Curso de Post-grado
"Biología Celular del RNA"
Coordinadora: Graciela L. Boccaccio

Programa 2017

CONTENIDOS TEÓRICOS:

Módulo I: Introducción: Proteínas de unión a RNA: generalidades y mecanismos de interacción. Relevancia global de la regulación de y por RNAs: la red Enzima-Metabolito-RNA. El ER y el citoesqueleto como plataformas para el aparato traduccional.

Módulo II: *foci* de silenciamiento de RNA. Gránulos de Estrés y P-Bodies. Silenciamiento traduccional por estrés celular. Mecanismos moleculares: inactivación del aparato traduccional, rol de 5' tRNAs. Dinámica de gránulos de estrés y PBs: motores moleculares y mecansimos de agregación proteína-proteína. Dominios prion-like, secuencias repetitivas y de baja complejidad. El proteosoma y la autofagia en la regulación de *foci* de silenciamiento de RNAs.

Módulo III: Transporte subcelular de RNA y polaridad ceular. Relevancia durante el desarrollo embrionario temprano. Polaridad neuronal: transporte de RNA a dendritas y axones. El transcriptoma sináptico. Modificaciones . Señales de reconocimiento. Exportación de RNAm por el poro nuclear y por *pathways* alternativos: evaginación de gránulos de RNA. Motores moleculares y adaptadores involucrados en el transporte de mRNA.

Módulo IV: Traducción localizada: relevancia en motilidad celular y en plasticidad sináptica. Mecanismos moleculares: Rol de RNAs pequeños no codificantes: BC-RNAs, miRNAs y piRNAs. Regulación por polyadenilación citoplasmática. Regulación de ARC mRNA: decaimiento disparado por traducción ectópica (*translation-dependent decay)*. Autoagregación de RBPs regulatorias, CPEB y Pumilio.

Módulo V: Desregulación y patogénesis. FMRP y Síndrome de "Fragil X Mental Retardation". Agregación de proteínas de unión a RNA en neurodegenaración. RNA con repeticiones en patologías: toxicidad del RNA y traducción independiente de ATG (*RAN translation*).

Módulo VI:
Tópico especial I: estructura y dinámica de los ARN virales. Elementos de estructura secundaria. Relevancia en la replicación y traducción virales.
Tópico especial II: splicing y regulación por vías de regulación
Tópico especial III: Regulación post-transcripcional en tripanosomas
Tópico especial IV: Función y metabolismo de RNAs pequeños. Regulación en cis por modificaciones post-transcripcionales y en trans por sus propios targets

TRABAJO PRÁCTICO:

-Microscopía confocal de PBs y SGs en células de mamífero, insecto y levaduras y en cortes de tejido. Knockdown por RNAi de componentes estructurales y catalíticos de PBs
-Análisis cuantitativo automatizado de imágenes mediante algoritmos de MATLAB.

SEMINARIOS:

Cada estudiante realizará una presentación oral (45 min) de un artículo indicado por los docentes y relacionado a los temas teóricos.

Bibliografía –Tópicos de Biología Celular Avanzada-Biología Celular del ARN 2017:

Clases teóricas y de seminarios

Modulo I:

Ray et al. A compendium of RNA-binding motifs for decoding gene regulation. ***Nature***. 2013;499(7457):172-7.

Castello et al. RNA-binding proteins in Mendelian disease. ***Trends Genet***. 2013 29(5):318-27.

Castello et al. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. ***Cell***. 2012 Jun 8;149(6):1393-406.

Hentze and Preiss. The REM phase of gene regulation. ***Trends Biochem Sci***. 2010 35(8):423-6.

Ascano et al. FMRP targets distinct mRNA sequence elements to regulate protein expression. **Nature**. 2012 492(7429):382-6.

Jia et al., Reversible RNA adenosine methylation in biological regulation.***Trends Genet***. 2013 29(2):108-15.

Squires et al.,Widespread occurence of 5-MeC in human coding and non-coding RNA. ***NAR***, 2012 40: 5023-5033 *(paper a discutir)*

Modulo II:

Franks TM, Lykke-Andersen J (2008) The control of mRNA decapping and P-body formation. ***Mol Cell*** 32 (5):605-615

Buchan JR, Parker R (2009) Eukaryotic stress granules: the ins and outs of translation. ***Mol Cell*** 36 (6):932-941

Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. ***Cell*** 125 (6):1111-1124

Thomas et al (2011) RNA granules: the good, the bad and the ugly. ***Cell Signal*** 23 (2):324-334.

Kato M, et al (2012) Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. ***Cell*** 149 (4):753-767.

Han et al. Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. ***Cell***. 2012 May 11;149(4):768-79.

Loschi et al. Dynein and kinesin regulate stress-granule and P-body dynamics. J Cell Sci. 2009 122:3973-82.

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Arribere et al. Reconsidering movement of eukaryotic mRNAs between polysomes and P bodies. ***Mol Cell.*** 2011 44(5):745-58.

Ivanov et al. Angiogenin-induced tRNA fragments inhibit translation initiation. ***Mol Cell.*** 2011 43(4):613-23.

Yang and Schimmel. Functional expansion of the tRNA world under stress. ***Mol Cell.*** 2011;43(4):500-2.

Modulo III:

Lécuyer et al. Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. ***Cell*** (2007) 131: 174–187.

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Dix CI et al. Lissencephaly-1 promotes the recruitment of dynein and dynactin to transported mRNAs. ***J Cell Biol.*** 2013 ; 202(3):479-94.

Speese SD, et al (2012) Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. ***Cell*** 149 (4):832-846.

Udagawa et al. (2012) Bidirectional control of mRNA translation and synaptic plasticity by the cytoplasmic polyadenylation complex. ***Mol Cell*** 47 (2):253-266.

Huang YW et al (2012) Dual Regulation of miRNA Biogenesis Generates Target Specificity in Neurotrophin-Induced Protein Synthesis. ***Cell*** 148 (5):933-946.

Giorgi C, et al (2007) The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. ***Cell*** 130 (1):179-191.

Baez MV et al Smaug1 mRNA-silencing foci respond to NMDA and modulate synapse formation. ***J Cell Biol*** 2011 195 (7):1141-1157.

Pascual M. et al Synaptic activity regulated mRNA-silencing foci for the fine tuning of local protein synthesis at the synapse. ***Commun Integr Biol*** (2012) 5 (4):388-392.

Majumdar et al (2012) Critical role of amyloid-like oligomers of Drosophila Orb2 in the persistence of memory. ***Cell*** 148 (3):515-529

Kruttner et al (2012) Drosophila CPEB Orb2A mediates memory independent of Its RNA-binding domain. ***Neuron*** 76 (2):383-395.
Salazar AM, (2010) Regulation of synaptic Pumilio function by an aggregation-prone domain. ***J Neurosci*** 30

Modulo V:

Mori K et al: The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. ***Science***. 2013 339(6125):1335-8.
Reddy and Pearson RAN translation: fragile X in the running. ***Neuron***. 2013 78(3):405-8.
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Todd et al. CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome. ***Neuron***. 2013 78(3):440-55.

Módulo VI:

Villordo SM and Gamarnik AV. Differential RNA sequence requirement for dengue virus replication in mosquito and Mammalian cells. ***J Virol.*** 2013 87(16):9365-72.
Gebhard LG et al. Functional RNA elements in the dengue virus genome. ***Viruses***. 2011 (9):1739-56.
Iglesias NG and Gamarnik AV. Dynamic RNA structures in the dengue virus genome. ***RNA Biol***. 2011 8(2):249-57.
Villordo SM, et al A balance between circular and linear forms of the dengue virus genome is crucial for viral replication. ***RNA***. 2010 16(12):2325-35.
Samsa MM, et al. Dengue virus capsid protein usurps lipid droplets for viral particle formation. ***PLoS Pathog***. 2009 Oct;5(10):e1000632.
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De Gaudenzi JG, Noé G, Campo VA, Frasch AC, Cassola A. Gene expression regulation in trypanosomatids. ***Essays Biochem.*** 2011;51:31-46. doi: 10.1042/bse0510031. Review. PubMed PMID: 22023440.
Jäger AV, De Gaudenzi JG, Cassola A, D'Orso I, Frasch AC. mRNA maturation by two-step trans-splicing/polyadenylation processing in trypanosomes. ***Proc Natl Acad Sci U S A***. 2007;104(7):2035-42. Epub 2007 Jan 31. PubMed PMID: 17267594; PubMed Central PMCID: PMC1892994.
De Gaudenzi J, Frasch AC, Clayton C. RNA-binding domain proteins in Kinetoplastids: a comparative analysis. ***Eukaryot Cell***. 2005 Dec;4(12):2106-14. PubMed PMID: 16339728; PubMed Central PMCID: PMC1317496.
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Cassola A, De Gaudenzi JG, Frasch AC. Recruitment of mRNAs to cytoplasmic ribonucleoprotein granules in trypanosomes. ***Mol Microbiol***. 2007 Aug;65(3):655-70. PubMed PMID: 17635187.
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Risso G, Pelisch F, Pozzi B, Mammi P, Blaustein M, Colman-Lerner A, Srebrow A.Modification of Akt by SUMO conjugation regulates alternative splicing and cell cycle. ***Cell Cycle***. 2013 Oct 1;12(19):3165-74. doi: 10.4161/cc.26183. Epub 2013
Blaustein M, Pérez-Munizaga D, Sánchez MA, Urrutia C, Grande A, Risso G, Srebrow A, Alfaro J, Colman-Lerner A. Modulation of the Akt pathway reveals a novel link with PERK/eIF2α, which is relevant during hypoxia. ***PLoS One***. 2013 Jul 29;8(7):e69668. doi: 10.1371/journal.pone.0069668. Print 2013.
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Trabajo Práctico:

Perez-Pepe et al: BUHO: a MATLAB script for the study of stress granules and processing bodies by high-throughput image analysis. **PLoS One.** 2012;7(12):e51495.

Kedersha N, et al. Real-time and quantitative imaging of mammalian stress granules and processing bodies. ***Methods Enzymol.*** 2008;448:521-52.

Thomas MG, Luchelli L, Pascual M, Gottifredi V, Boccaccio GL. A monoclonal antibody against p53 cross-reacts with processing bodies. ***PLoS One.***2012;7(5):e36447. doi: 10.1371/journal.pone.0036447. Epub 2012 May 10.