

Genética Molecular e Populacional

Course: Regulation of Gene Expression

Date: 10-14 February 2014

Location:

IBMC – Auditorium C.

Coordinator: Alexandra Moreira

Faculty and invited speakers: Alexandra Moreira, Andrea Cruz, Isabel Castro, Natalia Gromak, Ana Pombo.

Objectives of the course:

The life of an mRNA involves many steps that are tightly controlled and often interconnected. Gene expression thus depends on the fate of the mRNA, which is finely determined by a panoply of processes and factors, including pre-mRNA processing, *cis* regulatory elements, RNA binding proteins, miRNAs and mRNA localization. In this course international experts in the area will present state-of-the-art research on the molecular basis of gene expression regulation and mRNA processing during the physiological events that occur in different types of cells and in different systems. Scientific challenges in the field and how they are experimentally addressed will be discussed with the students.

Outline of the course: Lectures, tutorials, lab meetings, research seminars, students presentations.

Monday 10th – Alexandra Moreira

Morning – Alexandra Moreira

1. Introduction
2. Lecture 1: Overview of transcription and RNA processing; integration of co-transcriptional events.
3. Lecture 2, research seminar: Mechanisms of mRNA 3' end formation in Eukaryotes.
 - a. Transcription termination
 - b. Pre-mRNA cleavage and polyadenylation: Signals and molecular mechanisms; Regulation of mRNA 3' end formation
 - c. Poly(A) signals and diseases
 - d. Alternative polyadenylation

Afternoon

4. Paper dissection:
Pinto, PAB, Henriques, H, Freitas, MO, Martins, T, Domingues, RG, Wyrzykowska, PS, Coelho, PA, Carmo, AM, Sunkel, CE, Proudfoot, NJ and Moreira, A (2011) RNA polymerase II kinetics in *polo* polyadenylation signal selection, The EMBO Journal, 30: 2431–2444

Suggested reading:

- 1) Lutz CS, Moreira A (2011) Alternative mRNA polyadenylation in eukaryotes: and effective regulator of gene expression. Wiley Interdisciplinary Reviews – RNA, 2 : 23-31
- 2) Moreira, A (2011) Integrating transcription kinetics with alternative polyadenylation and cell cycle control. Nucleus, 2(6)
- 3) Henriques T, Ji, Z, Carmo, A, Tian, B, Proudfoot, NJ, Moreira, A (2012) Transcription termination between *polo* and *snap*, two closely spaced tandem genes of *D. melanogaster*, Transcription, 3(4): 198-212.

Tuesday 11th – Isabel Castro & Andrea Cruz

Morning – Isabel Castro and Gene Regulation group

1. GR Lab meeting: The students will participate in the weekly lab meeting of the Gene Regulation group. Isabel Castro will present her work on: "Global analysis of alternative polyadenylation regulators in T lymphocytes". GABBA students are expected to actively participate in the group scientific discussions.

Afternoon – Andrea Cruz

2. Research seminar: “Transcription Regulation of RhoGTPases during Myelination: Role of alternative polyadenylation and RNA Binding proteins”

Wednesday 12th - Natalia Gromak

Morning

1. Lecture (Part 1): Principles of gene expression - Polymerase II CTD code and histone code.
2. Lecture (Part 2): miRNAs and their role in the regulation of gene expression

Afternoon

1. Lecture: A. Inter-connection between gene expression steps.
 - a. Transcriptional termination
 - b. RNA/DNA hybrids (R-loops) in health and disease.
2. Paper dissection:
K Skourti-Stathaki, N. J. Proudfoot and N. Gromak (2011) Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. *Molecular Cell* 42(6): 794-805

Thursday 13th – Preparation of students presentations & Ana Pombo

Morning – Preparation of the students projects to be the discussed on Friday afternoon. Teachers will be available.

Students should arrange in 3 groups. Each group will make an oral presentation of a research project based on a topic that has been discussed this week. A formal presentation is not required, but it should be well structured, with a clear rationale. The students should focus on making a strong and solid proposal addressing some open questions in this field.

Afternoon

Lecture (Part 1): Interplay between Polycomb repression and co-transcriptional RNA processing in mouse ES cells?

Suggested reading:

- 1) Brookes E, Pombo A (2012) Code breaking: the RNAPII modification code in pluripotency. *Cell Cycle* 11, 1267-8.

- 2) Brookes E, de Santiago I, Hebenstreit D, Morris KJ, Carroll T, Xie SQ, Stock JK, Heidemann M, Eick D, Nozaki N, Kimura H, Ragoussis J, Teichmann SA, Pombo A (2012) Polycomb associates genome-wide with a specific RNA polymerase II variant, and regulates metabolic genes in ES cells. *Cell Stem Cell* 10, 157-70.
- 3) Stock JK, Giardrossi S, Casanova M, Brookes E, Vidal M, Koseki H, Brockdorff N, Fisher AM, Pombo A (2007) Ring1B-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in ES cells. *Nature Cell Biology* 9, 1428-1435.
- 4) Brookes E, Pombo A (2009) Modifications of RNA polymerase II are pivotal in regulating gene expression states. *EMBO Reports* 10, 1213-9.

Friday 14th - Ana Pombo & Students presentations

Morning

Lecture (Part 2): Chromatin 'communities' associated with RNA polymerase II

We have developed an unbiased approach called Proteome Chromatin Immunoprecipitation (pChIP) to dissect the proteins that co-associate with RNAPII bound to chromatin at different stages of the transcription cycle. After chromatin isolation from murine Embryonic Stem cells grown in SILAC (Stable Isotope Labelling by Amino acids in Cell culture) conditions, ChIP was performed with antibodies against RPB1-S5p, -S7p and -S2p. Using a multi-combinatorial design, the protein enrichment associated with each phosphorylation state was quantified by mass spectrometry. We identify several new protein associations with specific phosphorylation of RNAPII, expand the interactome of chromatin-bound RNAPII, and provide novel insights into the transcription process.

Afternoon – Students projects presentation and discussion with all the teachers.

Closing session and evaluation

6pm - Drinks with students

Teachers contacts list:

<p>Alexandra Moreira Isabel Castro Andrea Cruz</p> <p>Gene Regulation – IBMC, Univ Porto alexandra.moreira@ibmc.up.pt</p>	<p>Natalia Gromak</p> <p>Sir William Dunn School of Pathology University of Oxford, UK natalia.gromak@path.ox.ac.uk</p>
	<p>Ana Pombo</p> <p>Max Delbrück Center for Molecular Medicine, Berlin ana.pombo@mdc-berlin.de</p>