Ribonucleoproteins in retrotransposon and viral host-pathogen interactions

NYC RNA Salon

April 1, 2019
1pm - 6pm

Retrotransposon Mini-Symposium

The Rockefeller University
1230 York Ave
Weiss Research Building 301

Organizers

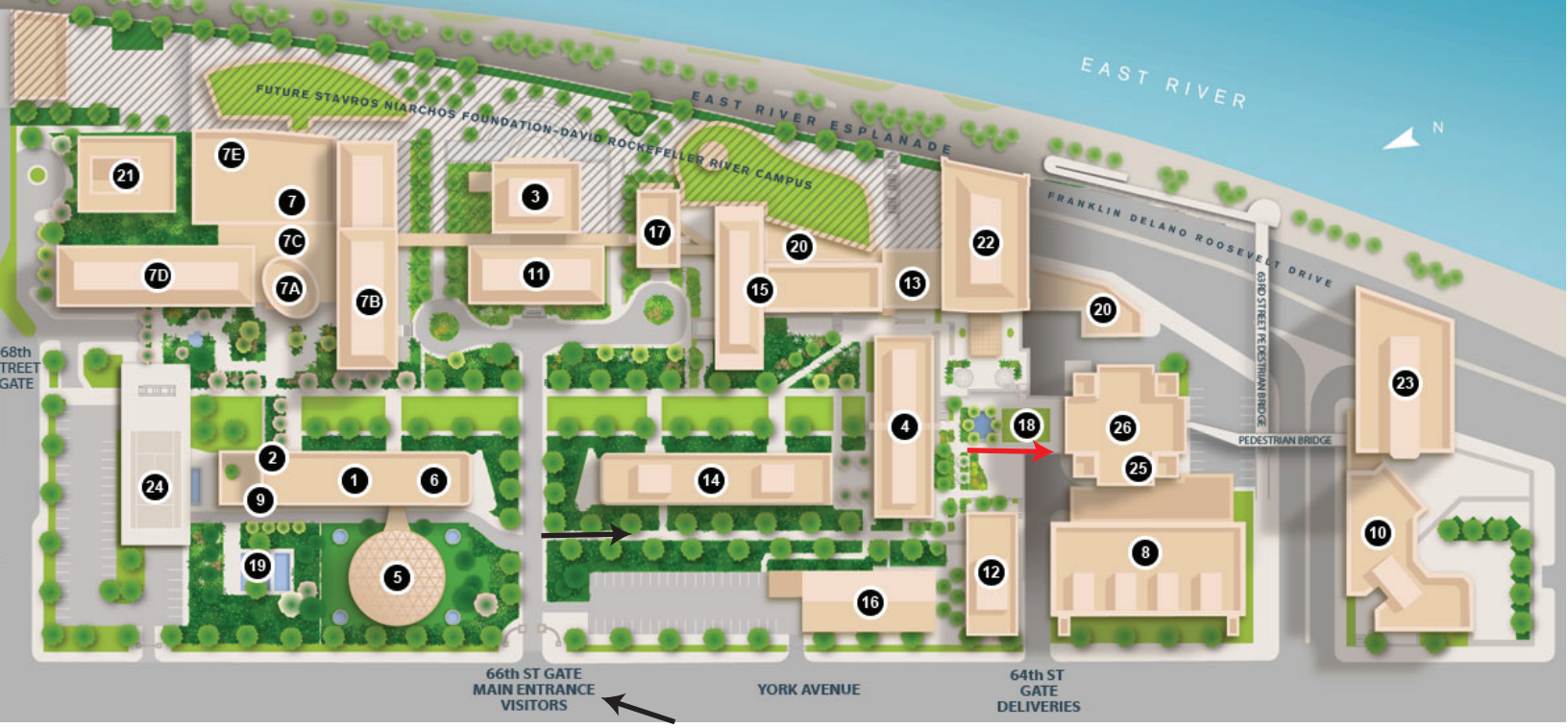
John LaCava
jlacava@rockefeller.edu
Erica Jacobs
ejacobs@rockefeller.edu

NCDIR
National Center for Dynamic Interactome Research

LEXOGEN

NYU Langone Health
Institute for Systems Genetics

The Rockefeller University 1901



*Enter here - 1230 York Ave.
*Follow marble path to right.
*Enter Weiss Building (red arrow, #26).
*Take elevator to 3rd floor - Rm. 301.

Schedule

1:00 - 1:10 pm
Welcome & brief introduction

1:10 - 2:00 pm
Prof. Kathy Burns - Johns Hopkins University School of Medicine
Our conflict with transposable elements and its implications for human disease.

2:00 - 2:50 pm
Dr. Jan Attig - The Francis Crick Institute
Degenerate retrotransposons increase transcriptomic diversity.

2:50 - 3:40 pm
Dr. Miguel Branco - Queen Mary University of London
Transposable elements as a source of regulatory elements in development and disease.

Break - 40 min

4:20 - 5:10 pm
Dr. John LaCava - The Rockefeller University
Interactomic and Enzymatic Analyses of Affinity Isolated Human LINE-1 Retrotransposons.

5:10 - 6:00 pm
Dr. Jose Luis Garcia-Perez - The University of Edinburgh
LINE-mediated brain somatic mosaicism in zebrafish targets, especially the motoneuron network.

Our conflict with transposable elements and its implications for human disease.

Kathleen Burns, M.D., Ph.D.
McKusick-Nathans Institute of Genetic Medicine
Professor of Pathology

Johns Hopkins University School of Medicine, Baltimore MD, USA.

Interspersed repeat sequences derived from mobile genetic elements make up much of our DNA. In this presentation, I will introduce the concept that transposable element insertions deliver repressed functions to genomic locations – *trans*-activating functions bound by repressive silencers, and splicing functions masked by RNA binding proteins. These antithetical properties are the result of complex relationships pitting the intrinsic activities of these retroelements against the host factors that evolve to mitigate their effects. Insertions provide a unique substrate for evolutionary innovation since either of the opposing functions can be made permanent.

Our lab studies how the contours of this conflict impact human disease. First, I will describe how specific transposable element loci 'escape' silencing in diseased tissues. For example, long interspersed element-1 (LINE-1) sequences are commonly de-repressed in human cancers, resulting in somatic retrotransposition events and potentially creating unique molecular requirements for cancer cell growth. Second, I will describe how rare and commonly-occurring inherited insertion alleles can affect mRNA splicing and lead to genetic disease. Examples include highly penetrant disease alleles, as well as variants with lesser effects that are detectable by genome-wide association study (GWAS). We have found many *Alu* insertion variants at GWAS loci in linkage disequilibrium (LD) with trait-associated single nucleotide polymorphisms (SNPs). One of these is an *Alu* insertion at the *CD58* locus on a haplotype that affects multiple sclerosis (MS) susceptibility (OR=1.3 p=3x10-10). We found perfect LD between the trait-associated SNP (rs2300747) and the intronic *Alu* insertion variant. We went on to show that the *Alu* alters splicing of the *CD58* mRNA, promoting exon 3 skipping in splice reporter assays, and creating a splice quantitative trait locus (sQTL) in lymphoblastoid cell lines. The aberrant *CD58* isoform is frame-shifted and presumed to be non-functional. Together, these data demonstrate a mechanism by which a commonly-occurring *Alu* insertion could compromise *CD58* expression and generate an abnormal protein product, which may be important in the pathogenesis of MS.

Payer LM, Steranka JP, Ardeljan D, Walker J, Fitzgerald KC, Calabresi PA, Cooper TA, Burns KH. *Alu* insertion variants alter mRNA splicing. Nucleic Acids Res. 2019 Jan 10;47(1):421-431.

Payer LM, Steranka JP, Yang WR, Kryatova M, Medabalimi S, Ardeljan D, Liu C, Boeke JD, Avramopoulos D, Burns KH. Structural variants caused by *Alu* insertions are associated with risks for many human diseases. PNAS. 2017 May 16;114(20):E3984-E3992.

Burns KH. Transposable elements in cancer. Nat Rev Cancer. 2017 Jul;17(7):415-424.

Degenerate retrotransposons increase transcriptomic diversity.

Jan Attig, Ph.D.
Retroviral Immunology Laboratory
Postdoctoral Researcher

The Francis Crick Institute, London, UK

Retrotransposons are self-replicating genetic elements and are pervasive in mammalian genomes. Most insertions are passive bystanders of genome function. Yet, individual sequences have been adapted in diverse biological contexts, from neurogenesis to placentation. I will present transcriptome-wide analysis on exonization of Alu, LINE and LTR retrotransposons. I have demonstrated that retrotransposon sequences are poised with binding sites for splice-repressive proteins and that loss of these binding sites is a necessity for formation of novel, lineage-specific exons at retrotransposon-derived sequences. Alus and LINEs are recognized through multivalent binding sites for splice-repressive proteins, and as a consequence, exonization during genome evolution is a gradual process. More recently, I have catalogued extensive usage of LINE and LTR-elements as part of novel transcripts across the tumor types represented in the TCGA data. These transcripts are recurrently and exclusively expressed in tumors, and I will present a proof-of-principle study that they generate antigenic peptides. Altogether, I conclude that thousands of past retrotransposon insertions are in a dormant state, and I suggest that selective pressure acts on the host proteins and/or retrotransposon sequences to maintain repression in somatic cells.

Transposable elements as a source of regulatory elements in development and disease.

Miguel R. Branco, Ph.D.
Blizard Institute
Senior Lecturer

Queen Mary University of London, UK

Transposable elements (TEs) are thought to have contributed to the establishment of gene regulatory networks, namely in the context of early mouse development. Additionally, epigenetic alterations in cancer may unlock the regulatory potential of TEs, which could potentially be explored by cancer cells to drive an oncogenic expression programme. However, there is limited functional evidence to date supporting a role for TEs in gene regulation. We have been exploring to what extent TEs have contributed to the rewiring of transcriptional circuits in two contexts: early mouse development (as modelled by embryonic and trophoblast stem cells) and acute myeloid leukemia (AML) in humans. To this end, we combine extensive epigenomic and transcriptomic profiling with genetic and epigenetic editing approaches to establish causal links between enhancer-like TEs and gene expression. Our results suggest that a relatively small subset of TEs is important for gene regulation in early mouse development, highlighting the importance of functional experiments when evaluating gene regulatory roles of TEs. In AML we uncovered regulatory roles for TEs of families that are frequently bound by transcription factors that play key roles in the pathogenesis of AML. Alterations in cancer can therefore uncover the enhancer capacity of TEs, which may help to drive oncogenesis.

Interactomic and Enzymatic Analyses of Affinity Isolated Human LINE-1 Retrotransposons.

John LaCava, Ph.D.
Laboratory of Cellular and Structural Biology
Research Assistant Professor

The Rockefeller University, New York NY, USA

LINE-1 (L1) retrotransposons are catalysts of evolution and disease, and L1 sequences compose a significant proportion of the human genome. Despite tremendous influence on genome composition, L1 ribonucleoproteins remain poorly characterized. Nevertheless, L1 RNAs are known to assemble with a combination of permissive host factors that are essential to their lifecycle, as well as repressive factors that constitute defenses against L1's mutagenic activity. Building on our prior analyses, we have completed a series of experiments to create a multi-dimensional interactomic characterization of affinity isolated L1s as expressed in HEK-293T cells. Our results are consistent with the presence of multiple transposon-related macromolecules, likely poised at distinctive stages in the L1 lifecycle.

In an effort to translate our findings to human health, we have turned to affinity proteomic analyses of L1s as expressed in resected human colorectal cancers (CRCs). While L1s are expressed in roughly half of all cancers, they are expressed in $\gtrsim$90% of CRCs, suggesting that many CRCs evolve in the presence of L1. In contrast, ectopic L1 expression in L1-negative model cells, such as HEK-293T or HeLa, is toxic and leads to apoptosis. L1s in patient CRCs may therefore have different interactomes than L1s in model cells, and/or L1s expressed in CRCs may exist in a distinctive cellular context. I will present our latest findings on L1 interacting proteins from these two systems.

Taylor, M. S. et al. Dissection of affinity captured LINE-1 macromolecular complexes. Elife 7**,** e30094 (2018).

LINE-mediated brain somatic mosaicism in zebrafish targets, especially the motoneuron network.

Jose Luis Garcia-Perez, Ph.D.
MRC Institute of Genetics & Molecular Medicine
Chancellor's Fellow

The University of Edinburgh, UK

The human brain contains about 100 billion “neuronal” and the same number of “non-neuronal” cells, with trillions of connections among them. During this vast cellular expansion, errors occur during DNA replication and cells differ in indels, copy number variants (CNVs), etc. A recently discovered additional source of genomic variation in the brain is driven by the activity of mobile DNA elements (MEs), as these generate new somatic insertions in selected brain cells. In the human genome, 80-100 active MEs continue to impact our genome. Although greater than half of our DNA is made of ME-derived sequences, only elements from the retrotransposon class known as Long Interspersed Element class 1 (LINE-1 of L1s) are currently active in our genome. It is though that, especially during neurogenesis, MEs are reactivated, generating perhaps millions of somatic new insertions in our brain. Genomic alterations generated by ME insertions could influence the transcriptome of brain cells, which ultimately could impact behaviour and cognition.

Here, we have generated a zebrafish model to study the impact of MEs on brain biology, focusing on active LINE elements from zebrafish. The zebrafish genome contains several active LINE elements from the class 2 (i.e., LINE-2 elements), which are closely related to human LINE-1s. As in humans, we found that LINE derived RNAs are ubiquitously expressed during early embryonic stages. Similarly, during organogenesis/gastrulation, LINE expression becomes restricted to neural tissues, especially to Neural Progenitor Cells (NPCs) and neurons, including Hb9+ moto neurons. To analyse retrotransposition of zebrafish LINEs, we developed a new engineered mobilization assay, which revealed high retrotransposition rates during embryonic development. Furthermore, we have mapped and characterized >30,000 insertions, revealing a random insertion pattern into A/T rich regions of the zebrafish genome during embryonic development. Using an inducible version of the engineered LINE mobilization assay developed, we detected abundant somatic retrotransposition of zebrafish LINEs in the brain of adult individuals. Time-controlled retrotransposition assays further revealed that retrotransposition preferentially targets NPCs and mature neurons, especially moto neurons and interneurons of motor control circuits in the ventral half of the spinal cord. Overall, these data suggest that LINEs might impact motility control of zebrafish, and in current experiments we are exploring the in vivo impact of somatic brain retrotransposition.