TITLE

Genome-wide identification of protein-RNA interactions using **i**ndividual-nucleotide resolution UV **C**ross**L**inking and **I**mmuno**P**recipitation (iCLIP) sequencing

Overview of the course

The central dogma of molecular biology has been profoundly revisited since its first definition. While being still broadly known that the flow of genetic information follows the "DNA – RNA – protein" model, it is also true that the regulation of gene expression is not as linear and simple as it seems, instead, it relies on an integrated and complex network of self-regulatory loops and interconnections between the different classes of molecules involved, with RNA being one of the major characters on stage. Indeed, once a pre-messenger RNA (mRNA) emerges from RNA Pol-II, it quickly assembles with a large number of proteins – therefore named RNA-binding proteins (RBPs) – and other RNA species, to form ribonucleoprotein complexes, whose remodeling influence every step of the mRNA life cycle: from splicing, polyadenylation and export to cytoplasmic localization, translation and finally degradation, each representing a further layer of complexity in the regulation of gene expression (1). The relevance of such interactions is quite obvious since dysregulation in RBP-RNA networks and related post-transcriptional mechanisms, is at the onset of several pathological conditions, including metabolic disorders, neurodegenerative diseases, and cancer (2). Therefore, understanding RBP-RNA interactions is crucial to unravel post-transcriptional networks whose targeting could help to achieve more personalized treatments. Individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) has emerged as a leading method to map the RNA interaction sites of a single RBP-of-interest with high resolution and in a quantitative manner (3). The objective of this course is to provide students the biological questions iCLIP can help to answer. The iCLIP protocol will be extensively discussed, including the most recent variants of the technique, its pitfalls, and the critical optimization steps. Publicly available iCLIP data set will be explored. Finally, recent literature supporting the clinical potential of the technique will be presented.

References

1. Hentze MW, Castello A, Schwarzl T, Preiss T. A brave new world of RNA-binding proteins. Nat Rev Mol Cell Biol. 2018 May;19(5):327-341.
2. Gebauer F, Schwarzl T, Valcárcel J, Hentze MW. RNA-binding proteins in human genetic disease. Nat Rev Genet. 2021 Mar;22(3):185-198.
3. Huppertz I, Attig J, D'Ambrogio A, Easton LE, Sibley CR, Sugimoto Y, Tajnik M, König J, Ule J. iCLIP: protein-RNA interactions at nucleotide resolution. Methods. 2014 Feb;65(3):274-87.

Schedule (~6 hours)

The course will include three lessons (**~**2 hours each), as it follows.

Programme

Lesson one (Dr. Rosario Avolio, DMMBM): May 3, Tuesday, 3:00 p.m.

Seminar room 4th floor Torre Biologica

- Overview of the iCLIP technique
- Biological questions iCLIP can help answer
- Step by step revision of the protocol, with special attention to the critical optimization points

Lesson two (Dr. Rosario Avolio, DMMBM): May 4, Wednesday, 3:00 p.m.

Seminar room 4th floor Torre Biologica

- Main variants of the iCLIP technique (irCLIP, eCLIP, HITS-CLIP and so on)
- Brief introduction to iCLIP data analysis
- Exploring iCLIP data sets

Lesson three (Dr. Elias Bechara, IIT): May 6, Friday, 3:00 p.m.

Seminar room 4th floor Torre Biologica or via Zoom

- Analysis and applications of iCLIP
- iCLIP and diseases
- iCLIP as a tool for RNA therapy
- RNA therapy in various diseases