

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

Programa 2016

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 mecanismos 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 celular. 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 neurodegeneración. 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

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 -Biología Celular del RNA 2016:

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 occurrence 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.
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- 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.
- Cajigas IJ, et al (2012) The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron* 74 (3):453-466.
- 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 FTL/ALS. *Science*. 2013 339(6125):1335-8.
Reddy and Pearson RAN translation: fragile X in the running. *Neuron*. 2013 78(3):405-8.
Ash et al. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron*. 2013. 77(4):639-46.
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.
Filomatori et al. A 5' RNA element promotes dengue virus RNA synthesis on a circular genome. *Genes Dev*. 2006; 20(16):2238-49.
De Gaudenzi JG, Carmona SJ, Agüero F, Frasch AC. Genome-wide analysis of 3'-untranslated regions supports the existence of post-transcriptional regulons controlling gene expression in trypanosomes. *PeerJ*. 2013 Jul 30;1:e118. doi: 10.7717/peerj.118. Print 2013. PubMed PMID: 23904995; PubMed Central PMCID: PMC3728762.
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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.
D'Orso I, De Gaudenzi JG, Frasch AC. RNA-binding proteins and mRNA turnover in trypanosomes. *Trends Parasitol*. 2003 Apr;19(4):151-5. Review. PubMed PMID:12689640.
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/eIF2 α , 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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- Pelisch F, Khauv D, Risso G, Stallings-Mann M, Blaustein M, Quadrana L, Radisky DC, Srebrow A. Involvement of hnRNP A1 in the matrix metalloproteinase-3-dependent regulation of Rac1 pre-mRNA splicing. *J Cell Biochem*. 2012 Jul;113(7):2319-29. doi: 10.1002/jcb.24103. PubMed PMID: 22345078; PubMed Central PMCID: PMC3927408.
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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.