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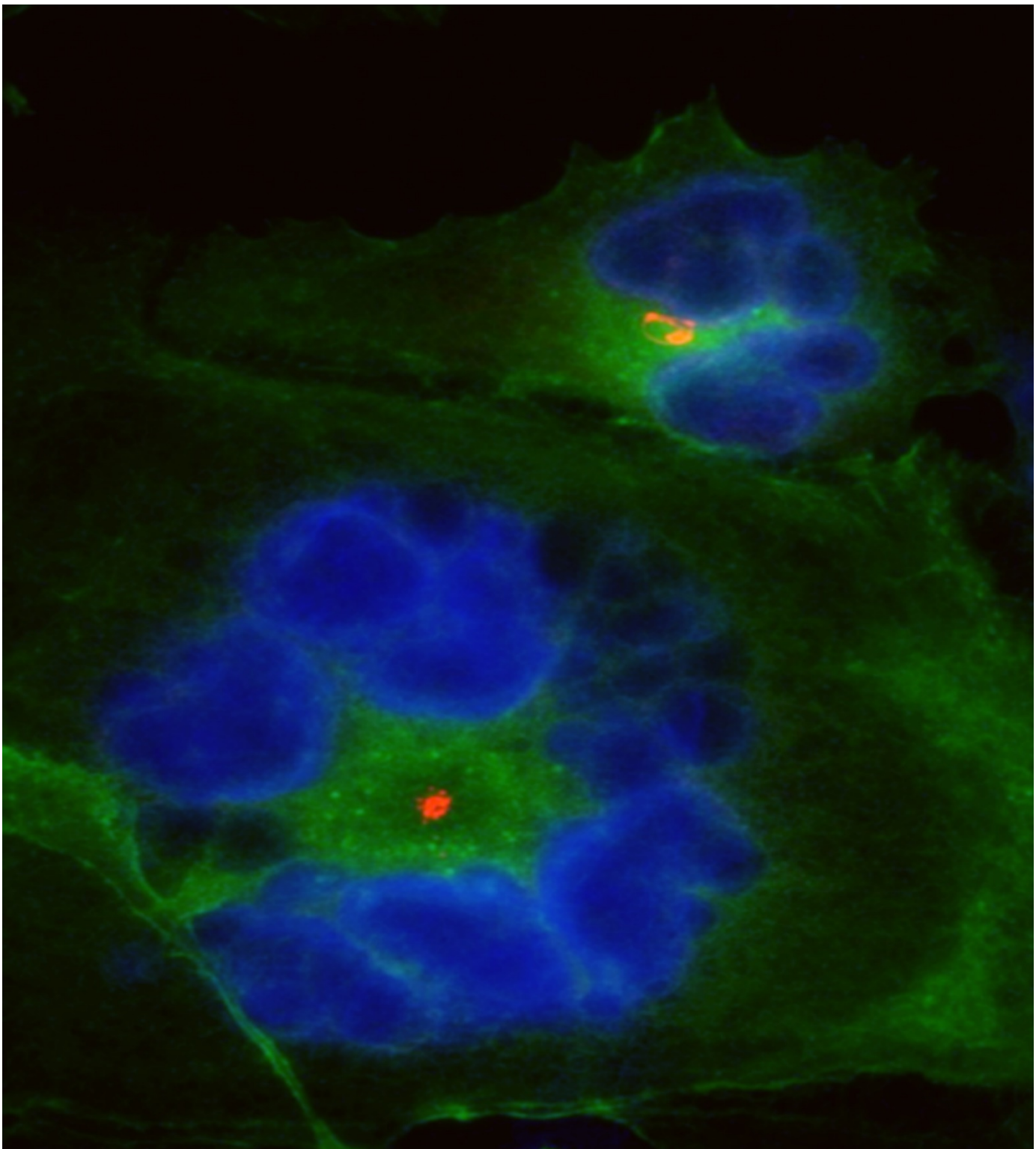

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SAIB 2019

TUESDAY November 5 2019

WORKSHOPS

WORKSHOP ON DRUG DISCOVERY

08:30-09:00 REGISTRATION

09:00-11:00 ORAL COMMUNICATIONS 1

Room Jacaranda

9:00-9:20
WELCOME BY ORGANIZERS

Ricardo Biondi
Instituto de Investigacion En Biomedicina de Buenos Aires - Instituto Partner De La Sociedad Max Planck (IBIOBA), Buenos Aires, Argentina
Hugo Gramajo
Instituto de Biologia Molecular y Celular de Rosario (IBR), Rosario, Argentina
Gaston Soria
Centro de Investigacion En Bioquimica Clinica e Inmunologia (CIBICI), Córdoba, Argentina

9:20 – 9:35
WS-C05
USE OF *IN VIVO* IMAGING SYSTEM FOR PRECLINICAL EVALUATION: EXAMPLES OF ITS APPLICATION IN DRUG DEVELOPMENT
Salinas FJ, Berengeno AL, Santiago G, Marelli BE, Baravalle ME, Salvetti NR, Ortega HH. Centro de Medicina Comparada, ICiVet-Litoral (UNL-CONICET), Esperanza, Santa Fe, Argentina. Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina. E-mail: hhortega@fcv.unl.edu.ar

9:40 - 9:55
WS-C07
HYDROXYLAMINE CHEMICALLY ENGINEERED EXTRACTS AS SOURCE OF ANTIMYCOBACTERIAL COMPOUNDS
Ramallo IA[1], Parés VS[1], Gago G[2], Gramajo H[2], Furlan RLE[1]. [1]Fac. de Ciencias. Bioquímicas y Farmacéuticas, UNR-CONICET. [2]IBR-CONICET, Rosario. E-mail: aramallo@fbioyf.unr.edu.ar

Wu Q [1], Medina S [1], Gopal Kushawah[1], DeVore M [1], Castellano L [1], Hand J [1], Wright M [1], Bazzini AA [1,2]
[1]Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA,[2]Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA. E-mail: arb@stowers.org

mRNA translation decodes nucleotide into amino acid sequences. However, translation has also been shown to affect mRNA stability depending on codon composition in model organisms, although the universality of this mechanism remains unclear. Our results show that in human cells, translation strongly affects mRNA stability in a codon dependent manner, impacting the homeostatic mRNA and protein levels. Using three independent approaches to measure exogenous and endogenous mRNA decay, we defined the regulatory identity of the 61 coding codons in 4 different human cell lines. We demonstrate that the regulatory information affecting mRNA stability is encoded in codons and not in nucleotides. We found that stabilizing human codons (optimal codons) tend to be associated with higher tRNA levels and higher charged/total tRNA ratios. These results suggested that the 'tRNA ready to go' level (quantity and quality) may be serving as a determinant of codon optimality. The molecular mechanism is still unclear, and while we observe that in human lines the poly(A)-tail length correlates with the codon-mediated mRNA stability (similarly to other species), we demonstrate that the poly(A)-tail is not required by this mechanism in both human and zebrafish embryos. And likely, the shortening of the poly(A)-tail in genes enriched in non-optimal codons is an indirect consequence of decreased stability rather than a required step in the codon-mediated mechanism. This mechanism depends on translation; however, the number of ribosome loads into an mRNA modulates the codon-mediated effects on gene expression. Therefore, this result leads us to explore that *trans*-regulatory elements and physiological conditions where mRNA translation is globally affected, may also impact the codon-mediated effects on gene expression. In sum, our work provides definitive evidence that translation strongly affects mRNA stability in a codon-dependent manner in human cells.

RN-02
DYNAMICS AND FUNCTIONAL RELEVANCE OF RIBONUCLEOPROTEIC MEMBRANE-LESS ORGANELLES

Boccaccio GL
Instituto Leloir (FIL) and IIIBBA-CONICET. E-mail: gboccaccio@leloir.org.ar

The dynamic formation of stress granules (SGs), processing bodies (PBs) and related RNA membrane-less organelles, regulates diverse cellular processes, including the coordination of functionally linked messengers, and the translational regulation at the synapse among others. The formation of these cellular bodies is governed by liquid-liquid phase separation (LLPS) processes, and their dysregulation may provoke pathogenic aggregation. LLPS *in vitro* depends on the thermal diffusion of macromolecules, which is limited inside cells, where the condensation and dissolution of membrane-less organelles (MLOs) would be helped by energy-driven processes. We found that the active transport by the retrograde motor dynein helps SG assembly, whereas the anterograde motor kinesin mediates SG dissolution, and a tug of war between these molecular motors allows transient SG formation. As in the case of PBs, SGs contain repressed mRNAs but are not required for their silencing, and the contribution of SGs to the protective response triggered upon stress remains elusive. In addition to SGs and PBs, several RNA granules and related MLOs are present in neurons. We found that distinct subsets of PBs and additional RNA bodies located at dendrites and synapses respond selectively to specific synaptic stimuli, which promote their rapid assembly or disassembly, thus controlling the release of bound mRNAs. This modulates the local transcriptome and allows fine-tuning of the translation at the post-synapse. More recently, we focused on Smaug MLOs. Smaug orthologs are highly conserved in the animal kingdom and recognize a wide variety of stem-loops termed Smaug Recognition Elements (SREs), which are present in a large number of mRNAs including nuclear transcripts that encode mitochondrial enzymes. We performed time-lapse confocal microscopy and found that Smaug1 MLOs are highly motile and frequently contact mitochondria, speculatively coordinating the transport and/or the translation of nuclear-encoded mRNAs at the mitochondrial periphery.

RN-03
FRAGMENTATION OF EXTRACELLULAR RIBOSOMES AND tRNAs SHAPES EXTRACELLULAR SMALL RNA PROFILES

Tosar JP[1,2], Gámbaro F[1], Fagúndez P[2], Segovia M[1,3], Hill M[1,3], Ivanov P[4], Cayota A[1,3]
[1]Institut Pasteur de Montevideo, Uruguay. [2]Facultad de Ciencias y [3]Facultad de Medicina, Universidad de la República, Uruguay [4]Brigham and Women's Hospital, Harvard Medical School, USA. E-mail: jptosar@cin.edu.uy

Extracellular RNAs have attracted increasing interest in recent years. This is explained by their role in intercellular communication and their use as biomarkers in minimally invasive liquid biopsies. Most studies performed thus far have focused on extracellular microRNAs and their presence inside extracellular vesicles (EVs), of which exosomes are the most renown type. It is generally accepted that the mechanisms for sorting small RNAs to the extracellular milieu are selective (i.e., RNAs harboring specific sequence motifs are recognized by molecular machinery that induces their release from cells). However, studies from our group have challenged this model. Our work suggests that RNAs are mainly released as a consequence of their steady-state levels inside the cells and that RNA intracellular and extracellular stability is a key variable influencing this process. Identification of RNAs capable of forming oligomeric structures that render them resistant to extracellular RNases encouraged us to characterize the extracellular non-vesicular RNaome, both in the presence and absence of externally added ribonuclease inhibitor. Inhibition of extracellular RNase A-family members enabled us to identify and purify extracellular tRNAs, ribosomes and even polysomes. Density gradient centrifugation provided robust separation between EV-associated and extravesicular tRNAs. The latter being highly sensitive to fragmentation in an anticodon sequence-dependent manner and are probably the source of highly abundant non-vesicular tRNA halves identified by us and others, both in cell culture and human biofluids such as plasma, serum, saliva, urine, and cerebrospinal fluid. Thus, relative extracellular enrichment of these fragments can be explained by a combination of their differential extracellular stability and the differential sensitivities of their parental