Emerging Roles for the Nucleolus 2019

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The nucleolus is the central organelle within eukaryotic cells whose primary function is to generate ribosomes, the major protein producing machines within all cells. New roles for the nucleolus are continuously emerging as we explore its molecular intricacies. Despite the central and fundamental role of the nucleolus in cell biology, there has previously been no single official meeting that enables the gathering of scientists whose research converges on the nucleolus. As a result, the community of researchers who study this organelle risks fragmentation across disciplines. The Emerging Roles for the Nucleolus Symposium, which has now taken place twice on a biennial basis, first in 2017 (1) and again in 2019, therefore, represents the first of its kind. The overarching goals of this symposium are (*a*) to convene researchers who study the nucleolus across model systems (yeast, nematodes, fruit flies, mouse, human cell lines) and biological perspectives (structural, biophysical, molecular, cellular, pathophysiology), (*b*) to share and disseminate the latest research breakthroughs in nucleolar biology, (*c*) to promote interaction, engagement, and collaboration centered on the nucleolus across disciplines, and (*d*) to provide trainees and early career investigators with an organelle-specific scientific community of support.

The second Emerging Roles for the Nucleolus meeting was sponsored by the American Society for Biochemistry and Molecular Biology and was held at the Stowers Institute for Medical Research in Kansas City, MO, from October 24 to October 27, 2019. It was organized by Jennifer Gerton (Stowers Institute), Francesca Duncan (Northwestern University Feinberg School of Medicine), and Craig Pikaard (Indiana University).

Here, we highlight the state of the field as discussed during the meeting with respect to nucleolar structure and integrity, nucleolar organizing regions, nucleolar DNA damage, rDNA copy number, dynamics and transcription, rRNA processing, ribosome biogenesis, ribosome dynamics in translational control, the role of nucleoli and ribosomes in cell division and organism development, nucleolar stress, nucleolar impact on the genome, and nucleoli in special cell types (*e.g.* stem cells, cancer, oocytes, and early embryos).

rDNA dynamics

The meeting's opening session focused on the chromosomal architecture surrounding the rDNA repeats within nucleolar organizers, the molecular mechanisms of nucleolar dominance and silencing, and the dynamics of rDNA copy number stability. **Brian McStay** (National University of Ireland, Galway) described nucleolar formation and subsequent fusion in human cells from a chromosomal perspective. Using monochromosomal cell hybrids to engineer individual NOR[3]-bearing acrocentric chromosomes, he showed that their p-arms with fused proximal and distal junctions lacking intervening rDNA still associate with nucleoli. He also showed that individual NORs occupy distinct territories within large mature nucleoli. **Olivier Gadal** (Toulouse University, France) used live cell imaging in yeast to quantitate the dynamic three-dimensional behavior of the nucleolus during synchronized cell cycles. Whereas overall nucleolar appearance remained constant through interphase, the geometry of interphase rDNA changed from short zig-zag configurations to elongated linear structures with cohesin regulating rDNA organization during mitosis. **Craig Pikaard** (Indiana University, Bloomington) described genetic approaches in *Arabidopsis* that have shown that the on or off states of different rRNA gene subtypes depend on the NOR in which they are clustered, not on the sequences of the individual genes themselves. Using chromosome-engineering techniques, he showed how his group is zeroing in on the minimal cis-acting sequences that confer NOR silencing or dominance. **Yukiko Yamashita** (University of Michigan, Ann Arbor) provided an overview of rDNA copy number loss in *Drosophila* germline lineages during aging, subsequent magnification of rDNA copies in the progeny, and the role that rDNA loci with the nucleolar Indra protein have in nonrandom sister chromatid segregation during asymmetric male germline stem cell division.

Transcription and the rDNA

The second session focused on transcription of the rDNA, specifically transcription elongation by RNA polymerase I (Pol I) and its specific inhibition by drugs intended for cancer therapeutics. **David Tollervey** (University of Edinburgh) has discovered experimentally and by mathematical modeling that Pol I elongation is influenced by DNA torsion, local RNA folding, and GC content in the transcription bubble. **Soma Dash** (Paul Trainor's laboratory, Stowers Institute for Medical Research)

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[3] The abbreviations used are: NOR, nucleolar organizing region; UAF, upstream activating factor; Pol I, RNA polymerase I; RNAP, RNA polymerase.

presented the consequences for nucleolar number and composition upon conditional knockout in mouse embryo fibroblasts of factors that cause hereditary genetic disorders of craniofacial development. Using *Escherichia coli*–expressed UAF complex and biophysical methods, **Bruce Knutson** (SUNY Upstate Medical University) has determined that the RNAP I transcription factor complex, UAF, forms a tetrasome-like structure when it binds to the rDNA. **Tamara Popova** (Jennifer Gerton's laboratory, Stowers Institute for Medical Research) applied a nucleolar normality score based on the measured nucleolin/UBF ratio in RPE1 cells after CX5461 treatment to carry out a drug screen on known anti-cancer compounds. **Baoshun Xu** (Sun Yat-Sen University, China) has found that a combination of an rDNA inhibitor (CX5461) and a TOR pathway inhibitor (INK128) synergize to combat oral cancer. **Marikki Laiho** (Johns Hopkins University School of Medicine) shared her outstanding progress toward bringing the RNAP I inhibitor, BMH21 (and related compounds), to the clinic for novel cancer therapies.

Ribosome dynamics and translational control

New paradigms continue to emerge describing how ribosome biogenesis factors possess alternative nuclear functions, how specific cells manufacture specialized ribosomes, or how select transcripts are preferentially translated by modified ribosomes. **Susan Baserga** (Yale University) shared how the Fanconi anemia protein, FANCI, involved in double-strand break repair, also plays a critical role in ribosome biogenesis (2). **Vassie Ware** (Lehigh University) presented her results on how specialized ribosomes, assembled with different ribosomal protein paralogues eRPL22 and eRPL22-like, function in *Drosophila* spermatogenesis (3). **Haiwei Pi** (Chang Gung University, Taiwan) conveyed how Arp6 transports Rpl10A to the cytoplasm to participate in preferential translation for *Drosophila* neurogenesis. **Rajeev Samant** (University of Alabama, Birmingham) explored how hypoxia in cancer cell lines drives increased RNAP I transcription, internal ribosome entry site translation, differential rRNA methylation, and changes in the ribosome-associated proteome. **Amanda Haupt** (Allen Institute for Cell Science, Seattle) shared her team's efforts to establish endogenously tagged nucleolar proteins in hiPSC cells for analysis of nucleolar structure and for CRISPi screening to discover proteins required for nucleolar structure and function.

Nucleoli and ribosomes in cell division and development

Nucleolar or ribosomal proteins are now known to regulate the cell cycle by blocking the E3 ubiquitin ligase, MDM2, to thus stabilize p53 and induce cycle arrest. New hypotheses continue to emerge regarding nucleolar functions in gamete formation or in maternal *versus* zygotic ribosome contributions during early development. **Helena Fulka** (Czech Academy of Sciences Institute of Experimental Medicine) shared her work on an essential role for nucleolar precursor bodies in chromatin maintenance in embryos from mouse and pig during embryonic development distinct from a role in ribosome biogenesis (4). Using the *Caenorhabditis elegans* system, **Elif Sarinay Cenik** (University of Texas, Austin) presented her work elucidating the principle that maternal ribosomes are sufficient for embryonic development and ribosomal insufficiency in a subset of cells triggers whole-organism arrest (5). **Kristin Watt** (Paul Trainor's laboratory, Stowers Institute for Medical Research) shared how mutations in *polr1c* in zebrafish disrupt RNAP I and III function and lead to increased cell death in the neural tube. **Karla Terrazas-Falcon** (Paul Trainor's laboratory, Stowers Institute for Medical Research) presented the consequences of disrupting RNAP I function during mouse neural crest cell development. **Xiaochun Yu** (City of Hope) revealed that the pre-rRNAs coat chromosomes during mitosis for chromosome segregation in 293T cells. **George Watase** (Yikiko Yamashita's laboratory, University of Michigan) shared that the rRNA loci (IGS) are responsible for nonrandom sister chromatid segregation of X and Y chromosomes in *Drosophila* male germline stem cells via the Indra protein. **Susan Gerbi** (Brown University) presented evidence of a long interruption (50 kb) bisecting the tandem array of rRNA genes in the fly *Sciara*. This interruption coincides with the "controlling element" that governs X chromosome nondisjunction in *Sciara* male meiosis II.

Nucleoli under stress

Various forms of cellular stress (heat shock, hypoxia, oxidative stress) can block nucleolar function in ribosome production and redistribute nucleolar or ribosomal proteins to the nucleoplasm to affect cycle arrest. On the other hand, nucleolar stress can be caused by the loss of normal ribosome biogenesis or function due to gene mutation. Stem cells and progenitor cells particularly sensitive to these mutations die by apoptosis, leading to several human ribosomopathies. **Patrick DiMario** (Louisiana State University, Baton Rouge) described how heat shock induces Pol I transcription of *R1* retrotransposons within *Drosophila* rDNA units (6) and how ribosome biogenesis factors with RGG peptide domains associate with chromosome loci that encode ncRNAs upon heat shock. **Anjana Saxena** (Brooklyn College) found that hypophosphorylated mutants of nucleolin cause nucleolar stress in human cell lines and activate p53 gene expression post-transcriptionally (7). **Jacob Gordon** (Robin Stanley's laboratory, NIEHS, National Institutes of Health) studied how a nucleolar localization signal on the polynucleotide kinase Nol9 also orchestrates transport of the endoribonuclease Las1L into the granular component of the nucleolus in mammalian cells for processing of the ITS2 within the pre-rRNA transcript (8). **Emily Sutton** (Victoria DeRose's laboratory, University of Oregon) has explored the structural properties of Pt(II) compounds that induce nucleolar stress in cultured mammalian cells (9). **Michael White** (Richard Kriwacki's laboratory, St. Jude Children's Research Hospital) used biophysical methods to examine how nucleophosmin's interaction with Arg-rich peptides encoded in *C9orf72* hexanucleotide repeat expansion forms and dissolves biomolecular condensates *in vitro* and nucleoli in cells (10). **Shannon Weeks** (Rajeev Samant's laboratory, University of Alabama, Birmingham) highlighted the close relationship of nucleolar abnormalities and cancer (11) and reported the effect of inhibition of Wnt signaling in triple-negative breast cancer on the nucleolar proteome, identifying Las1L as a good candidate for targeting the nucleolus in triple-negative breast cancer.

Nucleolar impacts on the genome

Whereas previous sessions within the conference focused on nucleolar roles in metabolic homeostasis, the last session concentrated on emerging properties of the nucleolus in regulating genome stability. **Adam Antebi** (Max Planck Institute for Biology of Aging) presented his results on the inverse correlation between nucleolar size and activity with longevity and immunity in *C. elegans* through NCL-1/TRIM2 modulation of fibrillarin expression. **Lesley Stark** (University of Edinburgh) presented evidence for cross-talk between TIF-IA and NF-κB in cellular and animal models of senescence. **Natalie Warsinger-Pepe** (Yukiko Yamashita's laboratory, University of Michigan) reported how cis elements on the Y chromosome dictate nucleolar dominance during development in *Drosophila* in a tissue-specific manner. **Elizabeth Kwan** (Bonny Brewer's laboratory, University of Washington) demonstrated that a minimal rDNA array replicates early and competes with genome replication in *S. cerevisiae*. **Jonathan Nelson** (Yukiko Yamashita's laboratory, University of Michigan) described how rDNA-specific retrotransposons *R1* and *R2* are required to maintain rDNA copy number in the *Drosophila* male germ line: derepression (transposition) of *R1/R2* elements in male germline stem cells creates rDNA double-strand breaks that facilitate unequal crossing over, leading to expansion of rDNA copy number. **Ashley Hall** (Christine Queitsch's laboratory, University of Washington) described experiments in progress indicating that variation in rDNA copy number alone does not affect lifespan in *C. elegans*.

Challenges and opportunities

With ongoing research, it is becoming clear that the nucleolus has a role that well-transcends that of a "housekeeping" organelle. At the conclusion of the meeting, there was a symposium-wide discussion to identify challenges and opportunities in the field. As more and more researchers find themselves "stumbling into the nucleolus" from diverse angles, we will need to evaluate and integrate the data across model organisms and systems to truly understand the dynamic role of the nucleolus. Currently, there are multiple models for nucleolar structure, with concepts of dynamic self-assembly, liquid-liquid phase separation, nuclear condensates, and heterochromatic shells at the heart of discussion and controversy. Reconciling these models will require careful consideration of specific cell types and whether data are derived from *in vitro* or *in vivo* scenarios. Understanding how the physical, biological, and morphological properties of the nucleolus relate to or drive physiology and pathology are important research frontiers. For example, investigating the relationship between rDNA copy number variation and cancer represents a clinically significant opportunity. Moreover, how the nucleolus interacts with other cellular structures and organelles is an emerging area of discovery, with new players such as nuclear actin emerging as novel regulators. New cell biology and imaging tools will greatly enable these studies. As the field grows, more resources need to be dedicated to it. From a scientific perspective, a major unmet need is the incorporation of rDNA sequences into organismal reference genomes and making them readily accessible. There are also practical challenges, with a need for more funding mechanisms focused on this essential organelle. Better awareness and education among scientists across disciplines and the public will aid in this effort, and at the meeting, we started this process through a STEAM (science, technology, engineering, art, and mathematics) project in which we created a montage of nucleolus images submitted by meeting participants. The "show us your best nucleolus" montage prompted buzz across social media, bringing much needed attention to this beautiful, diverse, multifunctional, and central organelle.

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References

1. Baserga, S. J., and DiMario, P. J. (2018) Emerging roles for the nucleolus 2017. *Mol. Biol. Cell* **29**, 773–775 CrossRef Medline
2. Sondalle, S. B., Longerich, S., Ogawa, L. M., Sung, P., and Baserga, S. J. (2019) Fanconi anemia protein FANCI functions in ribosome biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 2561–2570 CrossRef Medline
3. Mageeney, C. M., and Ware, V. C. (2019) Specialized eRpL22 paralogue-specific ribosomes regulate specific mRNA translation in spermatogenesis in *Drosophila melanogaster*. *Mol. Biol. Cell* **30**, 2240–2253 CrossRef Medline
4. Fulka, H., and Aoki, F. (2016) Nucleolus precursor bodies and ribosome biogenesis in early mammalian embryos: old theories and new discoveries. *Biol. Reprod.* **94**, 143 CrossRef Medline
5. Cenik, E. S., Meng, X., Tang, N. H., Hall, R. N., Arribere, J. A., Cenik, C., Jin, Y., and Fire, A. (2019) Maternal ribosomes are sufficient for tissue diversification during embryonic development in *C. elegans*. *Dev. Cell* **48,** 811–826.e6 CrossRef Medline
6. Raje, H. S., Lieux, M. E., and DiMario, P. J. (2018) *R1* retrotransposons in the nucleolar organizers of *Drosophila melanogaster* are transcribed by RNA polymerase I upon heat shock. *Transcription* **9**, 273–285 CrossRef Medline
7. Zhang, X., Xiao, S., and Rameau, R. D., Devany, E., Nadeem, Z., Caglar, E., Ng, K., Kleiman, F. E., and Saxena, A. (2018). Nucleolin phosphorylation regulates PARN deadenylase activity during cellular stress response. *RNA Biol.* **15**, 251–260 CrossRef Medline
8. Gordon, J., Pillon, M. C., and Stanley, R. E. (2019) Nol9 is a spatial regulator for the human ITS2 pre-rRNA endonuclease-kinase complex. *J. Mol. Biol.* **431**, 3771–3786 CrossRef Medline
9. Sutton, E. C., McDevitt, C. E., Prochnau, J. Y., Yglesias, M. V., Mroz, A. M., Yang, M. C., Cunningham, R. M., Hendon, C. H., and DeRose, V. J. (2019) Nucleolar stress induction by oxaliplatin and derivatives. *J. Am. Chem. Soc.* **141**, 18411–18415 CrossRef Medline
10. White, M. R., Mitrea, D. M., Zhang, P., Stanley, C. B., Cassidy, D. E., Nourse, A., Phillips, A. H., Tolbert, M., Taylor, J. P., and Kriwacki, R. W. (2019) C9orf72 poly(PR) dipeptide repeats disturb biomolecular phase separation and disrupt nucleolar function. *Mol. Cell* **74**, 713–728.e6 CrossRef Medline
11. Weeks, S. E., Metge, B. J., and Samant, R. S. (2019) The nucleolus: a central response hub for the stressors that drive cancer progression. *Cell Mol. Life Sci.* **76**, 4511–4524 CrossRef Medline

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