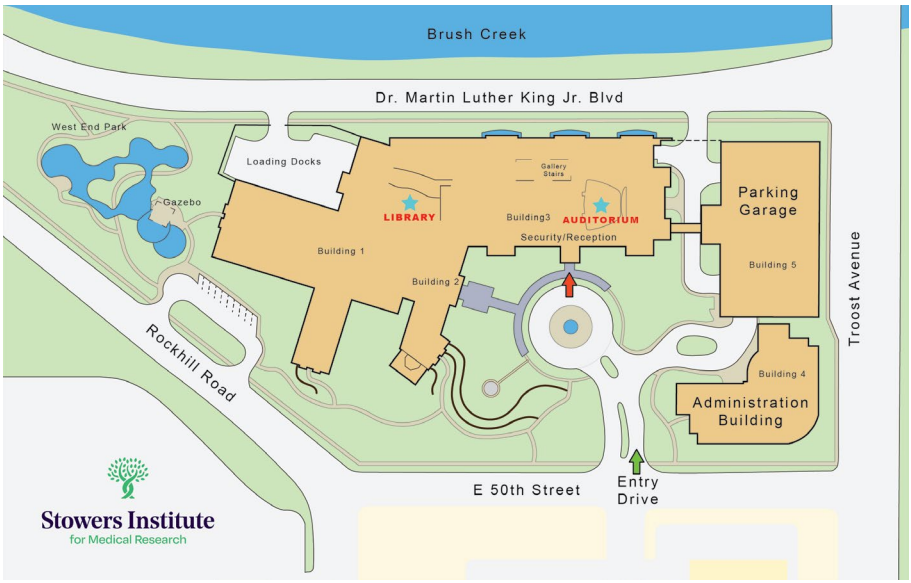
Use hashtag **#oddpols25**

Attendee Resources

Location: Stowers Institute for Medical Research – 1000 E. 50th Street, Kansas City, MO 64110
General Phone Number: 816-926-4000
Driving directions from Kansas City International Airport:
(approximately 30 minutes under normal driving conditions)

1. Follow the signs on International Circle to the airport exit.
2. Take Cookingham Drive to I-29 South (right ramp), heading toward Kansas City.
3. Continue to follow I-29 South as it merges into I-29 South 71 (merge in left lane), then I-35South (merge in left lane again).
4. Cross the Paseo Bridge and get in the lane for I-70 South 71 (Exit 3)
5. Merge right and follow sign to South 71 Highway (Exit 2M).
6. Take the Emmanuel Cleaver II Boulevard exit and turn right onto Emmanuel Cleaver II Boulevard.
7. Follow Cleaver II to Troost Avenue and turn left on Troost.
8. Turn right on 50th Street and take another immediate right at the Stowers Institute's entrance (1000 E. 50th Street, Kansas City, MO 64110).

Campus Map



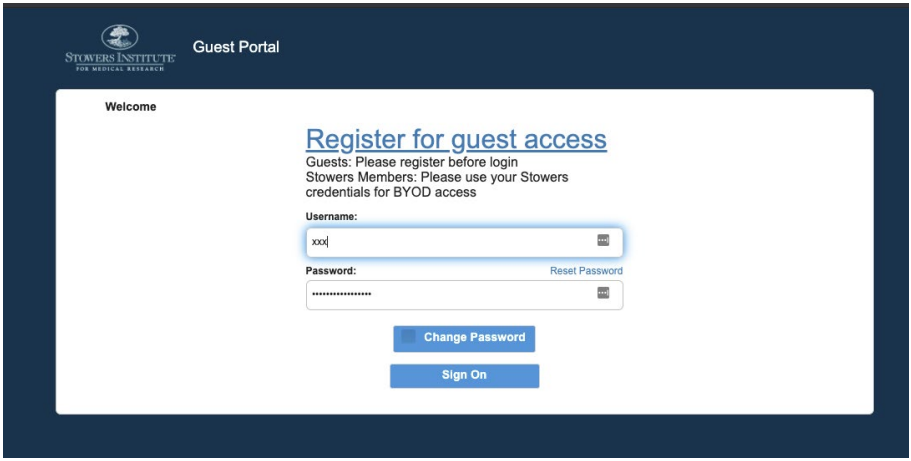
Parking: Visitor parking is available in the parking structure located between the Administration Building (to your right as you enter the campus) and the Research Building (to your left). Please park on the 5th floor of the parking garage and take the elevator down to the 1st floor to enter the Research Building.

Restrooms: Located by the two large planters on the 1st floor of the Research Building. All-gender restrooms are available at the base of the stairs on the B1 level.

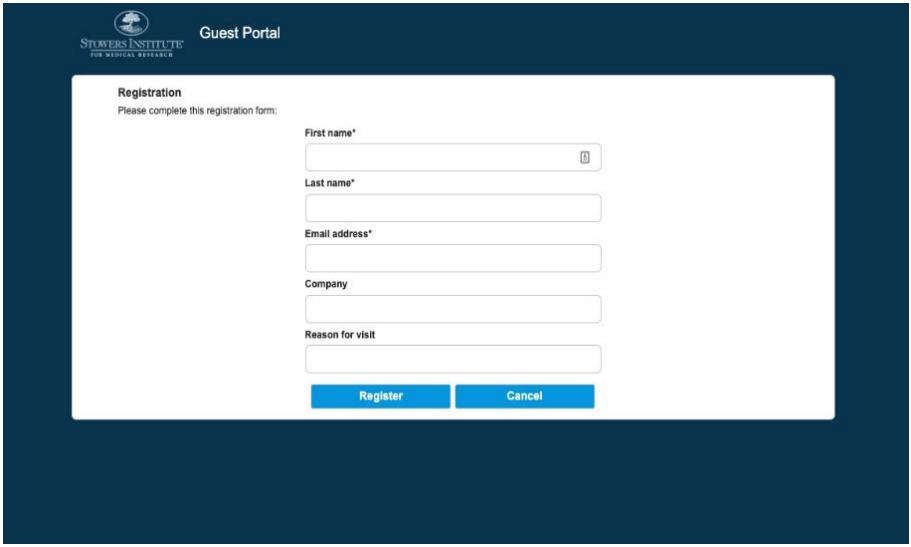
Mother's Room: At the base of the stairs on the B1 level, located next to the all-gender restrooms. An entry key will be provided by security to guests that request to use it.

Tobacco Free Campus: Tobacco of any kind is prohibited in all the Institute facilities and on the Institute grounds. The Institute does not offer any designated smoking areas.

Wireless Access: Stowers Guests may connect to the **stowers_guest SSID**
When connected, you will see a captive portal. Click on the link at the top of the page to register for guest access.

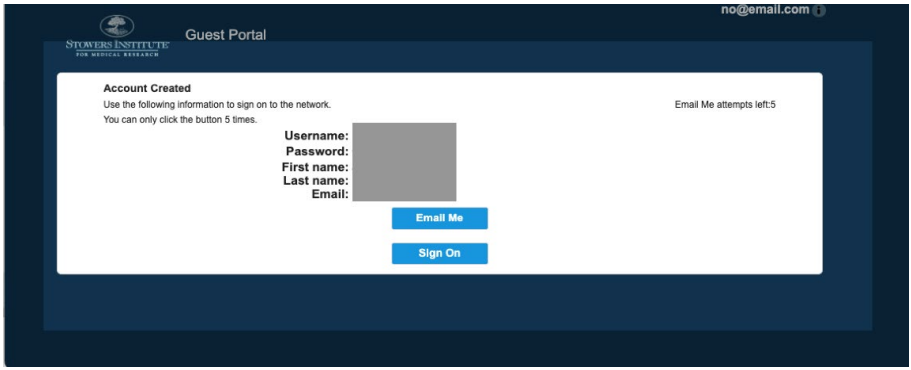


- You will be prompted to enter their information. Name and email are the only required fields.
- After entering the required information click Register.



- You will see a page like this with sign on information. You can use the userid and password to connect other devices without going through the registration, by entering the userid and password in the first page of the captive portal.

- Click the "Sign On" button.



After clicking sign on, you will be prompted to accept the "Acceptable use Policy". After clicking accept you should now have internet access.

If you have any questions, please contact the Stowers Help Desk at 816-926-4150.

Kansas City Fun: The Kansas City area offers a wealth of cultural, educational, and entertaining opportunities to explore, many of which are inexpensive or free of charge. The following is a brief listing of local attractions, event calendars, and resources.

Useful Resources
Information about Kansas City and the surrounding area can be found at kcmo.gov or at visitkc.com. Two publications to help you discover things to do in the area are: "Insider's Guide to Kansas City" by Katie van Luchene and "Day Trips from Kansas City" by Shifra Stein. Both can be found in local bookstores.

Linda Hall Library
5109 Cherry Street, 816.363.4600
The Linda Hall Library in Kansas City is one of the world's foremost independent science research libraries. Founded in 1946 through a philanthropic bequest, it houses vast collections spanning science, engineering, and technology, serving researchers, students, and the public. Known for its international research programs and collaborations, the Library promotes lifelong learning with innovative programming, digital access, and educational resources. The Library also maintains its grounds as a public urban arboretum, offering both scholarly and community engagement opportunities.
www.lindahall.org

Nelson-Atkins Museum of Art
4525 Oak Street, 816-751-1278
Opened in 1933, the Nelson-Atkins Museum has more than 50 galleries and several period rooms. The museum's outstanding feature is its collection of Asian art. The collection of Chinese landscape paintings is one of the finest in the West, and the museum's holdings of Chinese ceramics and decorative arts are also noteworthy. Besides European paintings from the Renaissance on, the museum also has notable collections of ancient Egyptian sculpture, Japanese porcelains and lacquer, and English pottery. The E.F. Pierson Sculpture Garden was dedicated in 1972, and the Henry Moore Sculpture Garden opened in 1989. Admission is free.
nelson-atkins.org

Kemper Museum of Contemporary Art
4420 Warwick, 816-753-5784
Founded in 1994, the Kemper Museum of Contemporary Art presents modern and contemporary art of the highest quality and significance. It collects, preserves, documents, interprets, and exhibits a growing permanent collection; develops and presents special exhibitions; and offers a variety of educational programs. Admission is always free, and the Museum serves a diverse and inclusive public population.
kemperart.org

Union Station
30 West Pershing Road, 816-460-2020
This fully restored 1914 landmark is Kansas City's most prominent destination for entertainment and cultural activities. The Station is home to a permanent rail exhibit with vintage rail cars, an interactive science center, a vibrant Theater District featuring giant screen movies and live theater, fine restaurants, unique shops, spaces for meetings and events and much more. Of course, you can still catch the train at Union Station, once again among Amtrak's busiest stops.
unionstation.org

Loose Park
Intersection: Wornall Road and 51st Street, 816-784-5300
Loose Park is one of Kansas City's most beautiful parks. The park is home to a lake, a walking path, a shelter house, Civil War markers, tennis courts, a wading pool, picnic areas and the famous Rose Garden. The Rose Garden is popular for all types of outdoor special events including theatrical performances and wedding ceremonies.

Westport
Westport is one of Kansas City's premier destinations for dining, shopping, site seeing and is the heart of the city's nightlife. Located in the midtown, Westport is just north of the Country Club Plaza and a few miles south of downtown Kansas City. Historically, Westport was built along the Santa Fe Trail as an outfitting center for wagon trains heading west. Today the area is filled with renovated and new buildings housing trendy shops, restaurants, and nightspots.

ABSTRACTS – ORAL PRESENTATIONS

Structural biology of RNA polymerase I and III transcription

Christoph W. Müller[1]

[1]European Molecular Biology Laboratory (EMBL), Molecular Systems Biology Unit, Heidelberg, Germany

Eukaryotic gene expression requires transcriptional activities of RNA polymerase (Pol) I, Pol II and Pol III with Pol I synthesizing pre-ribosomal RNA, Pol II synthesizing mRNAs and various non-coding RNAs, and Pol III synthesizing small, structured RNAs including tRNAs and 5S ribosomal RNA [1]. During the last decades, structural biology has provided a wealth of insights into the structure and function of Pol I and Pol III and their general transcription factors during transcription initiation, elongation and termination. Initial analyses had been mainly focusing on Pol I and Pol III transcription complexes from S. cerevisiae, but are now extended towards other organisms including human. In fact, detailed structural information about human Pol I and Pol III transcription complexes help targeting them by small molecules as possible anti-cancer drugs. In addition, cryo-correlative light/electron-microscopy (cryo-CLEM) and cryo-electron tomography (cryo-ET) show promise analyzing Pol I and Pol III transcription complexes in the cellular context. Our presentation will summarize recent advances in the structural biology of Pol I and Pol III transcription including our in situ work on Pol I transcription and ribosome biogenesis in the nucleolus of HeLa cells.

[1] Girbig M., Misiaszek A.D., Müller C.W. (2022). Structural insights into nuclear transcription by eukaryotic DNA-dependent RNA polymerases. Nat. Rev. Mol. Cell. Biol. 23, 603-622.

Why RNA polymerase I can make Christmas trees

Herbert Tschochner[1], Katrin Schwank[1], Catharina Schmid[1], Tobias Fremter[2], Olivier Gadal[3], Felix Grünberger[4], Philipp Milkereit[5], Joachim Griesenbeck[5]

[1]Regensburg Center of Biochemistry (RCB) Universität Regensburg, 93053 Regensburg, Germany, [2]Biochemistry III, Regensburg Center of Biochemistry (RCB) Universität Regensburg, 93053 Regensburg, Germany, [3]Molecular, Cellular and Developmental Biology Unit (MCD), Centre de Biologie Intégrativ (CBI), Université de Toulouse, CNRS, UPS, 31062 Toulouse, France, [4]Microbiology, Regensburg Center of Biochemistry (RCB) Universität Regensburg, 93053 Regensburg, Germany, [5]Biochemistry III, Regensburg Center of Biochemistry (RCB) Universität Regensburg, 93053 Regensburg

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 subunit 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 opimize 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 domain of Rpa34.5 and Rpa49 support cleavage and transcription fidelity. We peresent a model, how the dynamic interaction of lobe binding domains mediates optimal RNA synthesis adapted to different transcription conditions.

Single-molecule FRET experiments with fluorescently labeled human RNA polymerase I reveals dynamics of the A49 subunit

Andreas Schmidbauer[1], Julia L. Daiß[2], Gerti Beliu[3,4], Christoph Engel[2], Dina Grohmann[1]

[1]Institute of Microbiology & Archaea Centre, Single-Molecule Biochemistry Lab, University of Regensburg, [2]Regensburg Center for Biochemistry, University of Regensburg, [3]Rudolf Virchow Center for Integrative and Translational Bioimaging, Chemical Biology and Biochemistry, University of Würzburg, [4]Department for Chemistry and Pharmacy, Bioimaging, University of Regensburg

Ribosomal RNAs (rRNA) are the main structural and catalytic component of ribosomes. RNA polymerase I (Pol I) is highly specialized on producing rRNA making it a vital factor in cellular homeostasis. A unique feature of the Pol I enzyme are the two subunits A49 and A34 that are stably integrated into the RNA polymerase core enzyme that is shared between Pol I, II and III. A49 is an essential subunit of Pol I that stabilizes the formation of the transcription initiation complex and drives transition from initiation to the elongation phase of transcription, ensuring efficient rRNA synthesis. Single-molecule FRET (smFRET) studies on transcriptional systems using prokaryotic and lower eukaryotic RNA polymerases have been proven as highly useful to gain a molecular understanding of the complex transcription process and the dynamic aspects of transcription. However, single-molecule studies of the human Pol I transcription process are lacking, as direct labeling of large human protein complexes with fluorescent dyes is technically extremely challenging. Here, we established a method to site-specifically incorporate organic fluorescent dyes into the human Pol I complex rendering it suitable for fluorescence-based single-molecule studies.
We combined genetic code expansion (GCE) with a CRISPR-Cas9 engineered HEK cell line harboring a FLAG-sfGFP-tagged Pol I. GCE enables incorporation of unnatural amino-acids (uaa) into individual Pol I subunits that were additionally expressed in this cell line. We show that the overexpressed uaa-containing subunits incorporate into the endogenous Pol I complex. Utilizing click chemistry, the modified Pol I complex can be site-specifically coupled to an organic fluorescent dye at the exact position of the uaa. This workflow yields fluorescently labeled Pol I which maintains catalytic activity. This methodological advancement allowed us to map the position of A49 in a transcription elongation complex. Furthermore, intramolecular FRET measurements with a doubly labeled Pol I variant revealed the dynamic behavior of the tandem winged helix domain of A49. Our approach therefore opens up the possibility to monitor the conformational transitions of A49 during different stages of the transcription cycle with single-molecule resolution and helps to elucidate the role of the crucial built-in transcription factor unique to Pol I.

Structural reorganization of the nucleolus and change in ribosome assembly upon Pol I inhibition by Actinomycin D

Xiaohan Zhao[1], Herman Fung[2], Yuki Hayashi[1], Sara Cuylen-Häring[1], Julia Mahamid[1], Christoph Müller[1]

[1]EMBL, [2]University of Michigan

Biogenesis of ribosome is one of the most active processes in cells. Effective ribosome biogenesis is essential for cell growth, proliferation and differentiation, and make this process also a drug target of great interest [1]. Eukaryotic ribosomal RNA transcription and ribosome biogenesis happen in the nucleolus, a phase-separated condensate. The rDNA is transcripbed at the interface of the fibrillar center (FC) and dense fibrillar component (DFC), the initial transcripts are then processed, modified, and assembled into pre-ribosomes with the integration of around 80 ribosomal proteins taking place 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, nucleolar morphology undergoes a process known as 'nucleolar segregation,' where the nucleoli become spherical and the transcription machinery accumulates at the periphery of the GC, forming distinct structures called 'nucleolar caps' [2]. Actinomycin D, a chemotherapeutic agent used to treat various cancers, intercalates into the GC-rich regions of rDNA and inhibits Pol I transcription, leading to the formation of these "nucleolar caps" and defection of rRNA processing [3]. What drives this condensation process and how this nucleoli deformation affects pre-ribosome assembly is still unclear. Our interest is to investigate how does the pre-ribosome assembly in GC change after nucleolar cap formation. We performed cryo-ET and on-lamella CLEM to visualize the nucleolar molecular landscape in situ and found that the nucleolar reorganization induced by ActD changed the distribution of pre-60S ribosomes. Specifically, the absence of the Rixosome binding state of pre-60S ribosome within the "nucleolar cap" GC region provides a mechanistic explanation for the stalling of 32S RNA processing. More notably, we observed a marked increase in the abundance of late pre-60S ribosomes, suggesting a defect in transporting 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.

1. Dörner K, Ruggeri C, Zemp I, Kutay U. Ribosome biogenesis factors-from names to functions. EMBO J. 2023;42(7):e112699.

2. Hori Y, Shimamoto A, Kobayashi T. The human ribosomal DNA array is composed of highly homogenized tandem clusters. Genome Res. 2021;31(11):1971-1982.

3. Szaflarski W, Leśniczak-Staszak M, Sowiński M, et al. Early rRNA processing is a stress-dependent regulatory event whose inhibition maintains nucleolar integrity. Nucleic Acids Res. 2022;50(2):10331051.

Mechanisms by which RNA polymerases Pol IV, Pol V and RDR2 program transcriptional gene silencing in plants

Craig Pikaard[1], Akihito Fukudome[1], Feng Wang[2], Wei Zong[1], Zheng Tian[3], Jasleen Singh[4]

[1]HHMI, Indiana University, [2]HHMI, Indiana University, Purdue University, [3]Indiana University, [4]Indiana University, University of California, Berkeley

RNA-guided gene silencing occurs in virtually all organisms to defend against selfish genetic elements. These include external invaders, such as viruses, as well as endogenous elements, such as retrotransposons whose proliferation must be held in check. Selfish elements can be neutralized either post-transcriptionally, by slicing or sequestering their RNAs, or transcriptionally, using host noncoding RNAs to guide chromatin modifications that repress gene activity within the elements.

In plants, the major transcriptional gene silencing process for genome defense is RNA-directed DNA methylation. Key to the pathway are three plant-specific RNA polymerases: Nuclear DNA-dependent RNA POLYMERASE IV (Pol IV), Nuclear DNA-dependent RNA POLYMERASE V (Pol V), and RNA-DEPENDENT RNA POLYMERASE 2 (RDR2). Pol IV and RDR2 physically interact, and their reactions are coupled, generating double-stranded RNAs of ~30 bp that are then trimmed by DICER-LIKE3 to generate short-interfering RNA (siRNA) duplexes composed of 23 and 24 nt strands. The siRNA duplexes are then loaded into ARGONAUTE 4 (AGO4), with 23 nt RNA strands serving to specify that the paired 24 nt strands become stably associated with AGO4. These 24 nt “guide strands” then basepair with long noncoding RNAs synthesized by Pol V at loci to be silenced. AGO4 slices these Pol V transcripts, retains the cleaved fragments, and somehow recruits chromatin modifying activities to the adjacent chromatin, resulting in DNA methylation by the cytosine methyltransferase, DRM2 and associated repressive histone post-translational modifications. In our work to define the biochemical details of how RNA-directed DNA methylation occurs we have recapitulated in the testtube the enzymatic reactions of Pol IV, Pol V, RDR2, DCL3 and AGO4. Our studies have revealed how the three RNA polymerases endow their transcripts with sequence and structural features that collectively serve as an RNA code that specifies how the transcripts will be processed and used to specify the silencing of corresponding intervals of DNA.

Evidence for inside-out upregulation of nucleolus organizers in Arabidopsis

Ramya Enganti[1,2], Dalen Fultz[1,2], Anastasia McKinlay [1,2], Craig Pikaard[1,2]

[1]Indiana University, [2]Howard Hughes Medical Institute

Ribosomal RNA (rRNA) gene repeats span megabases in regions of the genome known as Nucleolar Organizer Regions (NORs). Arabidopsis has two NORs, one each on chromosomes 2 and 4, of which *NOR2* is selectively silenced whereas *NOR4* is mostly active, a phenomenon known as nucleolar dominance. The exact mechanism underlying this is not well understood partly due to NOR sequences being excluded from genomes. Using ONT sequencing, we recently assembled *NOR2* and *NOR4* sequences in a wild-type stock and showed that rRNA gene expression inversely correlates with CG methylation levels.

By assembling NORs from an additional eleven wild-type stocks, we show that NORs are highly dynamic. The overall copy number (CN) varies among the stocks with variation occurring primarily in the active NOR. Methylation profiles of the NORs show that despite the variable CN, the number of active rRNA gene copies remains relatively constant. Within a given stock, a loss of gene copies on *NOR4* is compensated for by either partial de-repression of *NOR2* or demethylation of the telomere and centromere-proximal ends of *NOR4* which tend to be heavily methylated. Overall, our data shows that NORs are highly dynamic and highlights the spread of NOR epigenetic regulation from the central region (inside) outward towards the edges.

Host tRNA(Gln) acts as an assembly chaperone for the poxviral early transcription machinery

Clemens Grimm[1], Julia Bartuli[1], Stefan Jungwirth[1], Takumi Okuda[2], Claudia Höbartner[1,3]

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Poxviruses replicate exclusively in the cytoplasm of infected cells and are therefore highly independent of the host's nuclear transcription apparatus. The heart of this virus-encoded transcription machinery is a multi-subunit polymerase (vRNAP) similar to Pol I-III. Unlike these polymerases, which have different enzymatic cores adapted to their respective roles in cellular RNA metabolism, poxviral transcription has only one vRNAP for early, intermediate and late transcription. This is enabled by a set of dedicated viral transcription factors that target the vRNAP core enzyme to cognate promoters. We have recently reported a genuine strategy to isolate and structurally dissect poxviral transcription complexes. We identified a 0.85 MDa vRNAP complex (complete vRNAP), which is the major polymerase entity of infected cells. Complete vRNAP brings together the core polymerase and all factors necessary for early transcription. Remarkably, this complex also contains host tRNAGln, whose function was previously unknown. We found that the tRNAGln lacking the prominent mcm5s2U34 anticodon modification is essential for the assembly of complete vRNAP. Cryo-EM analysis of assembly intermediates illustrates how tRNAGln orchestrates the recruitment of transcription and mRNA processing factors to vRNAP where it ultimately controls the transition to the pre-initiation complex (PIC). This is achieved by a unique induced-fit mechanism that internalizes the anticodon base G36 into the anticodon stem creating a non-canonical tRNA structure and selecting a defined tRNA modification pattern.

Eventually, the tRNAGln-induced complete vRNAP is packaged en block into virions and enables the initiation of the early transcription program upon infection. To our knowledge this is the first example for a specific RNA acting as assembly chaperone. The role of tRNA as an assembly chaperone extends also to Mpox virus and likely other orthopoxviruses with highly similar transcription systems.

Rules of RNA Polymerase V Transcription Elongation

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In plants, silencing of transposons and repetitive elements by RNA-directed DNA methylation (RdDM) requires “scaffold” transcripts, synthesized by nuclear DNA-dependent RNA polymerase V (Pol V), to which AGO4-associated 24-nt siRNAs basepair. However, full-length Pol V transcripts remain undefined and their synthesis has yet to be recapitulated in vitro. We defined full-length Pol V transcripts in vivo by ligating their 5’ and 3’ ends to form circles and then amplifying the junctions by RT-PCR. These experiments show that Pol V transcripts are typically 150~300 nt in length and have variable endpoints at any given locus, indicating that initiation and termination positions are not fixed. Next, we developed a biochemical assay for Pol V transcription elongation in vitro, revealing template features required for Pol V processivity. When incubated with a single-stranded DNA template strand and an RNA primer, Pol V can extend the primer by only ~7 nucleotides before arresting and terminating. Similarly, only short extension products are generated from an RNA primer basepaired to a DNA template strand within an artificial bubble (non-basepaired nucleotides) region of an otherwise double-stranded DNA template. However, if the primer is annealed to the template DNA strand in the presence of a fully complementary nontemplate DNA strand, long RNA transcripts are generated. These and other experiments indicate that bubble closure and bubble translocation need to occur in concert with ribonucleotide addition in order for processive Pol V elongation to occur. We also find that Pol V is sensitive to homopolymeric runs of adenines or thymines (dA:dT-tract) that are downstream from the active site, suggesting that bent DNA (or other structures) can jam the DNA entry channel, causing Pol V arrest. We speculate that Pol V’s sensitivity to transcription bubble dynamics and DNA structure may allow its transit time to be modulated to optimize RNA-directed DNA methylation.

Transcriptional Sabotage: Nucleotide Analog Drugs vs. Mtb RNAP

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Mycobacterium Tuberculosis (MTB) is the causative agent of the highly infectious disease tuberculosis. According to the World Health Organization, 1.5 million people die from tuberculosis (TB) each year, making TB the top infectious killer worldwide. Although there are drugs used to treat TB, about 8.5% of patients are diagnosed with drug-resistant TB indicating there is a need for new treatment options. In bacteria, RNA Polymerase (RNAP) is the enzyme that transcribes DNA to RNA. Rifampin, a highly effective treatment for tuberculosis, targets RNAP by binding and inhibiting extension of RNA transcripts. The selectivity and potency of rifampin demonstrates that RNAP is an excellent target for treatment of TB. This project explores alternative strategies to inhibit RNAP. It has been shown that MTB RNAP's binding affinity for nucleotide analogs is much higher than canonical nucleotides. This contrasts with eukaryotic RNA Polymerase II, which demonstrates no preference for binding analogs versus natural nucleotides. Here, we use rapid mixing techniques for transient state kinetic study of clinically used nucleotide analog drugs, Tenofovir and Acyclovir, on transcription elongation. By evaluating the effect of these compounds on nucleotide addition by MTB RNAP, we may identify these clinically used drugs as potential therapeutic strategies for further development in the battle against TB.

Tight coupling of transcription and translation regulates archaeal gene expression.

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Archaea encode only a single multi-subunit RNA polymerase (RNAP) and the lack of a nuclear compartment in these prokaryotic progenitors of Eukarya allows for immediate engagement of ribosomes with nascent transcripts. Like the coupling demonstrated in many Bacteria, the coupling between the archaeal transcriptional and translational apparatus (known as the transcription-translation complex, or TTC) protects the elongating RNAP from factor-dependent transcription termination, provides additional protections from retrograde or backtracking movements of the transcription elongation complex (TEC), and coordinates the speed of transcription to match translation. We have now resolved the atomic structure of the archaeal TTC, employing components from the model hyperthermophilic archaeon THermococcus kodakarensis and high-resolution cryo-EM. Our complete archaeal TTC structures - including nascent RNA stretching from teh active center of RNAP to the active center of the ribosome with bound tRNAs - structurally defines the contact surfaces between ribosomal proteins (and rRNA) with the archaeal RNAP and the conserved elongation factors Spt4/5. To validate our structural efforts, we have introduced site-specific amino acid changes to enhance or weaken the coupling of the ribosome and TEC to establish how the stability of the TTC impacts intrinsic and factor-mediated termination of transcription, polar repression of operon expression, and overall fitness. The combined results reveal contrasts and commanalities of transcription coupling in Bacteria and Archaea, key TTC contacts that are critical to archaeal fitness, and validate the structural resolution of the TTC.

A Unique Mechanism of Promoter Recognition Enables Poxvirus Intermediate Transcription

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Poxviruses express and replicate their genomes exclusively in the cytoplasm of their hosts. Consequently, the virus has only limited access to cellular enzymes for transcription, RNA maturation and DNA replication, which are mostly sequestered in the nucleus. Poxvirus propagation therefore critically depends on a virus-encoded gene expression machinery consisting of a multi-subunit RNA polymerase (vRNAP) as well as virus-specific transcription and mRNA processing/maturation factors. Poxviral transcription is temporally separated in early, intermediate and late phases and depends on dedicated factors that direct vRNAP to the respective promoter elements. Here, we describe a previously unknown mechanism of intermediate promoter recognition by the poxviral intermediate transcription factor VITF-3l/s. This heterodimeric complex comprises an atypical transcription factor pair, which is homologous to the general eukaryotic transcription factors TBP and TFIIB but acts in a radically different manner. We find that VITF-3l/s forms a stable ring-like structure that remains inert toward DNA in the absence of viral RNA polymerase (vRNAP). Cryo-EM analysis of the intermediate pre-initiation complex (iPIC) reveals that promoter recognition is directly mediated by vRNAP, rather than VITF-3l/s. Only after vRNAP binding can the VITF-3l/s ring be loaded onto a defined upstream promoter position. This anchoring mechanism positions vRNAP at the transcription start site and establishes vRNAP as a clamp loader for VITF-3l/s. Our findings uncover a novel mode of transcription initiation and emphasize the importance of TBP/TFIIB homologs in poxviral transcription.

Evidence for TFIIB and TFIIH-like activities in the RNA Polymerase V transcription factor, DRD1

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In plants, gene silencing by RNA-directed DNA methylation (RdDM) involves long noncoding RNAs synthesized by DNA-dependent RNA polymerase V (Pol V), an evolutionary derivative of RNA polymerase II (Pol II). We show that DRD1, a Pol V helper protein of unknown function, interacts with Pol V via a zinc ribbon domain with structural homology to the Pol II transcription factor, TFIIB. DRD1 is also a DNA-dependent ATPase whose DNA translocase activity renders double-stranded DNA partially sensitive to the single-strand specific DNA endonuclease, P1. Mutational knock-out of these activities disrupts RdDM and Pol V association with target loci, genome-wide. Collectively, our findings suggest that DRD1 has functions analogous to those of two different Pol II transcription factors, carrying out TFIIB-like recruitment of Pol V to DNA and ATP-dependent DNA translocation, like TFIIH, that transiently melt the DNA to allow transcription initiation.

A single-molecule platform for dissecting the molecular mechanisms of transcription

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Biological processes such as DNA transcription, gene regulation or chromatin remodeling are notoriously complex and tightly regulated in time and space. What if you could dissect these biological processes at the single molecule level?

Single-molecule technologies allow direct, real-time manipulation, observation and measurements of processes at the molecular level. And only by actively studying these individual molecules in real time, one can finally validate and/or complete current biological models.

Here, we present how dynamic single molecule techniques can be used to elucidate key processes in transcription and chromatin remodeling. For example, we will show how frequent RNA polymerase collisions, paradoxically, can solve traffic jams and lead to efficient transcription termination of convergent gene pairs by direct visualization of the mechanism; a work led by the group of Prof. Shixin Liu [1].

These examples illustrate how hybrid single-molecule methods can give unique insights in the many mechanisms involved in polymerase activity and other DNA-protein interactions involved in gene regulation.

[1] L. Wang et. al., Head-on and codirectional RNA polymerase collisions orchestrate bidirectional transcription termination, Molecular Cell; 83, 1153- 1164.e4 (2023).

Recruitment of Fpt1 to tRNA genes in budding yeast independently requires the RNA Polymerase III general transcription factors TFIIIB and TFIIIC

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Upon recognizing the promoter regions of tRNA genes, assembly factor TFIIIC facilitates the recruitment of initiation factor TFIIIB, thereby providing the platform for recruitment of RNA Polymerase III to initiate transcription. In repressive conditions, the repressor protein Maf1 translocates to the cell nucleus where it directly interacts with RNA Polymerase III and TFIIIB to prevent their assembly onto the DNA. We recently applied Epi-Decoder to decode the local proteome of one tRNA gene in budding yeast using DNA barcode sequencing and high throughput yeast genetics. This led to the discovery of a novel tDNA binding protein called Fpt1. Fpt1 occupancy varies between tRNA genes, with genes harboring low Fpt1 occupancy being less regulated than high occupancy genes. Moreover, in the absence of Fpt1, the rewiring of the transcription machinery on tRNA genes is partially compromised. To understand how Fpt1 affects RNA Polymerase III on tRNA genes, we investigated how Fpt1is recruited to tRNA genes in living cells. Using acute protein depletion (Anchor Away), we determined that Fpt1 recruitment independently requires TFIIIB and TFIIIC, under conditions of high expression (glucose) as well as repressive conditions (glycerol). Taking advantage of AlphaFold predictions, we identified a small predicted alpha helix in Fpt1 that is particularly critical for the increased occupancy of Fpt1 in repressive conditions. Mutations in a central subunit of TFIIIC predicted to interact with Fpt1 phenocopied the deletion of the alpha helix of Fpt1. These findings provide support for a model in which Fpt1 directly interacts with TFIIIC but independently depends on TFIIIB. Finally, we observed that the dependencies between Fpt1, TFIIIB, TFIIIC, and RNA Polymerase III vary between tRNA genes. Our future efforts will be focused on unravelling the mechanisms behind the variation in dynamics between tRNA genes. For this purpose, we are developing a semi-automated barcoding platform to enable Epi-Decoder analysis to multiple tRNA genes in parallel.

Genomic metamap of RNA Polymerase I, II and III, reveals RNA Polymerase III occupancy and transcription at protein coding genes promoter.

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The transcriptional interplay of human RNA polymerase I (RNA Pol I), RNA Pol II, and RNA Pol III remains largely uncharacterized due to limited integrative genomic analyses. Thus, we applied a uniform framework to quantify global RNA Pol I, II and III occupancies and found unexpected RNA Pol III recruitment at promoters of specific protein-coding genes. We show that such RNA Pol III-occupied promoters are enriched for small nascent RNAs terminating in a run of 4 Ts—a hallmark of RNA Pol III termination. Further, RNA Pol III disruption reduced the expression of RNA Pol III-occupied protein-coding genes, suggesting RNA Pol III recruitment and transcription enhance RNA Pol II activity. Similarly, scarce proteomic studies on Pol III have hindered our understanding of Pol III interactome. Thus, we utilize the concept of effective conductance on over 2 million protein-protein interactions(PPI) information to measure the effective closeness between proteins. Such large scale PPI networks are flawed with the popularity bias. We have developed a novel statistical method to overcome the popularity bias and find the genuine interactors of RNA Pol III. Overall these findings advance our ability to better understand the mechanisms and consequences of Pol III transcription.

Structural insights into distinct mechanisms of RNA polymerase II and III recruitment to snRNA promoters

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RNA polymerase III (Pol III) is specialised in the transcription of short, essential RNAs, including the U6 small nuclear RNAs (snRNAs). At U6 snRNA genes, Pol III is recruited by the snRNA Activating Protein Complex (SNAPc) forming, together with a Brf2-containing TFIIIB complex, a non-canonical yet transcriptionally competent pre-initiation complex (PIC). Addtionally, SNAPc is responsible for the recruitment of Pol II at the remaining spliceosomal snRNA genes (U1, 2, 4 and 5), representing a unique example of a multi-subunit transcription factor shared between different RNA polymerases. Despite recent structural characterisation, the mechanism of SNAPc cross-polymerase engagement and the role of SNAPC2 and SNAPC5 subunits in transcription remain poorly defined. Here, we present cryo-EM structures of the full-length SNAPc-containing human Pol III PIC assembled on the U6 snRNA promoter in the open and melting states at 3.2-4.2 angstrom resolution. Comparative structural analysis revealed unexpected differences with the yeast PIC and the molecular basis of selective and structurally distinct SNAPc engagement within Pol III and Pol II PICs. Harnessing crosslinking mass spectrometry, we also localise the SNAPC2 and SNAPC5 subunits in proximity to the promoter DNA, expanding upon existing descriptions of snRNA Pol III PIC structure.

An expanding universe of RNA polymerase III transcription, regulation, and function

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RNA polymerase III (Pol III) transcription and production of small noncoding RNAs (ncRNAs) essential for protein accumulation and cell growth is well established. However, in cancer, Pol III activity also expands to context-restricted genes and their products, such as snaR-A – a hominid-specific, cancer-emergent ncRNA implicated in cell proliferation and tumorigenesis. To better understand the breadth of Pol III expansion and its significance, we revisited the extent, mechanisms, and consequences of Pol III transcription in cancer using a combination of biochemical and large-scale genomic analyses that innovatively leverage the current Pol III-relevant dataverse. At the transcriptional level, our studies uncover evidence of Pol III activity at specific protein-coding gene promoters, revealing novel crosstalk between Pol III and Pol II beyond the canonical Pol III transcriptome. We extend these analyses further by establishing a *first-of-its-kind* atlas of Pol III patterns across human tissues and tumors, revealing both context-specific transcription patterns and unexplored disease signatures. At the gene regulatory level, we demonstrate that Pol III participates in macromolecular interactions with the NuRD chromatin remodeling complex, which directly targets and transcriptionally regulates a subrepertoire of Pol III-transcribed genes. At the RNA level, we demonstrate that the Pol III-derived snaR-A ncRNA interacts with U2 snRNP splicing factors and thereby perturbs efficient splicing of mRNA subpopulations with weak 3' splice site signatures. We propose that snaR-A-related splicing disruption may phenocopy previously reported splicing defects and consequences attributed to mutations in U2 snRNP in cancer, eliciting an alternative, non-mutational pathway driven by Pol III overactivity during tumorigenesis. Taken together, these findings address important gaps in our understanding of Pol III transcription, regulation, and downstream ncRNA function, and further highlight the need for future exploration of Pol III as an important player in cancer.

The NuRD chromatin remodeling complex regulates tRNA genes through direct interactions with RNA polymerase III

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RNA polymerase III (Pol III) synthesizes noncoding RNAs essential for translation, RNA processing, transcription regulation, and other core cellular processes. Pol III transcription levels are coordinated with nutrient availability for cellular growth, and its dysregulation is linked to disease, such as breast cancer and leukodystrophy. Despite its importance, human Pol III regulatory mechanisms remain largely uncharacterized, in part due to limited exploration of Pol III-centered protein-protein interactions (PPIs). To address this gap, we surveyed the Pol III complex interactome by leveraging a series of Pol III-specific subunit pull-down and mass spectrometry experiments. We identified over 300 novel Pol III interactors, including Pol II-related transcription factors, nuclear import factors, and splicing factors. Most notably, we uncovered complex-complex interactions between Pol III and a Pol II co-repressor, the NuRD (Nucleosome Remodeling and Deacetylase) complex. By generating a large-scale genomic atlas of NuRD binding patterns, including ChIP-seq experiments for previously unmapped NuRD subunits in human THP-1 monocytes, we found that NuRD localizes to Pol III-transcribed tRNA genes and exhibits a Pol III-dependent preference for the most highly active Pol III genes. Functionally, we show that inhibiting NuRD deacetylase activity increases Pol III transcription and the dynamic range of the Pol III transcriptome, suggesting NuRD mechanistically restricts Pol III transcription at highly active genes. Through nucleosome-level chromatin analyses, we additionally uncover evidence that NuRD contributes to nucleosome positioning through its ATP-dependent remodeler activity. Together, these findings advance our understanding of the Pol III interactome, uncovering new layers of transcriptional control and unexpected functional consequences of Pol III interactions in human cells.

RNA polymerase III transcription-associated polyadenylation promotes the accumulation of noncoding retrotransposons during herpesviral infection

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The accumulation of RNA Polymerase III (Pol III) transcribed noncoding retrotransposon RNA is a hallmark of various cellular stressors, including DNA virus infections, although the mechanisms driving this induction are largely undefined. Using the model murine gammaherpesvirus 68 (MHV68), we sought to define DNA sequence elements that underly viral induction of Pol III transcribed loci. We find that there is a prominent increase in the occupancy of Pol III in response to infection at thousands of B2 short interspersed nuclear element (SINE) retrotransposon genes. Using DNA motif analyses in combination with a convolutional neural network (CNN)-based model, we subsequently reveal that sequences associated with polyadenylation are enriched in infection-induced B2 SINE loci. Depletion of polyadenylation factors prevents both B2 SINE RNA polyadenylation and accumulation during infection. Thus, these normally lowly expressed or silent transcripts accumulate in response to herpesvirus infection due to a combination of transcriptional induction and polyadenylation-induced stabilization. Notably, we find Pol III-dependent recruitment of CPSF30, a component of the mRNA polyadenylation machinery, to virus-induced Pol III loci. This includes B2 SINE and tRNA genes, which have the same type II promoter. This uncovers an inducible, coupled relationship between Pol III transcription and mRNA-like polyadenylation.

5S rRNA regulatory changes in fission yeast are dynamic and rapid

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Ribosome biogenesis is a critical and metabolically expensive cellular process, with ribosomal RNA (rRNA) transcription making up a significant portion of this expense. Every Ribosome needs a stoichiometric ratio (1:1:1:1) of rRNA molecules, so it remains mysterious why eukaryotes divided rRNA transcription between two distinct regulatory pathways: RNA polymerase I (PolI) transcribes the 45S precursor rRNA (35S in plants), and RNA polymerase III (PolIII) transcribes 5S rRNA. Furthermore, while PolI activity is localized to the nucleolus, the spatial organization of PolIII transcription is less well understood. Compounding this mystery, the genomic organization of 5S rRNA genes is highly variable between species. 5S copies can be dispersed throughout the genome or clustered into arrays, sometimes associated with 45S rRNA genes. We decided to explore the question: how are 5S genes regulated, despite all this variation? We utilized the fission yeast *Schizosaccharomyces pombe*, a unique model where 5S genes are dispersed throughout the genome at 37-40 independent loci. We leveraged single nucleotide polymorphisms to assess individual 5S rRNA gene contributions to the overall rRNA pool. We found that in standard culture conditions, single 5S genomic copies can contribute anywhere from 0% to 21% of cellular 5S rRNA. In one isolate, two out of thirty-nine 5S copies contribute 50% of the 5S rRNA, suggesting that the cell could potentially survive with only four 5S copies. Deletion of these two copies has no impact on cellular fitness, and under rapid growth conditions, their contribution to the 5S rRNA pool decreases. This indicates that 5S rRNA regulation is dynamic, quickly responding to internal and external changes. We are now investigating the hypothesis that a PolIII transcriptional hub exists, akin to the nucleolus for PolI, and that localization of 5S genomic copies to this region may be responsible for the observed biases, and for rapid changes in 5S rRNA regulation.

Nucleolar dynamic during zebrafish oogenesis

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Oogenesis is the developmental process by which oocytes are formed, matured, and equipped to support fertilization and early embryonic development, making it essential for female fertility and species propagation. It is characterized by dramatic cellular growth as the oocyte accumulates the molecular components needed to support early embryonic development. This increase in cell size requires a massive upregulation of protein synthesis, which in turn depends on robust biogenesis of ribosome, the molecular machines responsible for protein synthesis. Central to this process is the nucleolus, a membraneless organelle that serves as the site of ribosomal RNA transcription, processing, and ribosome subunit assembly. However, how the nucleolus changes during oogenesis to meet the high demand for ribosome assembly is poorly characterized. Here, we use hybridization chain reaction (HCR) to visualize rRNA synthesis during zebrafish oogenesis. We discover regulated changes in the number, localization, and architecture of nucleoli across different stages of oogenesis. These findings suggest that the nucleolus is highly dynamic and undergoes developmentally programmed remodeling to support the biosynthetic demands of the growing oocyte.

Chromosome-Specific Regulation of Great Ape rRNA Arrays Shapes rRNA Pools and Genome Organization

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Ribosomal RNA (rRNA) genes, transcribed exclusively by RNA polymerase I (Pol I), are encoded in large tandem arrays located on Great Ape acrocentric chromosomes. Pol I transcription of these genes drives ribosome biogenesis and nucleolar formation, serving as both the rate limiting step in the energetically demanding formation of ribosomes and as a key determinant of nuclear architecture through the spatial organization of rDNA within nucleoli. Despite this, the regulation of rRNA output remains poorly understood, as past studies have analyzed the hundreds of gene copies in aggregate. Leveraging long-read sequencing technology, we uncover a new layer of regulation at the level of entire rDNA arrays. Specifically, we have found that entire arrays exist in distinct transcriptional states, either active or inactive. We demonstrate that active arrays harbor rRNA genes that undergo robust Pol I transcription, with highly accessible chromatin based on Fiber-seq, and lack DNA methylation. Inactive arrays contain rRNA genes that are not transcribed by Pol I, are inaccessible, and show high DNA methylation. Furthermore, these molecular features drive changes in chromosome behavior, with inactive arrays no longer organizing nucleoli. Our findings demonstrate that Pol I transcription of rRNA genes can be regulated at the level of whole rDNA arrays, revealing a previously unappreciated layer of control. By integrating long-read sequencing methods, additional imaging and rRNA expression analyses, we have identified the molecular features of active and inactive rDNA arrays and link them to nuclear organization and ribosome biogenesis potential. This conserved array-level regulation impacts chromosome positioning in the nucleus and the pool of rRNAs available for ribosome biogenesis. This work elucidates the molecular features of rDNA array activity, contributes to our understanding of how Pol I activity is coordinated across the genome, and provides a baseline for understanding its regulation in human health and disease.

Higher-order chromatin organizations of highly repetitive NOR, 5S gene and centromeric loci in Arabidopsis

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In eukaryotes, the higher-order organizations of centromeres, nucleolus organizer regions (NORs) and 5S ribosomal RNA gene loci are understudied at a molecular level due to the technical challenges posed by their size and repetitive nature. However, long-read DNA sequencing technologies have recently allowed the reconstruction of these loci by revealing sets of position-specific sequence polymorphism that could be connected into full assemblies. In this study, we used non-standard assays compatible with long-read sequencing technologies to probe the chromosome conformations, chromatin accessibility, histone modification and DNA methylation landscapes for the two NORs, three 5S rRNA gene loci and five centromeric regions of *Arabidopsis thaliana*, collectively accounting for approximately 20% of the genome. Using Oxford Nanopore Pore-C analysis to conduct chromatin conformation capture, we find that centromere conformations are unique to each chromosome and that NORs and 5S rRNA gene loci form discrete territories with limited interactions with flanking sequences. At loci where 5S gene repeats are interrupted by a block of unrelated DNA, the 5S genes loop out from the intervening sequence. Chromatin accessibility and histone post-translational modification landscapes were revealed by methods involving adenosine methylation and long-read sequencing. At the NORs, silenced *NOR2* DNA is predominantly cytosine hypermethylated and inaccessible to m6A modification whereas the active central region of *NOR4* is cytosine hypomethylated, accessible to m6A modification and H3K4me3-enriched. At 5S gene loci, genes displaying ‘silent’ or ‘active’ chromatin features were interspersed at the loci on chromosomes 4 and 5 but not at chromosome 3, where only silent marks were enriched. Collectively, our results reveal new insights into previously understudied, highly repetitive genomic regions made possible by assays dependent on long-read sequencing.

Dysregulated ribosomal transcription due to reduced levels of WSTF in the chromatin remodelling complex B-WICH induces the integrative stress response.

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Ribosomal gene expression is essential for cell growth and tightly regulated by environmental cues. The B-WICH complex, comprising WSTF, the ATPase SNF2h and nuclear myosin, activates rRNA transcription in response to glucose by modifying the chromatin state at the promoter. Here,we show that WSTF knock down reduced cell viability, without inducing a clear nucleolar stress and apoptosis. WSTF siRNA knock down did not lead to a p-53 dependent nucleolar stress, but instead resulted in reduced translation and induction of the Integrated stress response. In HeLa cells, which have a p53 deficiency, the integrated stress response with phosphorylation of EIF2α and HSP60 in the mitochondrial Unfolding stress response were activated. The mitochondria were not dysregulated; we observed a slight upregulation of mitochondrial oxygen response, similar to glucose starved cells, and the mitochondria responded to glucose refeeding equally well. In conclusion, WSTF knock down results in a p53-independend stress response mediated by integrated stress response pathway.

IGS38s ncRNA from human rDNA intergenic spacer regulates rDNA chromatin organization and 45s rRNA transcription

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Eukaryotic ribosomal DNA (rDNA) is a multi-copy gene, with only about one-third actively transcribed in differentiated cells. In this study, a novel noncoding RNA, IGS38, is introduced, which positively regulates rDNA transcription by modulating the accessibility of the rDNA promoter. IGS38s interacts with WSTF, a component of the B-WICH chromatin-remodelling complex, to keep the rDNA promoter region accessible for UBF binding and the recruitment of the RNA Pol I machinery. A reduction in IGS38s levels leads to decreased transcription of 45S rRNA and prevents the binding of UBF and RNA Pol I accessory factor RRN3 to the rDNA promoter. This also results in chromatin compaction at the promoter, causing an inactive chromatin state. Furthermore, the decrease in IGS38s induces an indirect, mild innate immune response, reflected by higher expression of OAS2 and CXCL11 transcripts. Overall, this study describes a novel mechanism through which IGS38s maintains the accessibility of rDNA repeats in human cells.

Ribosomal RNA synthesis by RNA polymerase I is subjected to premature termination of transcription

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The RNA polymerase I (Pol I) enzyme that synthesizes large rRNA precursors, exhibits high rate of pauses during elongation, indicative of a discontinuous process. We show here that Premature Termination of Transcription (PTT) by Pol I is a critical regulatory step limiting rRNA production *in vivo*. The Pol I mutant, SuperPol (RPA135-F301S), produces 1.5-fold more rRNA than the wild type (WT). Combined CRAC and rRNA analysis link increased rRNA production in SuperPol to reduced PTT, resulting in shifting polymerase distribution toward the 3' end of rDNA genes. *In vitro*, SuperPol shows reduced nascent transcript cleavage, associated with more efficient transcript elongation after pauses. Notably, SuperPol is resistant to BMH-21, a drug impairing Pol I elongation and inducing proteasome-mediated degradation of Pol I subunits. Compared to WT, SuperPol maintains subunit stability and sustains high transcription levels upon BMH-21 treatment. These comparative results show that PTT is alleviated in SuperPol while it is stimulated by BMH-21 in WT Pol I.

Identifying factors involved in the codon optimality mechanism in human cells.

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Codon composition strongly impacts mRNA stability and translation efficiency from yeast to human cells. Some codons enhance translation efficiency and stability of mRNAs (optimal codons), and others have the opposite effect (non-optimal codons). This phenomenon, known as codon optimality, is one of the strongest mechanisms regulating gene expression post-transcriptionally. Although translational levels and the tRNA supply have been shown to be important determinants of codon optimality in humans, the molecular mechanism remains a mystery.

To identify factors involved in the molecular mechanism of codon optimality in humans, we performed a genome-wide CRISPRi screening. We found that the top hit in the screening binds to elongating ribosomes independently of the nascent peptide or the mRNA, by analyzing previously published polysome profiling, CLIP and Mass spectrometry data. To validate the identified factors, inducible shRNA lines were used to, first, analyze reporter gene expression. And second, to analyze the stability of endogenous mRNA. The shRNA knockdown of one of our top candidates, reduced the expression of reporters enriched in non-optimal codons, without affecting those enriched in optimal codons.

In summary, our work shows that transcripts mostly enriched in non-optimal codons become stabilized in the absence of our top candidate factor from a CRISPRi screening, and we describe a factor influencing codon optimality for the first time in humans.

A ribosome-biogenesis-independent role of RNA Pol I and nucleolar integrity in the maintenance of proper germline H3K4 remodeling and oogenesis

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This study uncovers a novel, non-ribosomal role for RNA Polymerase I (Pol I) in regulating chromatin dynamics during oogenesis in *Caenorhabditis elegans*. Using the auxin-inducible degron system, we separately depleted a catalytic subunit of RNA Pol I and a ribosome assembly factor, allowing us to distinguish between the consequences of nucleolar disruption and reduced ribosome biogenesis during female germ cell maturation. While ribosome assembly disruption has minimal influence on oocyte production, disruption of nucleolar structure through RNA Pol I depletion leads to defects in meiotic chromatin organization, as well as fewer but significantly larger oocytes. This oogenesis defect coincides with increased chromatin accessibility at oogenesis-specific promoters, which are enriched for oogenesis-promoting EFL-1 transcription factor binding sites. Additionally, promoters with elevated accessibility are associated with H3K4me3 regions, canonically remodeled during the germ-to-oocyte transition. Consistently, RNA Pol I depletion dampened the distal-proximal H3K4me3 gradient through premature H3K4me3 deposition, an essential histone modification for shaping oocyte chromatin architecture. These findings reveal an essential and novel role for RNA Pol I activity and nucleolar integrity in epigenetic regulation during germ cell maturation, independent of ribosome production. Given the evolutionary conservation of nucleolar integrity and H3K4me3 remodeling during female gametogenesis, our results suggest that similar nucleolar functions may play a critical role in fertility, particularly in individuals experiencing nucleolar stress due to environmental factors, aging, or genetic disorders.

In vivo functions of UBF1 and UBF2 isoforms

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Upstream Binding Factor (UBF) is a cytoarchitectural transcription factor that plays a pivotal role in RNA Polymerase (Pol) I -mediated transcription of ribosomal RNA genes (rDNA). UBF binds at the regulatory regions as well as throughout the coding region of the rDNA repeats, in a sequence-independent manner, and remodels rDNA loci into an active conformation.

The levels of UBF-bound rDNA are directly related to the number of actively transcribing gene copies. UBF deletion in mice causes death during early embryonic development (blastocyst stage; embryonic day (e) 3.5) indicating that UBF is indispensable for ribosome biogenesis, and consequently life. Furthermore, UBF mutations have been identified in patients with neuro-regression syndrome and cancer.

UBF possess two isoforms due to alternative splicing: the longer isoform, UBF1, retains exon 8, which is absent in the UBF2 shorter isoform. Evidence suggests, that UBF1 binds specifically to the rDNA regulatory regions while UBF2 binds through the rDNA coding regions and regulates the expression of highly transcribed Pol II-dependent genes (e.g. histone clusters). It is therefore hypothesised that UBF1 has a more prominent role in regulating Pol I transcription. However, the unique and shared functions of UBF1 and UBF2 remain unclear.

We have generated mouse models expressing exclusively UBF1 or UBF2. Ubf2 KO mice are born at Mendelian ratios and develop normally. UBF1 binding throughout the rDNA regulatory and coding regions remains unchanged and consequently no significant changes in Pol I transcription levels were observed. Surprisingly, Ubf1 KO mice develop apparently normally but die at e9.5. This indicates that UBF2 is able to support Pol I-transcription and ribosome biogenesis through the early stages of embryonic development. Detailed molecular analysis of these models will provide insights into the functional interplay between the two UBF isoforms and their impact on growth and development through Pol I transcription regulation and/or other biological processes.

Ribosome Biogenesis is Essential for Neural Crest Cell Differentiation and Craniofacial Cartilage and Bone Development

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Neural crest cells (NCCs) are a migratory cell population specified during early embryogenesis that generate cartilage, bone, and connective tissue as well as neurons, glia and melanocytes in the head and face. Previous work in the lab has shown that RNA Polymerase I (Pol I)-mediated ribosomal RNA (rRNA) transcription, a rate limiting step in ribosome biogenesis, is elevated in NCCs compared to other tissues. Disruption of Pol I function in mice leads to increased pre-migratory and migratory NCC death, resulting in hypoplastic frontonasal prominences and pharyngeal arches, and consequently cranioskeletal hypoplasia, anomalies characteristic of craniofacial disorders such as Treacher Collins syndrome. Emerging evidence also supports the tight regulation of ribosome biogenesis in differentiating cells, suggesting post-migratory roles during NCC development. To examine the importance of ribosome biogenesis in later stages of craniofacial development, we deleted *Polr1a*, which forms part of the catalytic core of Pol I, with tamoxifen-inducible Cre^{ERT2} at E9.5-E13.5, timepoints during which NCCs begin to differentiate. *Polr1a* mutant embryos exhibited craniofacial anomalies such as micrognathia and cleft palate. To determine the mechanisms driving these changes, single-cell RNA sequencing and bulk proteomics were performed on dissected craniofacial tissues at E12.5, during the early onset of the phenotypes. Transcriptomic results revealed significantly altered cell type proportions in the mutants, with reduced mesenchymal cartilage and bone progenitors, and increased neuronal progenitor populations. Spatial transcriptomic data in wild type mice at E12.5 showed that the most affected mesenchymal progenitor cell types correlated with the anatomical structures most affected in *Polr1a* mutants. Differential protein analysis revealed impaired cartilage development in addition to metabolic changes in mutant tissues. Together, these results support a requirement for ribosome biogenesis in NCC differentiation during craniofacial development. Thus, even though ribosome biogenesis is a global molecular process, it is tissue specifically required during NCC development and in the pathogenesis of craniofacial disorders.

Getting More for Less: Synergistic Drug Combinations with RNA Polymerase I Inhibitors for Cancer Therapy

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Despite treatment advances, breast cancer (BC) remains a major global health challenge and there is an ongoing need for specific and effective BC therapies. Dysregulated ribosome biogenesis and hyperactivation of RNA polymerase I (Pol I) are critical features of breast cancer progression and aggression. Specific inhibition of Pol I is a promising new targeted therapy with high potential to reduce BC disease burden by impairing tumor proliferation, metastasis, and chemoresistance. Small-molecule Pol I inhibitors, such as CX-5461 and BMH-21, have been identified and are under investigation for their ability to treat BC. To enhance the efficacy of Pol I inhibitors in cancer therapy, we investigated the ability of Pol I inhibitors to synergize with each other. Our analysis showed that CX-5461 and BMH-21 synergize in reducing viability of MCF-7 BC cells. Compared to monotherapies, combination treatment enhanced activation of p53 and degradation of the Pol I large subunit, RPA194. Because Pol I inhibitors activate the transcription factor p53, and p53 is commonly mutated in BC, we investigated the ability for p53 reactivators to synergize with Pol I inhibitors in p53 mutant cells. We hypothesized that treating p53 mutant cells with p53 reactivators, which activate wild-type functions in mutant p53 proteins, would enhance sensitivity to Pol I inhibition. To this end, we evaluated synergy of drug combinations composed of one Pol I inhibitor (CX-5461 or BMH-21) and one p53 reactivator (ZMC-1 or APR-246). Synergy was highly variable between combinations, highlighting the importance of drug-specific effects in mediating synergy. Based on our observations, we performed further analysis of p53-mutant BC cells treated with BMH-21 alone and found that BMH-21 alone was sufficient to promote mutant p53 reactivation. Our findings demonstrate that Pol I inhibitors may synergize with other anti-cancer agents in treating BC, highlighting the usefulness of Pol I inhibitors in drug combinations. Furthermore, our approach allowed us to identify a previously unrecognized function of BMH-21 in reactivating mutant p53. These insights into BC treatment with Pol I inhibitor combinations may guide future development and clinical application of these exciting new therapies.

A spatiotemporal expression atlas of mouse tRNA genes

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Mammalian genomes encode more than 300 genes coding for tRNA which have been thought to be constitutively expressed in all cell-types. Recent studies have shown the possibility of differential expression of tRNA genes in different cell-types, but a comprehensive analysis of tRNA gene expression has not been done due to a lack of a robust method to quantify the expression of tRNA genes. Here we report PreT-seq, a simple, cheap, reproducible, scalable and quantitative method to analyze tRNA gene expression. We used PreT-seq to quantify tRNA gene expression across multiple mouse tissues from embryonic and adult stages. We find that 200 out of 300 tRNA genes exhibit tissue-restricted expression across mouse development raising the fundamental question about the mechanism of dynamic regulation of tRNA genes. Using machine learning, we develop a PreT-seq analysis platform to identify the tissue-source of cellular material providing a novel method for tissue fingerprinting. Using publicly available chromatin and gene expression datasets across mouse tissues, we identify potential regulators of tissue-restricted expression of tRNA genes. In summary, our study provides the first comprehensive, spatiotemporal atlas of tRNA gene expression across mouse development and provide mechanistic insights into the tissue-specificity of tRNA expression.

RNA Polymerase III subunit Polr3a is required for craniofacial cartilage and bone development

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Ribosome biogenesis and protein translation are essential processes required in all cells, yet disruptions in these processes lead to tissue-specific human phenotypes which frequently affect craniofacial development. Pathogenic variants in genes encoding subunits of RNA Polymerase (Pol) III, including *POLR3A*, lead to a variety of phenotypes including hypomyelination in the central nervous system, hypodontia, and other rare anomalies of the face and skull. Given the perturbed development of cranial neural crest cell (NCC)-derived tissues in humans with pathogenic variants in *POLR3A*, we hypothesized that Pol III-mediated transcription is required for craniofacial development through the regulation of ribosome biogenesis and translation in NCCs. To test this model, we established *polr3a* mutant zebrafish which displayed hypoplasia of NCC-derived craniofacial cartilage and bone by 5 days post fertilization (dpf). To determine how these malformations arise in *polr3a* mutants, we assessed NCC development, proliferation, and cell death from 1.5 - 5 dpf. Surprisingly, no significant changes were observed in NCC development, proliferation, or cell death prior to 2 dpf. However, at 3 dpf and beyond, both increased cell death and reduced proliferation were observed throughout the head. Interestingly, markers of cartilage and bone development were unchanged at this stage, suggesting that cartilage and bone hypoplasia at 5 dpf could be primarily the result of changes in cell proliferation and survival. Quantitative RT-PCR revealed reduced transcription of tRNAs and increased levels of *tp53* at 3 dpf, and we also observed diminished ribosome biogenesis and transcription of tRNAs at 5 dpf. Altogether, our data indicate that mutations in *polr3a* disrupt ribosome biogenesis and tRNA transcription, leading to increased cell death and reduced proliferation in cranial tissues, and hypoplasia of craniofacial cartilage and bone at 5 dpf. Current and future work aims to understand the mechanisms driving tissue-specific changes in Pol III-mediated transcription during craniofacial bone and cartilage differentiation.

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Loss of Pol III repressor Maf1 in neurons promotes longevity by preventing the age-related decline in 5S rRNA and translation

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Attenuating protein synthesis promotes longevity in multiple species. However, numerous observations that ageing drives a decrease in protein synthetic capacity hint at potential, unexplored benefits of stimulating protein synthesis in old age. Here we focus on Maf1, a repressor of protein synthesis genes transcribed by RNA Polymerase (Pol) III, such as the 5S rRNA and tRNAs; loss of *Maf1* is known to extend lifespan in mice. We find that knockdown of *Maf1* also extends lifespan in the fruit fly. *Maf1* limits longevity specifically from adult fly neurons, impacting age-related impairments in neuromuscular function as well as the function of a distal organ, the gut. Lifespan extension upon *Maf1* knockdown requires Pol III initiation on the *5S* rRNA. Indeed, reducing neuronal *Maf1* activity rescues the age-related decline in *5S* expression and protein synthesis in the brain. Hence, stimulating neuronal protein synthesis can promote healthy ageing. This effect appears cell-type specific, as reduced Pol III activity in the stem cells residing in the fruit fly gut also promotes longevity, in this case though altered tRNA expression.

Maf1 Cooperates with Progesterone Receptor to Repress RNA Polymerase III Transcription of tRNAs

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RNA Polymerase III (Pol III) transcribes small molecules necessary for translation including all transfer RNAs (tRNAs). Pol III activity is tightly regulated by tumor suppressors, the nutrient-sensing mTOR pathway, and the transcriptional repressor Maf1. Little is known of how steroid hormones regulate Pol III in mammalian cells, particularly since most studies on Pol III are in organisms lacking steroid receptors. Using breast cancer cells abundant for progesterone receptors (PR) and estrogen receptors (ER), we demonstrated that PR is associated with the Pol III complex at tRNA genes and that progestins downregulate tRNA transcripts in breast tumor models. To further elucidate the mechanism of PR-mediated regulation of Pol III, we studied the interplay between PR, the Pol III repressor Maf1, and TFIIIB, a core transcription component. ChIP-seq was performed for PR, the Pol III subunit POLR3A, the TFIIIB component Brf1, and Maf1 in breast cancer cells with or without progestin treatment. Upon progestin exposure, PR localized to approximately half of POLR3A-occupied tRNA genes, with Maf1 co-recruited to many of these PR-POLR3A sites. While progestin treatment did not significantly alter the number of tRNA genes occupied by Pol III or Brf1, Brf1 occupancy was stabilized, as indicated by increased peak amplitudes. Analysis of nascent tRNA transcription revealed a specific progestin-induced downregulation of approximately one-third of highly expressed tRNA genes. This repression was significantly reduced by Maf1 knockdown, indicating that Maf1 is necessary for PR-mediated tRNA transcription downregulation. Overall, these findings demonstrate a ligand-dependent PR-mediated repression of tRNA transcription through Maf1. We suggest this is one mechanism by which P indirectly suppresses estrogen-driven growth in breast cancer cells and has wider implications for hormone regulation of cell growth and differentiation.

Mouse Models of Polr3-related Disease Reveal Neurodevelopmental and Neurodegenerative Phenotypes

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Polr3-related disease is a debilitating neurologic syndrome caused by biallelic mutations in subunits of RNA polymerase III. Disease onset occurs from infancy to early adulthood and is associated with a variable range and severity of clinical features including hypomyelination, hypodontia, hypogonadotropic hypogonadism, cerebellar atrophy, myopia and short stature. Disease pathology is poorly understood and no treatments are available. Important progress in understanding the cellular and molecular mechanisms of *Polr3*-related disease has recently been made using mice expressing pathogenic mutations in *Polr3a.* Three different *Polr3a* mouse lines have been examined. These include (i) a developmental model targeting cells of the oligodendrocyte lineage responsible for axonal myelination; (ii) a second, more severe developmental model in which the mutation is expressed during embryogenesis in all neural cell populations and (iii) a postnatal whole-body inducible model that allows the impact of diminished Pol III transcription to be assessed in multiple tissues with a later onset of disease. Our current findings will be presented to inform how a deficiency in Pol III transcription leads to neurodevelopmental deficits and neurodegeneration.

Targeting the nucleoli as a strategy to treat ovarian cancer

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The nucleolus is the site for RNA Polymerase I (Pol I) transcription of ribosomal RNA (rRNA) genes and ribosome subunit assembly. Cancer cells exhibit enlarged nucleoli and hyperactivation of ribosome biogenesis to meet the high demand for protein synthesis. CX-5461 (Pidnarulex), a first-in-class selective inhibitor of Pol I transcript has demonstrated therapeutic benefits in various preclinical [1-5] and Phase I clinical trials of blood and solid cancers including ovarian cancer [6-7]. Our work has shown that CX-5461 activates a unique form of stress, termed nucleolar stress, leading to cell cycle arrest and cell death in ovarian cancer models [5]. CX-5461 exhibit a unique sensitivity profile compared with chemotherapeutics in patient-derived ovarian cancer cell models [5,8]. Thus, we proposed that activating nucleolar stress represents a promising new paradigm for cancer therapy.

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 types of distinct changes in nucleolar morphology that are linked to the inhibition of biological processes, including ribosome biogenesis, cell cycle and RNA metabolism. Our data highlight the tight coordination between these processes and nucleolar fidelity and identify specific pathways/factors as mediators of nucleolar stress and potential therapeutic targets. We have also identified and characterized a new role for protein phosphatases in the regulation of Pol I transcription.

To complement this screen and identify compounds that target the nucleoli, we completed a high-throughput drug screen of 24,000 compounds, including FDA-approved compounds, utilising the same screening approach, to identify compounds that trigger nucleolar stress and inhibit ovarian cancer cell growth. Cyclin dependent kinases (CDKs), topoisomerase, and other targets were identified as candidate hits on our screen. In summary, our work uncovered novel nucleolar stress pathways that can be harnessed as novel cancer therapy approaches.

References:

1. Bywater,M.J. et al. Cancer Cell 22, 51-65 (2012).
2. Devlin, J.R. et al. Cancer Discov 6, 59-70 (2016).
3. Hein, N. et al. Blood 129, 2882-2895 (2017).
4. Lawrence, M. G. et al. Mol Cancer Ther 20, 2140-2150 (2021).
5. Sanij, E. et al. Nat Commun 11, 2641 (2020).
6. Hilton, J. et al. Nat Commun 13, 3607 (2022).
7. Khot, A. et al. Cancer Discov 9, 1036-1049 (2019).
8. Yan, S. et al. British Journal of Cancer 124, 616-627 (2021).

Targeting ribosomal RNA biogenesis mitigates hypoxia-induced tumor progression and functionally reprograms tumor-associated macrophages in breast cancer

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The biological complexity of breast cancer, particularly in the context of its metastatic behavior and resistance to conventional treatments, highlights the need for a more detailed understanding of the mechanisms driving tumor progression. Intratumoral low oxygen tension sustains a tumor-promoting immune milieu and promotes cancer cell invasion and metastasis. Hypoxia-Inducible Factor 1-alpha (HIF1α) orchestrates cellular responses to hypoxic stress, mediating the regulation of genes implicated in adapting to perturbations in oxygen homeostasis. We identified a novel nucleolar localization domain in HIF1α that enables HIF1α to translocate to the nucleolus. Nucleolar HIF1α binds the rDNA promoter and upregulates RNA Polymerase I activity leading to dysregulated rRNA transcription and consequently enhanced ribosome biogenesis.

Quite unexpectedly, using gene set enrichment analysis (GSEA) of scRNAseq data encompassing patient breast tumors, we identified several significantly enriched pathways relevant to ribosome biogenesis and rRNA biosynthesis in the tumor-supportive M2-like macrophages in the tumor milieu. Aligned with this, we determined that M2-polarized macrophages support increased rRNA transcription. To understand the functional relevance of enhanced RNA Polymerase I in the tumor microenvironment, we inhibited RNA Polymerase I in mammary tumor model systems. Inhibition of RNA Polymerase I impeded aggressive traits of hypoxia-driven cancer progression. Concomitantly, inhibition of rRNA synthesis inflicted nucleolar stress in macrophages, altered the translation profile, and promoted a pro-inflammatory phenotype of M2-polarized macrophages resulting in a pro-inflammatory tumor microenvironment.

Cumulatively our work presents a tumor cell-intrinsic and tumor-extrinsic role for nucleolar activity in orchestrating cancer progression. As such, our work provides unexpected and unprecedented evidence that ribosome biogenesis is a targetable dependability of hypoxic tumor cells and alternatively polarized, tumor-supporting macrophages; inhibiting ribosome biogenesis is a therapeutic strategy to impede hypoxia-influenced tumor progression and functionally reprogram anti-inflammatory macrophages in breast cancer.

Expanding Horizons: Novel Pol I inhibitor PMR-116 demonstrates versatile anti-tumour activity across a broad-spectrum of high MYC malignancies

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Background:

Ribosome biogenesis (RiBi) is a fundamental determinant of cell growth and proliferation, frequently upregulated in cancer due to oncogenic activation, particularly by MYC. The first-generation RiBi inhibitor, CX-5461 (Drgyin et al., Cancer Research 2011; Bywater et al., Cancer Cell, 2011), showed clinical potential (Khot et al., Cancer Discovery, 2019; Hilton et al., Nat Commun. 2022) but also induced DNA damage through off-target inhibition of TOP2α (Cameron et al., Biomedicines, 2024; Koh et al., Nature Genetics, 2024), raising concerns about RiBi as a viable therapeutic target. A second-generation RiBi inhibitor, PMR-116, has been developed to overcome these limitations, offering improved drug-like properties and enhanced therapeutic efficacy.

Methods & Results:

We performed in-deps study of molecular mechanism of PMR-116 inhibitory activity and we found that unlike CX-5461, PMR-116 effectively inhibited Pol I transcription at stages subsequent to pre-initiation complex formation and moreover it inhibits RiBi without triggering global DNA damage signalling. We evaluated the anti-tumour activity of PMR-116 across a broad range of preclinical models, including both haematologic and solid malignancies. The inhibitor demonstrated potent anti-proliferative effects, particularly in cancers where MYC is a key driver or is highly expressed. Based on these data we have launched a phase I clinical trial of PMR-116, in patients with advanced solid tumours (CTRN12620001146987).

Conclusions:

Our findings establish RiBi as a genuine and exploitable therapeutic vulnerability in high-MYC cancers. PMR-116 exhibits robust efficacy in preclinical cancer models, with the potential to address the unmet need for effective treatments in MYC-driven malignancies that currently have poor clinical outcomes.

Genome-wide CRISPR screen identifies regulators of POLR1A turnover

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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 tumour 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 transuded 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 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. Under BMH-21 treatment, we further confirmed FBXL14 as top hit, that when depleted prevents loss of POLR1A. FBXL14 is a ubiquitin ligase potentially mediating POLR1A degradation as previously described by other team (Pitts et al, 2022). 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.

Unlocking the power of long read sequencing to study regulation of rRNA synthesis

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Eukaryotic cells contain at least three DNA-dependent RNA polymerases (Pols) that are responsible for the flow of genetic information from DNA to RNA through transcription. Pol I synthesizes the majority of the ribosomal RNAs (rRNAs), Pol II synthesizes messenger RNAs (mRNAs), and Pol III synthesizes transfer RNAs (tRNAs) and the 5S rRNA. The activities and regulation of these polymerases are intricately linked to cellular stress, development, metabolism, and overall growth. Notably, cancerous cells exhibit increased ribosome production, necessitating elevated rRNA synthesis by Pols I and III, alongside increased ribosomal protein synthesis by Pol II. Given that rRNA synthesis by Pol I is the initial and rate-limiting step in ribosome production, it is as a promising target for anti-cancer therapies.

Understanding the function and regulation of Pol I is pivotal for the development of effective therapeutic strategies. Significant progress has been made in this area, including research in our lab, where we employ various techniques to study Pol I transcription and the rapid, co-transcriptional processing of rRNA. Despite the expanding body of knowledge about Pol I mediated rRNA synthesis, numerous questions remain unanswered. Some of these questions are particularly challenging to investigate *in vivo* due to technological constraints. We are leveraging recent advancements in long-read, nanopore-based sequencing to overcome those limitations. Specifically, we are 1) addressing issues related to mature rRNA contamination in sample preparations, 2) making qualitative methods of rRNA processing analysis quantitative, and 3) developing a methodology to directly analyze *in vivo* co-transcriptional RNA structure dynamics. These *in vivo* approaches allow us to not only better understand the regulation of Pol I transcription and rRNA processing individually, but also to elucidate the interplay between Pol I transcription, rRNA processing and secondary structure.

Evolution of ribosome heterogeneity in teleosts

Michay Diez, Eric J. Ross, Dylan Tedder, Jennifer L. Gerton

Zebrafish (*Danio rerio*) display a remarkable developmental switch in the composition of their ribosomes, the machinery responsible for protein synthesis. This heterogeneity is partly driven by the presence of two subtypes of 45S ribosomal RNAs (rRNAs) that are transcribed during distinct developmental stages and display striking sequence divergence. The maternal rRNAs are expressed in oocytes and enriched in the egg and the early embryo. The somatic rRNAs are expressed after zygotic genome activation and are transcribed in virtually all adult tissues.

Zebrafish belong to the teleost clade of fishes, a group characterized by a whole-genome duplication (WGD) event leading to many vertebrate genes having duplicated orthologs in teleosts. To address whether the duplicated 45S subtypes in zebrafish originated with this event, we conducted a 45S evolutionary census of short-read whole genome sequencing data representing over 1500 teleost species. Our findings revealed the presence of multiple 45S paralogs in only 5% of teleosts. The low prevalence of nucleotide variation in the 45S of teleosts implies that the occurrence of zebrafish 45S subtypes did not originate with the teleost WGD event. Furthermore, most of the species in the 5% we detected belong to the *Danio* genus and its sister genus *Devario*. Hence, we hypothesized that zebrafish maternal and somatic 45S subtypes originated in the *Danio* and *Devario* common ancestor, 20 million years ago. To test this, we produced a de novo chromosome-level genome assembly of the fish *Devario devario* and confirmed the presence of two subtypes of 45S genes in this species. We are working to dissect the spatiotemporal expression and regulation of these subtypes in *Devario* and their role in development. Understanding the evolutionary origin of the maternal and somatic 45S subtypes of zebrafish will inform models for spatiotemporal control of 45S gene expression and the ribosomal developmental switch in zebrafish.

Inactivation of mTOR is a strategy for tumor resistance to ribosome biogenesis inhibition

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Increased ribosome biogenesis supports the need for high translation capacity and is a hallmark of cancer. Targeting this process presents a promising strategy for cancer therapy. We have developed first-in-class inhibitors of RNA polymerase I (Pol I) transcription that block the first and rate-limiting step of ribosome biogenesis, rRNA synthesis. We find that Pol I inhibition by BMH-21 leads to a significant decrease in active ribosomes. We found a broad, but heterogenous efficacy in 18 cancer cell lineages. To identify genes that drive potential resistance to Pol I inhibition, we performed genome-wide CRISPR-Cas9 screens in human colorectal carcinoma cells. These positive selection screens identified high-confidence hits leading to drug resistance and included all key positive regulators of the mTORC1 complex. These findings are striking and counterintuitive given that mTOR is a major driver of ribosome biogenesis and cellular translational programs. The findings were validated using chemical inhibition of mTOR by Torin-1, a catalytic mTOR inhibitor and genetic knockout of an activator of mTOR signaling. Consistently, compromised mTOR activity led to resistance to BMH-21. However, loss of mTOR activity did not abrogate Pol I transcription inhibition by BMH-21. To assess the impact on protein translation, we used polysome profiling and found that BMH-21 treatment caused a severe defect in ribosome biogenesis. Surprisingly, mTOR inactivation partially rescued the translation repressed by Pol I inhibition, suggesting that this rescue could be pivotal for cell survival. To profile translational events, we performed Ribo-seq and RNA-seq in the BMH-21 sensitive and resistant cells. These multi-omics results revealed that mTOR inactivation decreased polysome-based translation on 5'TOP mRNAs encoding ribosomal proteins, suggesting that mTOR inactivation reduces ribosome occupation on ribosomal mRNAs. We propose that mTOR inactivation switches off polysome translation to rearrange active ribosomes for translation of survival-essential mRNAs. We further quantified newly synthesized proteins by AHA-labeling combined with TMT-mass spectrometry. These data show that distinct proteins continue to be translated when both Pol I transcription and mTOR activity are blocked. Next, we will examine preferentially translated proteins in resistant cells, and explore combination therapies to circumvent the drug resistance. In summary, we uncover a new non-genetic model of cancer drug resistance termed as translational fitness that enables cells to survive severe translational repression. These findings reveal an unexpected complication by mTOR inhibitory strategies and have implications for exploring effective drug combinations in cancer therapy.

POSTER PRESENTATIONS

All posters will be displayed on designated poster boards beginning at the time of registration on Thursday, May 29, through the conclusion of the program on Saturday, May 31. Odd-numbered posters will be presented on Thursday, May 29, while even-numbered posters will be presented on Friday, May 30. However, all of the breaks and receptions during the meeting provide additional informal poster viewing time.

Defining R-Loop and G-Quadruplex-Forming Sequences in the Highly Repetitive Ribosomal DNA Locus

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Ribosomal DNA (rDNA) is the gene locus that encodes for ribosomal RNA (rRNA), which is essential for producing ribosomes and maintaining cellular protein synthesis. The rDNA locus is a tightly regulated and repetitive genetic element organized in a head-to-tail cluster of approximately 200–300 repeats. These repeats form nucleolar organizing regions (NORs) in acrocentric chromosomes. The rDNA locus is transcribed by RNA polymerase I to generate 18S, 5.8S, and 28S rRNA, which are processed and partially assembled in the nucleolus to form ribosomal subunits (ribosomes). rDNA transcription dysregulation or aberrant rRNA modification can lead to disease states, including craniofacial developmental disorders and cancer. Given the high transcriptional activity and GC-rich nature of this region, we hypothesized that rDNA locus favors the formation of non-canonical DNA structures, such as R-loops and G-quadruplexes (G4s). R-loops consist of an RNA-DNA hybrid with a displaced single-stranded non-template DNA strand. G4s form through Hoogsteen hydrogen bonding of guanine quartets and are stabilized by monovalent cations. Stable R-loops and G4s play diverse regulatory and pathological roles by influencing transcription, replication, and genome stability. However, experimental detection of these structures in rDNA is challenging due to the repetitive nature of rDNA, which complicates mapping and has been ignored in most genome-wide studies. While studies in yeasts and plants support the presence of R-loops and G4s in rDNA, comprehensive investigation of the human rDNA locus remains limited. This gap can be addressed using computational tools designed to predict non-canonical structures based on sequence features in nucleic acid sequences. Here, we show that R-loop forming sequences (RLFS) and potential canonical G-quadruplex forming DNA sequences (pG4-CS) are non-randomly distributed across the rDNA locus and are evolutionarily conserved, highlighting their potential role in maintaining rDNA stability. In conclusion, the rDNA locus is prone to form stable non-canonical DNA structures. The non-arbitrary distribution and evolutionary conservation of non-canonical DNA structures across the rDNA locus underscore their potential roles in regulating transcription, rRNA processing, and maintaining genomic stability.

Poster board #1

The membrane fusion protein NAPA (α-SNAP) is required for pre-rRNA transcription

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Ribosome biogenesis is a highly regulated cellular activity that requires a multitude of diverse protein and RNA factors to assist in maturation and assembly. Defects in this process are implicated in a variety of genetic human diseases. We have previously developed and utilized an siRNA screen to perform a genome-wide search for novel regulators of ribosome biogenesis in MCF10A human breast epithelial cells (Farley-Barnes et al., 2018). One hit identified in this screen is NSF attachment protein alpha (NAPA), also known as α-SNAP. NAPA is a key component of the cytoplasmic SNAP-SNARE vesicle fusion system, where it promotes the disassembly of the bound SNARE complex by NSF following membrane fusion (Jahn et al., 2023). Although NAPA's function within the vesicle fusion context has been well characterized, its involvement in ribosome biogenesis has not yet been explored. We have shown that siRNA depletion of NAPA decreases incorporation of 5-ethynyl uridine into pre-rRNA, reduces levels of the primary pre-rRNA transcript (47S/45S), and lowers global protein synthesis. These results point towards an integral role for NAPA in the pre-rRNA transcription step in ribosome biogenesis. Together, our work will provide insight into the action of the cytoplasmic protein NAPA in the nucleolar steps of ribosome biogenesis.

Poster board #2

Getting More for Less: Synergistic Drug Combinations with RNA Polymerase I Inhibitors for Cancer Therapy

Jonathan Y. Chung[1], Bruce A. Knutson[1]

[1]SUNY Upstate Medical University

Despite treatment advances, breast cancer (BC) remains a major global health challenge and there is an ongoing need for specific and effective BC therapies. Dysregulated ribosome biogenesis and hyperactivation of RNA polymerase I (Pol I) are critical features of breast cancer progression and aggression. Specific inhibition of Pol I is a promising new targeted therapy with high potential to reduce BC disease burden by impairing tumor proliferation, metastasis, and chemoresistance. Small-molecule Pol I inhibitors, such as CX-5461 and BMH-21, have been identified and are under investigation for their ability to treat BC. To enhance the efficacy of Pol I inhibitors in cancer therapy, we investigated the ability of Pol I inhibitors to synergize with each other. Our analysis showed that CX-5461 and BMH-21 synergize in reducing viability of MCF-7 BC cells. Compared to monotherapies, combination treatment enhanced activation of p53 and degradation of the Pol I large subunit, RPA194. Because Pol I inhibitors activate the transcription factor p53, and p53 is commonly mutated in BC, we investigated the ability for p53 reactivators to synergize with Pol I inhibitors in p53 mutant cells. We hypothesized that treating p53 mutant cells with p53 reactivators, which activate wild-type functions in mutant p53 proteins, would enhance sensitivity to Pol I inhibition. To this end, we evaluated synergy of drug combinations composed of one Pol I inhibitor (CX-5461 or BMH-21) and one p53 reactivator (ZMC-1 or APR-246). Synergy was highly variable between combinations, highlighting the importance of drug-specific effects in mediating synergy. Based on our observations, we performed further analysis of p53-mutant BC cells treated with BMH-21 alone and found that BMH-21 alone was sufficient to promote mutant p53 reactivation. Our findings demonstrate that Pol I inhibitors may synergize with other anti-cancer agents in treating BC, highlighting the usefulness of Pol I inhibitors in drug combinations. Furthermore, our approach allowed us to identify a previously unrecognized function of BMH-21 in reactivating mutant p53. These insights into BC treatment with Pol I inhibitor combinations may guide future development and clinical application of these exciting new therapies.

Poster board #3

Expansion Microscopy of the Nucleolus

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The nucleolus has a unique nanoscale structure whose organization is responsive to cell signals and disease progression. Expansion Microscopy (ExM) has potential to capture nanoscale insights into nucleolar morphology. We apply dual protein Expansion Microscopy (dual-proExM) in combination with click Expansion (click-ExM) and spinning disk microscopy to capture super-resolution nucleolus images at ~ 45 ± 2 nm resolution. Time-dependent changes in nucleolar structure and function under nucleolar stress induced by Actinomycin D, oxaliplatin, and other small molecules reveal new 'condensation' stages that occur prior to the complete sequestration of RNA Pol I into nucleolar caps. RNA transcription is linked to nanoscale protein rearrangements using a combination of click-ExM and pro-ExM, revealing locations of active transcripts during the early stages of nucleolar stress reorganization. With prolonged stress, fibrillarin and NPM1 are observed to segregate from the nucleolus into nucleoplasmic foci. These observations provide detail on the early and late stages of nucleolar stress response, and highlight utility of the expansion protocol for nanoscale nucleolar imaging.

Poster board #4

Biochemical Mechanisms Governing RNA Polymerase III and Herpesvirus Interaction

Fabiola Iommazzo[1], Mariavittoria Pizzinga[1], Fabio Pessina[1], Veronica La Rocca[1], Oliver Harschnitz[1], Alessandro Vannini[1]

[1]Human Technopole

RNA Polymerase III (POL III) is a multi-subunit complex involved in the transcription of small non-coding RNAs, including transfer RNAs (tRNAs), 5S ribosomal RNA (rRNA), and U6 small nuclear RNA (snRNA). POL III assembly starts in the cytoplasm, before being transported to the nucleus where it exerts its primary transcriptional function. While usually associated with nuclear activity, recent studies highlight a cytoplasmic activity connected to the innate immune response to foreign DNA. As such, POL III recognizes viral AT-rich DNA sequences and transcribes them in 5'-ppp RNAs, which activate the cytosolic sensor RIG-I and, in turn, the interferon response. Consistent with these findings, mutations in several POL III subunits (e.g., POLR3A, POLR3C, POLR3E, and POLR3F) have been associated with immunodeficiency and susceptibility to herpesvirus reactivation. However, the precise mechanism of POL III viral DNA recognition is still unknown.

My PhD project aims at filling several gaps in the field: i) whether POL III recognizes viral DNA via a canonical promoter or in a promoter-independent manner; ii) the pool of viral and host factors involved; iii) the cellular context of POL III anti-viral activity; iv) how this affects the innate immune response in both infected and neighboring cells.

Our preliminary data shows that infection of both transformed (A549) and untransformed (MRC5) cells with HSV-1 at 6 hours post-infection (hpi) causes the re-localization of the POL III subunits to a peri-nuclear region, forming distinctive "pearl necklace-like structures" that strongly co-localize with a marker for HSV-1 capsid. Additionally, co-staining with markers of the endomembranous systems, highlights POL III accumulation coincides with Golgi and trans-Golgi compartments, indicating an association with the viral egress pathway. This phenomenon is specific for HSV-1 but not for RNA viruses like influenza.

To understand the mechanism of POL III-mediated viral recognition, I will employ a combination of transcriptomics and proteomics analysis. Global RNA sequences will allow us to determine whether POL III functions as a primary viral DNA detector in the cytoplasm or post-genome release into the nucleus. Moreover, this analysis may also identify consensus viral sequences necessary for POL III recognition and transcription, as well as host reprogramming during the infection. For the proteomics part, I will use a combination of co-fractionation mass spectrometry (CoFracMS) and proximity labeling, to identify both stable and transient interactors of POL III during the infection. It will also allow us to uncover POL III complex composition changes, as well as host factors involved in the same functional network.

Overall, my PhD project integrates molecular biology and structural proteomics with immunology and virology to define POL III's role in antiviral immunity and host defense mechanisms. By defining the molecular mechanisms underlying POL III's antiviral function, we may identify new therapeutic targets for modulating immune responses against herpesviruses and broadly to other DNA viruses, as well as provide mechanistic significance into the molecular basis of viral susceptibility in affected individuals.

Poster board #5

Investigating glucocorticoid receptor regulation of tRNA transcription in breast cancer

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Most breast cancers are driven by estrogen receptor (ER) activity, and for decades this has been the basis of ER-targeting breast cancer therapies. The glucocorticoid receptor (GR) responds to endogenous corticosteroids, and its activity is associated with variable prognosis in breast cancer. In ER-dependent cancers, GR is associated with better outcomes whereas in ER-independent cancers it is associated with worse outcomes. Thus, there is a need to investigate the mechanisms of how GR might drive or inhibit breast cancer cell growth. We previously demonstrated an interaction between progesterone receptor (PR) and RNA Polymerase III (Pol III) in breast cancer cells, leading to decreased tRNA transcription. From this, we hypothesize that GR activity in ER+ breast cancer also results in decreased tRNA transcription and growth through association with Pol III. To test this, we first assessed GR expression and impact on tRNA expression in several breast cancer cell lines. Treatment with glucocorticoid agonists decreased expression of select pre-tRNAs. Chromatin immunoprecipitation (ChIP)-qPCR assays found glucocorticoids induced GR occupation at select tRNA genes. Ongoing studies are investigating how GR agonists and antagonists affect nascent tRNA transcription. Future work will include investigating the mechanisms of how GR and other nuclear receptors associate with the Pol III complex near tRNA genes, how these interactions alter tRNA pools, and whether GR regulates Pol III transcription of tRNAs in ER-negative breast cancers. These findings are expected to provide insight into the divergent roles of GR in breast cancer by revealing its regulation of Pol III-dependent transcription in addition to its known effects on Pol II-regulated genes.

Poster board #6

An RNA polymerase III tissue and tumor atlas delineates context- and cancer-specific transcription patterns and disease signatures

Simon Lizarazo[1], Sihang Zhou[2], Ruiying Cheng[2], Rajendra K C[3], Yawei Shen[4], Qing Liu[4], Kevin Van Bortle [2]

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RNA polymerase III (Pol III) produces a plethora of small noncoding RNA involved in nearly all cellular processes, from transcription regulation and splicing to RNA stability, translation, and protein turnover. Though Pol III activity is broadly coupled with cellular demands for protein synthesis and growth, a more precise understanding of gene-level dynamics and context-specific expression patterns remain missing, in part due to multiple challenges related to sequencing and mapping Pol III-derived small ncRNA species. Here, we establish a multi-tissue map of Pol III activity across 19 tissues and 22 primary cancers by comprehensively profiling the chromatin accessibility of Pol III-transcribed genes (e.g. tRNA, 5S rRNA, U6 snRNA, 7SL SRP RNA, etc.). Our framework relies on the unique relationship between gene accessibility and Pol III transcription and defines a binary gene “on” vs. “off” state across ~500 ATAC-seq datasets. Using an information entropy method to characterize multi-context uniformity, we provide a definition of the “core” Pol III transcriptome universally active in all specialized tissues and catalog genes with varied levels of uniformity (High, Intermediate, and Low Uniformity). Notably, our genomic atlas points to variable levels of restriction and expansion of Pol III activity across tissues, including sharp contraction of the Pol III transcriptome in heart tissues and frequent expansion across diverse cancers. We discover different sequence-based and epigenomic regulatory features at high-intermediate and low-uniformity genes, including evidence for multiple active and repressive mechanisms that likely contribute to the global expression and silencing of Pol III-transcribed genes respectively. These findings provide a resource for better understanding Pol III dynamics in tissues and the link between Pol III overactivity and small RNA biogenesis in cancer.

Poster board #7

Rsp5 ubiquitin ligase is implicated in the control of tRNA processing and RNA polymerase III transcription machinery

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Transfer RNA (tRNA) biogenesis in yeast involves the synthesis of the primary transcript by RNA polymerase III (Pol III), followed by processing to remove 5' and 3' ends, further maturation, and export to the cytoplasm. In this study, we found that the ubiquitin ligase Rsp5 influences both tRNA transcription and the initial processing of tRNA precursors. We observed high levels of unprocessed primary tRNA transcripts in rsp5 mutants at elevated temperature, which were reduced upon the overexpression of RPR1, the catalytic RNA subunit of RNase P. This observation suggests a role for Rsp5 in the maturation of 5' ends of tRNA precursors. Under the same conditions, in vivo labeling indicated that the amount of newly synthesized tRNA decreased. Furthermore, we found that Rsp5 directly interacted with the Tfc3 subunit of the TFIIIC transcription factor, which is modified by ubiquitination. The inactivation of Rsp5 catalytic activity affected the interaction between the general Pol III factors TFIIIB and TFIIIC and decreased the recruitment of TFIIIC to tRNA genes. These findings suggest that Rsp5 ligase is implicated in the control of Pol III transcription in yeast.

Poster board #8

Pol III Transcription in Mouse Models of Polr3-related Disease.

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Pathogenic variants in subunits of RNA polymerase (Pol) III cause a spectrum of *Polr3*-related neurodegenerative diseases including 4H leukodystrophy. Disease onset occurs from infancy to early adulthood and is associated with a variable range and severity of neurological features. The molecular mechanisms by which reduced Pol III transcription results primarily in central nervous system phenotypes are unknown.

We have developed three conditional mouse models that express the same pathogenic *Polr3a* mutation. Each model exhibits features of *Polr3*-related disease with varying severity of behavioral deficits and cerebral and cerebellar pathology. We will present comparative data on the consequences of defective Pol III transcription in developmental models targeting the oligodendrocyte lineage and neural stem cell-lineages, and in a postnatal whole-body model where the mutation is expressed across a range of adult tissues.

We find that different tissues, brain regions and brain cell-types vary in their sensitivity to defective Pol III transcription, responding with reductions in select Pol III transcripts, and variably inducing a Pol II stress response and activating an immune response.

Poster board #9

Disrupted ribosome biogenesis upon ribosomal DNA reduction triggers ribosomal DNA repeat expansion in Drosophila male germline stem cells

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Genomes' retention of hundreds of ribosomal DNA (rDNA) repeats throughout populations suggest the presence of germline mechanisms to maintain rDNA abundance as genomes are transmitted from generation to generation. In *Drosophila*, this maintenance is achieved by the rapid expansion of rDNA copies in male germline stem cells (GSCs) that restores lost rDNA. This rDNA expansion relies on the activity of the rDNA-specific retrotransposon R2, thus exposing the genome to deleterious retrotransposition when restoring rDNA copy number. Unsurprisingly, this rDNA expansion activity and coordinated R2 expression is typically repressed and is only activated when rDNA expansion is necessary, though it is unclear how GSCs sense rDNA copy number to regulate this process. Here we find that ribosome biogenesis serves as a cellular sensor to monitor rDNA copy number status within GSCs and regulate rDNA expansion activity. Transcriptomic analysis revealed reduced ribosomal protein gene expression in low rDNA GSCs, and we find low rDNA GSCs have cellular hallmarks of disrupted ribosome production and reduced translation. Curiously, these defects do not appear to be due to differences in rRNA transcription rate, but instead erroneous processing of normally silent rRNA variants that become transcribed in low rDNA GSCs. We further find that RNAi mediated knockdown of ribosomal proteins in GSCs induces rDNA expansion, suggesting that the disruption of ribosome biogenesis caused by reduced rDNA copy number acts as a proxy sensor for genomic rDNA status in GSCs. Interestingly, among the cells in low rDNA testes, ribosome biogenesis is most strongly obstructed in GSCs, and inhibition of ribosomal proteins in differentiating germ cells does not cause rDNA expansion. Thus, rDNA expansion may be limited to GSCs because the rDNA-dependent sensitivity and monitoring of ribosome biogenesis is restricted to these cells. We are now working to uncover these GSC-specific mechanisms of ribosomal monitoring and are investigating how rRNA sequence variants impact processing.

Poster board #10

Shared Blueprint, Custom Builds: Unraveling the Distinct Assembly Roles of the Alpha-like Subunits in RNA Polymerases I and III Biogenesis

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RNA Polymerases (Pols) I and III are large multi-subunit complexes that synthesize rRNA, which is crucial for ribosome production and subsequent protein synthesis. Perturbations in Pol I and III transcription lead to dysregulated ribosome biogenesis, which impacts protein synthesis and results in a cascade of downstream cellular defects that affect cell growth and division. Pols I and III encode and share two distinct alpha-like subunits, POLR1C and POLR1D, that are evolutionarily conserved across all life forms. These subunits form a heterodimer, which is proposed to be crucial for assembly. Dysregulation of these alpha-like subunits is linked to diseases such as cancer, developmental disorders, and neurological defects. While the effects of mutations in the alpha-like subunits have been widely characterized, the functional roles of these subunits in Pols I and III complex biogenesis remain largely unexplored. Given the evolutionary conservation of the alpha-like subunits, we used AC19 and AC40, the yeast orthologs of POLR1D and POLR1C, to decipher the role of these subunits in Pols I and III complex biogenesis. We utilized the Auxin-inducible degron (AID) system to trigger the proteasomal degradation of AC19 or AC40 and subsequently analyzed the impact of the loss of either subunit on the integrity of Pols I and III complexes, as well as the protein abundance of other subunits within these complexes. Our findings reveal that the alpha-like subunits play distinct roles in the subunit expression dynamics and integrity of these polymerases. Although Pols I and III share a similar subunit architectural blueprint, our results suggest that Pols I and III have distinct, if not custom, assembly pathways, diverging not only from each other but also from other Pols. These findings provide new insights into Pol subunit interactions and possibly points of regulation that could inform therapeutic strategies for diseases linked to Pol dysregulation.

Poster board #11

Regulation of RNA Polymerase I in Hematopoiesis

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Ribosomal RNAs (rRNAs) are the most abundant RNAs, and their transcription in mammals from 300-600 copies of rDNA repeats by RNA polymerase I (Pol I) is the most energy-intensive transcriptional process in the cell. Complex mammalian organ systems contain millions of stem, progenitor, and differentiated cell types that vary in size, function, and proliferation rates. A gap remains in our understanding of how the universal process of rRNA transcription is customized in a cell-type-specific manner to meet the specific ribosomal demands of complex multicellular tissues.

Using the mouse hematopoietic tree as a model system, we used FISH-Flow (fluorescent in-situ hybridization combined with flow cytometry) to construct a detailed map of nascent (47S) and mature (18S and 28S) rRNA levels in normal and leukemic hematopoiesis. Within normal hematopoiesis, we identified a 7-fold range of nascent rRNA levels and a 9-fold range of mature rRNA levels. These levels were tightly associated with cell identity and were largely independent of cell cycle status. Bone marrow from mice with acute myeloid leukemia exhibited elevated levels of nascent and mature rRNA compared to matched normal cell types.

We previously reported that the master myeloid transcription factor (TF) CEBPA binds to a canonical motif sequence on rDNA repeats (Antony et al, Molecular Cell 2022). We also reported that degradation of CEBPA in a mouse myeloid cell line led to rapid reduction (within hours) of Pol I occupancy on rDNA, followed by reduced 47S rRNA levels, ribosome subunit abundance, and growth. To further understand the steps of Pol I activity regulated by this cell-type-specific factor, we used live-cell single-molecule imaging of Pol I and its initiation factor RRN3. We generated a mouse cell line with GFP fused to the *Ubtf* locus, allowing us to constitutively visualize active rRNA repeats bound by UBTF protein. We also inserted a *Halo* sequence into the N-terminus of the endogenous *Polr1a* and *Rrn3* loci, enabling live-cell visualization of individual POLR1A and RRN3 molecules using low concentration of Halo fluorescent ligand and sparse photoactivation. Using imaging at 10 ms/frame, we observed that Pol I and RRN3 exist in two populations within the nucleus: (i) Slow-moving (co-localizing with UBTF, and therefore rDNA-loaded), and (ii) Fast-moving (freely diffusing). We observed that 70% Pol I molecules and 25% of RRN3 molecules are rDNA loaded at baseline. These findings support a model in which a majority of Pol I molecules are actively engaged in transcription, and unbound RRN3 is available in abundance to bind with free Pol I and facilitate its rDNA loading. Notably, non-DNA-bound Pol I and RRN3 molecules are not confined to the nucleolus but instead explore the entire nucleus.

We then degraded CEBPA protein (tagged with FKBP degron), and observed that both Pol I and RRN3 molecules showed a decreased proportion of the “slow-moving” population as early as 2 hours after CEBPA degradation. This indicates that CEBPA loss reduces loading of the Pol I-RRN3 complex on rDNA, consistent with our published findings of reduced Pol I and RRN3 ChIP-Seq signal. We further performed imaging at 500 ms/frame and captured long tracks of slow-moving molecules, allowing us to measure residence time on DNA. CEBPA degradation led to a progressive increase (within 2 hours) in the duration of slow-moving tracks of both Pol I and RRN3. These findings suggest that CEBPA loss increases the pausing of the Pol I-RRN3 complex at rDNA promoters.

In summary, our FISH-Flow studies provide a detailed map of rRNA transcription across the mouse hematopoietic tree. Our live-cell single-molecule tracking studies indicate both that Pol I-RRN3 pauses at promoters, and that CEBPA promotes loading and pause release. This illustrates how a lineage-defining TF can promote rRNA transcription in a cell-type-specific manner, a mechanism that may be broadly relevant across eukaryotic biology.

Poster board #12

Killing Two Birds with One Stone: Dual Inhibition of RNA Polymerases I and III as a Novel Strategy for Targeting Ribosome Biogenesis in Cancer

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Dysregulated ribosome biogenesis is a hallmark of cancer. RNA polymerase I (Pol I) and RNA polymerase III (Pol III) are essential for cellular growth, particularly in rapidly proliferating cancer cells. Pols I and III are upregulated in most if not all cancers, providing essential components for ribosome biogenesis and protein synthesis to meet the protein requirements of cancer cells. Given their central role in tumor cell proliferation, Pol I and Pol III represent attractive therapeutic targets. Pol I transcribes the majority of the ribosomal RNA (rRNA) that forms the structural and catalytic core of the ribosome. Likewise, Pol III transcribes the 5S rRNA and tRNAs necessary for translation of mRNA into mature proteins. Current inhibitors targeting Pol I (BMH-21) and Pol III (ML-60218) have shown promise individually, however no strategy exists to simultaneously inhibit both Pols I/III. The current bottleneck in the field is the limited understanding of the synergistic effects of combining the current Pols I/III inhibitors, as well as the absence of a dual inhibitor optimized to target both Pols I/III.

To address this gap, we will target POLR1D and POLR1C which are essential subunits that are shared between both Pols I and III. These two subunits form a heterodimer subcomplex which plays a critical role in Pols I/III complex assembly. To develop more specific and selective approaches to target Pols I/III dysregulation in cancer, we employ structure-based design to develop peptide inhibitors against the POLR1D/POLR1C subcomplex. Specifically, we utilize the Peptiderive application on the Rosetta Online Server that Includes Everyone (ROSIE). **We hypothesize that a novel peptide inhibitor that simultaneously targets both Pols I/III will disrupt Pols I/III complex integrity and function, inhibiting transcription and translation to maximize therapeutic efficacy.** Here, we present our current findings that the combined inhibition of Pols I/III and the development of a dual peptide inhibitor that targets both Pols I/III will show maximized therapeutic efficacy as a novel anti-cancer therapeutic.

Poster board #13

Structural basis of archaeal FttA-dependent transcription termination

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The ribonuclease FttA (also known as aCPSF and aCPSF1) mediates factor-dependent transcription termination in archaea. Here we report the structure of a *Thermococcus kodakarensis* transcription pre-termination complex comprising FttA, Spt4, Spt5 and a transcription elongation complex (TEC). The structure shows that FttA interacts with the TEC in a manner that enables RNA to proceed directly from the TEC RNA-exit channel to the FttA catalytic centre and that enables endonucleolytic cleavage of RNA by FttA, followed by 5'→3' exonucleolytic cleavage of RNA by FttA and concomitant 5'→3' translocation of FttA on RNA, to apply mechanical force to the TEC and trigger termination. The structure further reveals that Spt5 bridges FttA and the TEC, explaining how Spt5 stimulates FttA-dependent termination. The results reveal functional analogy between bacterial and archaeal factor-dependent termination, functional homology between archaeal and eukaryotic factor-dependent termination, and fundamental mechanistic similarities in factor-dependent termination in bacteria, archaea, and eukaryotes.

Poster board #14

The Human RNA Ligase RLIG1 Regulates Ribosome Biogenesis

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Ribosome biogenesis is a complex and highly regulated process essential for cell function, and aberrations in this process are associated with disease, including ribosomopathies and cancer. A genome-wide, imaging-based siRNA screen in MCF10A breast epithelial cells was developed by members of the Baserga laboratory to identify novel regulators of ribosome biogenesis. Using nucleolar number (nucleoli per nucleus) as an output, a decrease in the average nucleolar number from 2-3 to one upon siRNA knockdown indicates a role for the target gene in ribosome biogenesis. We identified RLIG1, a recently described RNA ligase, as a hit from this screen, and validated this hit via siRNA deconvolution screening. RLIG1 was largely uncharacterized until recently, when Yuan et al. 2023 and Hu & Lopez et al. 2024 found that it catalyzes RNA ligation through a self-AMPylating 5' to 3' mechanism. While this mechanism has now been rigorously established *in vitro*, these research groups have proposed different RNA substrates as primarily relevant for the biological role of RLIG1 – 28S rRNA or tRNA, respectively. Due to this lack of consensus on the specific role of RLIG1, we are interested in understanding of the biological functions of RLIG1 in cellular processes. In addition to reducing nucleolar number, we found that siRNA depletion of RLIG1 inhibits nucleolar ribosomal RNA (rRNA) biogenesis as measured by 5-ethynyluridine incorporation. I further validated this effect on early ribosome biogenesis by measuring levels of the primary pre-rRNA transcript with RT-qPCR and rDNA promoter activity with a luciferase reporter assay. I found that both were lower upon siRNA knockdown of RLIG1. I verified that siRNA-mediated knockdown of RLIG1 inhibits global protein synthesis measured by puromycin incorporation, as expected with defective ribosome biogenesis. Together, this suggests a role for RLIG1 in the earliest stages of ribosome biogenesis, pre-rRNA transcription by RNAPI. Our research will continue to explore the underlying mechanism of RLIG1 in ribosome biogenesis, including whether the catalytic domain critical for RNA ligase activity is essential for its nucleolar function.

Poster board #15

Probing Nuclear DNA-dependent RNA Polymerase IV subunit functions in vitro

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All eukaryotes have three essential DNA-dependent RNA polymerases—Pol I, II, and III—each composed of 12 to 17 subunits. Plants have two additional nuclear multi-subunit RNA polymerases, abbreviated as Pol IV and Pol V. Pol IV and V evolved as specialized forms of Pol II, sharing more than half of their non-catalytic subunits with Pol II. Pol IV and V synthesize noncoding RNAs that guide transcriptional gene silencing—primarily of transposable elements—via a process known as RNA-directed DNA methylation (RdDM). In the RdDM pathway, Pol IV generates RNA transcripts that are channeled into the associated RNA-dependent RNA polymerase, RDR2, which then synthesizes the complementary RNA strand. The subsequent double stranded RNA is then processed into a short interfering RNA (siRNA) duplex by DICER-LIKE 3 (DCL3).

Genetic evidence indicates that the individual loss of Pol IV's fourth (NRPD4), seventh (NRPD7), or ninth (NRPD9a orNRPD9b) subunits doesn't affect siRNA biogenesis in vivo. However, simultaneous loss of both the fourth (NRPD4) and seventh (NRPD7) subunits results in a loss of RdDM-associated DNA methylation. The biochemical basis for this loss of function is unknown. To address this gap, we developed a system for co-expressing all 12-subunits of Pol IV in cultured insect cells using baculovirus vectors. The *resulting* recombinant Pol IV contains all twelve subunits and is functional for Pol IV transcription and RDR2 coupling *in vitro*. We have also demonstrated that Pol IV activity is abolished when all ten non-catalytic subunits are absent. Using this system, we aim to further investigate the roles of individual non-catalytic subunits and subunit complexes by systematically removing them and assessing their impact on Pol IV function.

Poster board #16

Tissue-specific requirement of Polr1D in the Prothoracic Gland for ecdysone-mediated developmental transitions

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Ribosome biogenesis, the process of producing ribosomes responsible for protein synthesis, is a fundamental and highly regulated cellular activity. POLR1D is an essential and shared subunit of RNA Polymerases I and III (Pols), which preform the rate limiting step of ribosome biogenesis; the transcription of the rRNA incorporated into ribosomes. Mutations in POLR1D cause Treacher Collins Syndrome, a craniofacial disorder that arises from impaired ribosome biogenesis in neural crest cells. However, the precise role of POLR1D in development and disease pathologies remains less understood. Moreover, it is surprising that mutations in POLR1D that disrupt ribosome biogenesis globally result in cell and tissue-specific defects. Previous work from our lab using a Polr1D mutant *Drosophila melanogaster* model has uncovered a critical function of Polr1D in promoting larval development. Additionally, we found that RNAi knockdown of *Polr1D* in several non-neural *Drosophila* tissues caused developmental defects that phenocopy mutations affecting ecdysone signaling. Ecdysone is a steroid hormone produced in the prothoracic gland (PG) of insects that triggers developmental transitions. Here, we show that Polr1D is required for PG development and ecdysone production to facilitate larval developmental transitions. We found that *Polr1D* RNAi in the PG causes larval developmental arrest due to defective peripheral ecdysone signaling. We also found that Polr1D is required for the growth of PG cells, and for maintaining nucleolar structure. We found that Polr1D is required for the synthesis of mature ribosomes and the production of the Pol III-transcribed 7SK RNA. Furthermore, developmental arrest of *Polr1D* RNAi larvae and Polr1D mutant larvae was partially rescued by treatment with exogenous ecdysone. Given the parallels between ecdysone signaling and mammalian steroid hormone signaling, *Drosophila* offers a valuable model system for exploring the developmental consequences of disease-causing mutations in POLR1D, including potential endocrine dysfunctions seen in other diseases linked to Pol I and III, like 4H leukodystrophy. These results demonstrate a role for *Drosophila* Polr1D in PG development and suggests that disruptions in human POLR1D might impact additional cell types during development.

Poster board #17

Cancer-associated snaR-A noncoding RNA interacts with core splicing machinery and disrupts processing of mRNA subpopulations

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RNA polymerase III (Pol III) transcription in cancer is linked with the emergence of snaR-A (small NF90-associated RNA isoform A), a hominid-specific ncRNA previously shown to enhance cell proliferation, migration, and invasion. Here, we investigate snaR-A-protein interactions to delineate its role as a putative driver of cancer progression. At the molecular level, we discover that snaR-A interacts with mRNA splicing factors, including SF3B2, a core component of the U2 small nuclear ribonucleoprotein (snRNP), and localizes to subnuclear foci enriched for mRNA splicing factors. Ectopic snaR-A overexpression leads to increased intron retention, a hallmark of inefficient splicing, whereas depletion of endogenous snaR-A reduces intron retention in mRNA subpopulations associated with 3' intron-exon U2 snRNP residency and nuclear speckle proximity. We additionally show that chromatin patterns indicative of snaR-A gene activity in tumors are unfavorable signatures for cancer patients, providing further evidence that snaR-A emerges as a potential disease factor in cancer progression. Taken together, these findings establish snaR-A ncRNA as a molecular disruptor of splicing. We propose that snaR-A-related splicing disruption may phenocopy previously reported splicing defects and consequences attributed to mutations in U2 snRNP in cancer, eliciting an alternative, non-mutational pathway during tumorigenesis.

Poster board #18

Genetic and epigenetic requirements for RNA Polymerase V Localization at sites of RNA-directed DNA methylation

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RNA-directed DNA methylation is a complex process that silences retrotransposons, DNA viruses and other selfish genetic elements in plants. Loci silenced by the pathway are specified in an RNA-dependent manner, involving both short interfering RNAs (siRNAs) and long noncoding RNAs to which siRNAs basepair. These long noncoding RNAs are synthesized by nuclear DNA-dependent RNA Polymerase V (Pol V). How Pol V is recruited to its sites of action remains undefined at a biochemical level, but genetic and genomic studies have implicated pre-existing DNA cytosine methylation patterns, methylcytosine reader proteins (SUVH2 and SUVH9), Argonaute proteins (AGO4, AGO6, or AGO9), siRNAs, proteins comprising the ATP-dependent DNA translocase complex known as DDR, and the C-terminal domain (CTD) of the Pol V largest subunit. To assess the roles of these various activities in Pol V recruitment and localization, we performed genome-wide chromatin immunoprecipitation (ChIP) studies to determine sites of Pol V occupancy in wild-type plants and mutants missing one or more of the activities in question.

We find that Pol V localization is dependent on a specific subdomain within the Pol V CTD, the methylcytosine reader proteins (SUVH2 or SUVH9), and the DDR complex. In an *ago4 ago6 ago9* triple mutant, the majority of Pol V ChIP peaks are unaffected, suggesting that Argonaute proteins and the siRNAs they bind are not essential for Pol V recruitment. Intriguingly, in the absence of SUVH2, SUVH9 or DDR activity, Pol V mislocalizes to new chromatin regions not normally associated with RNA-directed DNA methylation, implying a shift in binding affinity when proper recruitment is compromised.

Together, our results provide new insights into the molecular architecture underlying Pol V targeting.

Poster board #19

A new promoter element for human tRNA genes

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RNA polymerase III (Pol III) transcription is usually regarded as housekeeping machinery only subjected to global regulation. However, growing evidence suggests that certain transfer RNA (tRNA) genes are indeed individually regulated. Most tRNA genes contain two conserved internal sequences as the only known regulatory elements, namely Box A and Box B, which drive the recruitment of the general transcription factor TFIIIC. Therefore, the mechanisms underlying gene-specific tRNA transcription regulation have still to be identified. To gain a comprehensive perspective of tRNA transcription regulation, we have revisited the promoter definition of human tRNA genes by performing massively parallel assays of human tRNA gene variants, with each of the nucleotides mutated to the other three nucleotides. In addition to the canonical Box A and Box B, our analyses uncovered a new element, termed "*Box H*", which is present in 57% of human tRNA genes and controls Pol III transcription independently of TFIIIC recruitment. Furthermore, we have identified putative regulators of tRNA transcription by identifying Box H-binding proteins and molecular mechanisms will be discussed. Importantly, Box H coincides with nucleotide positions in the mature tRNA molecule that ensure the accurate decoding of the mRNA codon during protein translation. Our results reveal a novel promoter element for gene-specific Pol III-dependent transcription that potentially couples tRNA expression to the fidelity of the genetic code.

Poster board #20

Nucleolar Dysfunction and RNA Polymerase I Inhibition in H3.3/ATRX-Mutated Paediatric Gliomas

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Heterochromatin formation and DNA stability within ribosomal DNA (rDNA) repeats are critically regulated by the deposition of histone H3.3 via the ATRX/DAXX chaperone complex. Our previous research demonstrated that ATRX deficiency leads to rDNA repeat instability, impairing ribosomal RNA (rRNA) transcription and sensitizing cancer cells to RNA Polymerase I (Pol I) inhibition (Udugama et al., 2018). Here, we expand these findings to paediatric high-grade gliomas (pHGGs) harbouring histone H3.3 mutations—specifically H3.3 K27M and G34R—highlighting their impact on rRNA transcription, nucleolar function, and RNA Polymerase I vulnerability.

We find that H3.3 mutations lead to reduced rRNA synthesis and nucleolar disorganization in both mutant mouse embryonic stem cells, patient-derived pHGG tumour cell lines, and patient tissue samples. Electron microscopy reveals structural defects in the fibrillar centre (FC), dense fibrillar component (DFC), and granular component (GC), consistent with impaired rRNA processing. These changes coincide with altered UBF1/2 binding at rDNA, further linking nucleolar architecture disruptions to transcriptional instability. As a consequence of impaired rRNA synthesis, H3.3-mutant pHGG cell lines and organoid models exhibit heightened sensitivity to the RNA Pol I inhibitors CX-5461 and PMR-116, highlighting a potential therapeutic vulnerability.

Furthermore, we propose that H3.3/ATRX status, combined with distinct UBF1/2 staining patterns, may serve as predictive biomarkers for Pol I inhibitor responsiveness. These findings underscore rRNA transcriptional instability as a hallmark of H3.3/ATRX-mutated gliomas, offering a rationale for targeting nucleolar dysfunction to improve treatment outcomes for these aggressive paediatric tumors.

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