Emerging Roles for the Nucleolus 2017

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The meeting "Emerging Roles for the Nucleolus," sponsored by the American Society for Biochemistry and Molecular Biology, was held at Stowers Institute for Medical Research in Kansas City, MO, from October 26 to October 29. It was organized by Jennifer Gerton (Stowers Institute) and Thoru Pederson (University of Massachusetts Medical School).

The symposium nicely blended the integrity and activity of the nucleolus in its primary role of ribosome biogenesis, ribosomal DNA (rDNA) copy number variation and its effects on cell viability and organism life span, nucleolar stress phenomena, specialized ribosomes, nucleolar function in aging, nucleolar epistatic regulation of the genome, and selective nucleolar organizer silencing and dominance.

Nucleolar integrity and activity

Cliff Brangwynne (Princeton) described phase transitions in nucleolar assembly and organization. Of particular interest was his data and model for multi-phase transitions to describe the formation of the granular component of the nucleolus. Related to multi-phasic assembly, **Kausik Si** (Stowers Institute) described polymerization of the neuronal RNA-binding protein, CPEB, as a potential mechanism for persistence of memory. **Susan Baserga** (Yale University) described a high-throughput genomewide small interfering RNA screen that reduced nucleolar numbers from two to three per nucleus to only one to identify novel regulators of human ribosome assembly and function. **David Schneider** (University of Alabama at Birmingham) described RNA Pol I elongation and its link to pre-rRNA processing by defining DNA sequence elements that elicit Pol I pausing. In vivo pausing could allow coordination between rRNA transcription and processing.

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rDNA copy number relates to organism viability and lifespan

Bonita Brewer (University of Washington) proposed that replication origins in rDNA units of *Saccharomyces cerevisiae* (one potential origin per unit) constrain the species specificity of Origin Recognition Complex (ORC) binding and that a balanced level of replication initiation within the rDNA imposes selection that drives ORC/origin interactions. **Devika Salim** (from the Gerton lab) described a yeast temperature-sensitive screen to identify genes that contract rDNA copy number. Reduced-copy-number mutants grew better than cells with normal rDNA copy number, suggesting that a loss of rDNA repeats confers an advantage under conditions of DNA replication stress. **Elizabeth Morton** (University of Washington) described novel technologies to determine rDNA copy numbers in *Caenorhabditis elegans* that may be used to link copy number and phenotypic variation. **Aline Marnef** (Université de Toulouse) described how double-strand breaks in human rDNA can be repaired within the nucleolus by nonhomologous end joining, while homologous recombination (HR) is restricted to nucleolar caps.

Nucleolar function in aging

Francesca Duncan (Northwestern University Feinberg School of Medicine), working in collaboration with Jennifer Gerton, described age-associated alterations in nucleolar structure and activity in mouse oocytes, with older animals displaying increased numbers of fibrillar centers, an increase in fibrillarin, and an enrichment in oocyte ribosome content. **Abigail Buchwalter** (from the Hetzer lab, Salk Institute) showed increases in protein synthesis, ribosome biogenesis, and nucleolar size in primary Hutchinson-Gilford progeria-derived cells and also found that primary human cells from elderly donors exhibit enlarged nucleoli that produce more rRNA. **Daniel Warmerdam** (University Medical Center Groningen) described how structural maintenance of chromosomes protein 5 (SMC5)-mediated HR that repairs breaks in rDNA leads to a loss of rDNA repeats and reduced cell viability, likely leading to enhanced aging and higher rates of cancer. The interplay between nucleolar function and aging is an intriguing and rapidly emerging question in nucleolar biology.

The nucleolus in signaling and development

Lesley Stark (University of Edinburgh) described functional cross-talk between NF-kB signaling and the nucleolus. Nucleolar sequestration of RelA mediated apoptosis in an atypical nucleolar stress response pathway. Depletion of Pol I complex components mimicked stress effects on NF-kB while aspirin-mediated degradation of TIF-IA correlated with activation of NF-kB in colorectal tumors. **Vandana Gupta** (Harvard Medical School) described a zebrafish mutation in *DDX27* that reduced myoblast proliferation and caused premature differentiation. ddx27 is a nucleolar DEAD-box RNA helicase required for rRNA maturation but is down-regulated in Duchenne muscular dystrophy. **Sonu Baral** (from the DiMario lab, Louisiana State University) showed that the relatively few but identifiable mushroom body neuroblast lineages in the *Drosophila* larval brain had greater resilience to nucleolar stress imposed by

Nopp140 depletion as compared with the majority of other neuroblast lineages in the brain. The human ribosomopathies arise from similar, yet poorly understood, differences in stem cell or progenitor cell sensitivities to nucleolar stress.

Emerging approaches to nucleolar stress

Amee George (from the Hannan laboratory, Australian National University) described a loss of function RNAi screen to identify genes and pathways that participate in or regulate the p53-mediated nucleolar stress pathway that leads to cell cycle arrest, apoptosis, or senescence depending on the cell type. **Po-Hsiang Chen** (from Szecheng Lo's lab, Chang Gung University) described the p53-caspase-USP33 pathway in *C. elegans*. USP33 and its human orthologue USP36 are ubiquitin specific peptidases that stabilize fibrillarin, and caspase-mediated cleavage of USP33/USP36 leads to a loss of fibrillarin and reduced nucleolar size. **Patrick DiMario** (Louisiana State University) described a p53-independent nucleolar stress pathway in *Drosophila* that leads to developmental deformities and subcellular anomalies. In a joint talk, **Kristin Watt** and **Karla Terrazas**, both from Paul Trainor's lab at Stowers, proposed that loss of proper spatiotemporal regulation of RNA Pol I and III activity revealed by several mutations in mouse and zebrafish may explain differential sensitivities in the neural crest cells and in bone and cartilage development during embryogenesis. **Sui Huang** (Northwestern University Feinberg School of Medicine) reported the development of a new therapeutic compound, metarrestin, that disrupts the integrity of the perinucleolar compartment often found associated with metastatic cancer cells. The drug has been shown to block metastasis of several human cancer cells in xenograft mice. These talks underscored the roles that ribosomes play in development and cancer, a robust focus in nucleolar biology.

Specialized ribosomes, an emerging nucleolar function

Vassie Ware (Lehigh University) described the ubiquitous expression of the *Drosophila* ribosomal protein eRpL22 and the restricted expression of its paralogue eRpL22-like in the testis, resulting in heterogeneous ribosome populations within the germline. **Maria Barna** (Stanford University) described a mass spec pipeline to reveal subsets of ribosomes that are heterogeneous for ribosomal protein composition in ES cells. Using CRISPR, she tagged and then purified heterogeneous ribosomes to show that clusters of specialized ribosomes are reserved for translating particular mRNAs whose protein products participated in specific signaling pathways. Continued advances in identifying a "ribosome code" associated with ribosome heterogeneity should reveal novel complexities in gene expression at the level of mRNA translation.

Nucleolar interactions with the genome

Ross Hannan (Australian National University) provided an update on CX-5461, the anti-cancer drug that blocks Pol I transcription and induces double-strand breaks selectively within the rDNA promoter. He also described 4C-seq experiments demonstrating increased association of genomic interactions with the activation of previously silent rDNA as cells transition to malignancy. The hypothesis is that these additionally activated rDNA repeats alter physical interactions with specific gene networks, some advancing to malignancy. **Marikki Laiho** (Johns Hopkins University) described BMH-21 as a potent drug that selectively blocks Pol I elongation by dislodging the enzyme. Depleting RPA135 or RPA12 abrogated the effects of BMH-21, indicating that these two subunits are likely responsible for a checkpoint activation of Pol I in a species-conserved manner. **Paul Kaufman** (University of Massachusetts Medical School) discussed Ki-67, a proliferation factor important for heterochromatin maintenance near the periphery of nucleoli. He described distinct classes of heterochromatin in murine embryonic stem cell nucleolus-associated domains (NADs) versus lamin-associated domains (LADs). Two subcategories of NADS exist in mouse embryonic fibroblasts; they differ in their histone marks, replication times, and their association with LADs. **Bernardo Lemos** (Harvard School of Public Health) showed >10-fold variations in rDNA copy numbers for both 5S and 45S rDNA repeats in different *Drosophila* and human genotypes. Hi-C maps in human K562 or LCL cells identified contacts between genome elements/regions and either 5S or 45S rDNAs. While genomic regions showed selective spatial proximity to the respective rDNAs, no direct contacts were apparent between 5S and 45S rDNAs. **Keith Maggert** (University of Arizona) showed that epigenetic effects of reduced rDNA copy number in *Drosophila* can enhance the phenotypic readout of a JAK/STAT signaling mutation (*Tumorous-larvae*) that affects several metabolic processes. His talk linked the epigenetic effects of rDNA copy number not only on nucleolar stability, signaling, and metabolism but also on heterochromatin formation, an overall driving theme throughout the meeting.

Emerging roles of nucleolar organizers

Brian McStay (National University of Ireland Galway) described distal and proximal junctions that flank nucleolar organizer regions on the short arms of the five acrocentric chromosomes in humans (13, 14, 15, 21, and 22). Proximal junctions are segmentally duplicated, while distal junctions are mostly unique to the individual chromosome but are marked by a large (100-kb) inverted repeat. Genome editing (tagging) of individual human nucleolar organizer regions (NORs) and their distal sequences now reveal emerging roles that the distal junctions and their Pol II long noncoding transcripts have on the integrity of rDNA and nucleolar formation. **Kannanganattu Prasanth** (University of Illinois) described a long noncoding, single nucleolus-associated repeat-containing RNA (SNARE RNA) that negatively regulates rDNA transcription; knockdown of SNARE-RNA resulted in increased rDNA transcription. **Qinyu Hao** from the Prasanth lab showed that the SNARE-RNA localized to nucleolar regions that are generally devoid of nascent or processed rRNA. **Tamara Potapova** from the Gerton lab described inter- and intrachromosomal linkages among nucleolar organizers during mitosis. These linkages consist of rDNA coated with upstream binding factor (UBF); the frequency of these linkages correlated with rDNA transcription. **Fernando Rabanal** (Max Planck Institute for Developmental Biology, Tübingen) described natural sequence variation in the rRNA genes in *Arabidopsis thaliana*, thus suggesting heterogeneous rRNA pools within cells. He also described cross-talk between NOR2 and NOR4 to induce NOR-specific silencing, where each cluster seems to act as a separate allele. **Craig Pikaard** (Indiana University) showed that silenced rRNA gene subtypes in the *Arabidopsis thaliana* ecotype Col-0 map to NOR2 on chromosome 2, whereas active rRNA gene subtypes map to NOR4. A mutation blocking H3K27 mono-methylation caused a multi-megabase conversion of NOR4 sequences to NOR2 sequences, but the NOR2 sequences now on chromosome 4 escaped silencing. Thus, NOR2 silencing is a function of chromosome position rather than subtype-specific sequence variation. Crosses between Col-0 and ecotype Sha, in which NOR2 is active, allowed genetic mapping of segregating loci required for

Col-0 NOR2 silencing. These studies revealed that NOR2 silencing occurs only if NOR4 is also derived from Col-0, suggesting regulatory cross-talk between active and silent NORs or closely linked loci. Clearly, nucleolar dominance, which occurs in eukaryotes as diverse as plants, flies, frogs, and human cells is an intriguing problem in epigenetic gene control—yet another emerging property of the nucleolus.

Postdocs and graduate students were in strong attendance and led much of the charge on emerging nucleolar biology, especially in the lively poster session. As the meeting adjourned, it was evident to all that a previously somewhat fragmented group of investigators had coalesced into a spirited nucleolus community, with abounding opportunities to push forward on the increasingly beguiling complexities of this organelle.

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