MULTIPLE ROLES OF RNAs

PROGRAM
2015-2016

From February 15th to 26th, 2016
INTRODUCTION

A few years ago, the RNA world was still divided in two categories: the protein coding RNA (mRNAs) and the non-coding RNAs such as tRNAs, snRNAs, snoRNAs and rRNAs. Several important concepts and discoveries such as splicing (Nobel Prize 1993, P.A. Sharp and R.J. Roberts), catalytic RNA (Nobel Prize 1989, T. Cech and S. Altman) have pointed out the major role of RNA in gene expression. Recently, the discovery of novel classes of RNA, such as siRNAs, miRNAs and RNA interference (RNAi) (Nobel Prize 2006, A.Z. Fire and C.C. Mello) shed light on novel major roles of RNA. It revealed a crucial role for RNA in many regulations of gene expression such as transcriptional and post-transcriptional control. More recently, deep-sequencing and high-resolution microarrays revealed the existence of a highly complex population of non-coding transcripts. It is now clear that a plethora of stable and unstable RNAs, generated by a pervasive transcription, exists. Many questions remain open about the regulation and function and molecular mechanisms involving these non-coding RNAs.

In order to sort out the relevant message from junk RNAs, the cell has developed several level of quality control. In the nucleus, most of the pervasive transcription products are degraded by the nuclear surveillance machinery. In the cytoplasm, other machineries are involved (Siwaszek A et al, RNA 2014 and Lykke-Andersen et al, Nature Reviews Molecular Cell Biology 2015 for recent reviews). The most studied of them is the “Nonsense mRNA Mediated Decay” (NMD). NMD monitors the capacity of mRNAs to be adequately translated into full-length proteins. NMD degrades mRNAs with premature termination codons (PTCs), a diverse class of RNA molecules that are by-products in the natural process of RNA synthesis because RNA splicing can be inefficient or RNA polymerase can often starts transcription after the position of the start codon (Malabat et al., eLife, 2015).

NMD is a fundamental process in eukaryotes. Even if the way this quality control works varies between different species, the main actors of this process, Upf1, 2, 3 and the translation machinery, are highly conserved in all eukaryotes. It is thus likely that the basic mechanisms of NMD have been conserved through evolution and that the study of the process in the yeast Saccharomyces cerevisiae can provide useful clues to understand RNA degradation mechanisms in eukaryotes in general.

Despite 30 years of efforts in this field, fundamental mechanisms of NMD remain largely unknown. Two key questions remain essentially unsolved: what distinguishes a PTC from a normal STOP codon and by which mechanisms mRNA degradation is triggered by the recognition of a PTC? Finally, how the constraints on NMD target recognition globally impact the transcriptome landscape in eukaryotic cells remains largely unexplored.
The "Multiple roles of RNA" practical course introduces tools allowing large-scale studies of NMD substrates and the biochemical mechanisms involved in coupling translation and RNA degradation.

During this course, the students will learn to perform transcriptome analysis and compare them to qRT-PCR and Northern blot experiments.

They will explore the global effects on mRNAs of a UPF1 deletion in S. cerevisiae, (RNAseq experiments) and test the association of the Upf1 protein to NMD substrates by combining affinity purification of RNA-protein complexes and sequencing (RIP seq experiments). The results of these experiments should highlight major features of NMD substrates in yeast and should allow a better understanding of the critical feature required for NMD dependent RNA destabilization. The tools used for exploring NMD substrates will be also useful for studying many other RNA-protein complexes in yeast or other species.

Public concerned

This course targets a public of highly motivated researcher, mostly master degree (M2) or PhD students, eager to enlarge their knowledge about the RNA world. Candidates should have strong basis in molecular biology at a graduate student level.
OBJECTIVES OF THE PRACTICAL COURSE

During the course, the students will:

• Purify the complexes associated with the Upf1 and Rpl16 proteins in cells, using Affinity Purification adapted from Rigaut G. et al. 1999 but with modifications to preserve the integrity of RNAs and extraction of the RNAs associated to these particles (Exp 1)

• Extract total RNAs as well as purify polyA RNAs from WT and Upf1 mutant strains (Exp 2)

• Prepare RNA seq libraries (using RNAs from the complexes and total RNAs), and analyse the data after sequencing (Exp 3)

• Validate candidates by qRT-PCR (Exp 4)

• Perform Northern blot experiments (Exp 5).

NB:
* RNA seq runs are performed at the Platform (PF2).
* These experiments require a large amount of yeast cells (6 litres of culture per 2 students). The cellular lysates will be prepared and stored at -80°C before the course. The experiments will be done with Upf1-HTP (test) and Rpl16A-TAP (reference) strains.

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Exp 1: Purification of RNAs associated to complexes

WT/ mutant

Exp 2: Extraction of total RNAs

WT/ mutant

Exp 3: RNA-seq

Exp 4: qRT-PCR

Exp 5: Northern
CONFERENCES

1. « RNA stability in eukaryotes: new concepts emerging from single nucleotide resolution sequencing and large-scale tests on reporter molecules »
   Cosmin SAVEANU
   (Institut Pasteur)

2. « RNAi-mediated antiviral immunity in insects »
   Carla SALEH
   (Institut Pasteur)

3. « Analysis of a nucleic acid population using qRT-PCR »
   Mathilde GARCIA
   (UPMC, Campus des Cordeliers)

4. « Dive into NGS data»
   Christophe MALABAT
   (Institut Pasteur)

5. « Differential analysis of RNA-Seq data: design, describe, explore and model »
   Hugo VARET
   (Institut Pasteur)

6. « Long non-coding RNAs and X-chromosome inactivation in mammals »
   Claire ROUGEULLE
   (Université Denis Diderot, Paris)

7. « Transcriptional diversification of unique gene loci: alternative splicing as a source of bifunctional RNAs and mirtrons »
   Claire FRANCASTEL
   (Université Denis Diderot, Paris)

PRACTICAL COURSE

EXP. 1 Purification
EXP. 2 Extraction of total RNA WT/mutant
EXP. 3 RNA seq
EXP. 4 qRT-PCR
EXP. 5 Northern
<table>
<thead>
<tr>
<th>Monday 15th</th>
<th>Tuesday 16th</th>
<th>Wednesday 17th</th>
<th>Thursday 18th</th>
<th>Friday 19th</th>
<th>Monday 22nd</th>
<th>Tuesday 23rd</th>
<th>Wednesday 24th</th>
<th>Thursday 25th</th>
<th>Friday 26th</th>
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<tbody>
<tr>
<td>T1</td>
<td>T2</td>
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<td>T7</td>
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**Exp 1: Purification of Complexes by TAP method and extraction of the RNA associated to the complex**

**Exp 2: Total RNA and PolyA Extraction**

**Exp 3: RNA seq library preparation and Analysis**

**Exp 4: qRT-PCR**

**Exp 5: Northern blot**

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T1 Cosmin Saveanu «RNA stability in eukaryotes: new concepts emerging from single nucleotide resolution sequencing and large-scale tests on reporter molecules.»

T2 Carla Saleh «RNAi-mediated antiviral immunity in insects»

T3 Mathilde Garcia «Analysis of a nucleic acid population using qRT-PCR»

T4 Christophe Malabat «Dive into NGS data»

T5 Hugo Varet «Differential analysis of RNA-Seq data: design, describe, explore and model»

T6 Claire Rougeulle «Long non-coding RNAs and X-chromosome inactivation in mammals»

T7 Claire Francastel «Transcriptional diversification of unique gene loci: alternative splicing as a source of bifunctional RNAs and mirtrons»
PLANNING WEEK 1

Monday February 15th 2016

9:00  Welcome to students  
Yannick ANDÉOL & Gwenaël BADIS-BRÉARD (Co-Directors of the Course)  
General presentation of the Course  
Claire TORCHET (Head of Studies)

10:00-10:30  General presentation of the room  
Isabelle LEQUEUTRE

10:30-12:30  
- Exp.2 : Culture of yeast strains  
- Exp.1 : TAP Protein A binding to IgG Dynabeads  
- Exp.1 : TAP IgG Dynabeads washing and TEV digestion

- Lunch -

13:30-16:00  
- Exp.1 : TEV elution  
- Exp.1 : Extraction of RNA associated to the complex and precipitation  
- Exp.2 : OD measurement of yeast strains and freezing of pellets

16:00-17:30  « RNA stability in eukaryotes: new concepts emerging from single nucleotide resolution sequencing and large-scale tests on reporter molecules »  
Cosmin SAVEANU  
(Institut Pasteur)

Tuesday February 16th 2016

9:00-12:00  
- Exp.2 : Total RNA extraction  
- Exp.1 : Measurement of the amount of RNA associated to the complex  
- Exp.2 : Measure of the amount of total RNA Agarose gel RNA

- Lunch -

13h00  
- Exp.3 and Exp.4 : DNAse treatment of total RNA

14:30-16:00  « RNAi-mediated antiviral immunity in insects »  
Carla SALEH  
(Institut Pasteur)

16:15-17:30  - Exp.3 Bioanalyzer

Wednesday February 17th 2016

9:00  Illumina libraries preparation:  
Caroline PROUX  
(Institut Pasteur)

- Exp.3 : PolyA purification  
- Exp.3 : Fragmentation  
- Exp.3 : First strand cDNA  
- Exp.3 : Second strand cDNA

- Lunch -

13:30  
- Exp.3 : AMPure beads purification  
- Exp.3 : Adenylate 3’ ends  
- Exp.3 : Adapters ligation + AMPure beads purification  
- Exp.3 : PCR amplification

Thursday February 18th 2016

9:00-9:45  - Exp.3 : AMPure beads purification  
- Exp.3 Bioanalyzer

10:00-11:30  « Analysis of a nucleic acid population using qRT-PCR »  
Mathilde GARCIA  
(UPMC, Campus des Cordeliers)

11:30-12:30  - Exp.3 : Quantification Qubit and sequencing preparation.

- Lunch -

13:30-14:45  - Exp.4 : cDNA for qRT-PCR

15:00-16:30  « Dive into NGS data »  
Christophe MALABAT  
(Institut Pasteur)

16:30-17:00  - Exp.4 : RT inactivation
Friday February 19th 2016
9:00  - Exp.3 : Data analysis: Bioinformatic (all day)
- Lunch -
13:30  - Exp.3 : Data analysis : Bioinformatic (all day)

PLANNING WEEK 2

Monday February 22nd 2016
9:30-12:00  - Exp.3 : Data analysis: Statistics
9:30-12:00  ☑ « Differential analysis of RNA-Seq data: design, describe, explore and model »  Hugo VARET  
(Institut Pasteur)
- Lunch -
13:00  - Exp.5 : Northern (agarose gel)
17:00  - Exp.5 : Transfert

Tuesday February 23rd 2016
9:00-13:30  - Exp.4 : qRT-PCR design and experiment
- Lunch
14:30-15:00  - Exp.5 : PCR DIG labelling, (run during conference)
15:15-16:45  ☑ « Long non-coding RNAs and X-chromosome inactivation in mammals »  Claire ROUGEULLE  
(Université Denis Diderot, Paris)
17:00  - Exp.5 : Probe labelling, verification on gel
- Exp.5 : Northern hybridization

Wednesday February 24th 2016
9:30-12:30  - Exp.3 : Data analysis: Statistics
- Lunch -
13:30  - Exp.5 : Northern Washing
- Exp.4 : qRT-PCR data analysis
16:30  - Exp.5 : Northern Revelation

Thursday February 25th 2016
9:00-12:30  - Assessment
11:00-12:30  ☑ « Transcriptional diversification of unique gene loci : alternative splicing as a source of bifunctional RNAs and mirtrons »  Claire FRANCASTEL  
(Université Denis Diderot, Paris)
- Lunch -
13:30-17:30  - Assessment

Friday February 26th 2016
9:00-12:30  - Assessment
- Lunch -
13:30-14:00  - Assessment
14:00-16:30  - Oral Examination
17:00  - Party
Organisation of the Examinations

Oral examinations (30/100)

- Continuous student assessment during the practical course:
  Based on the participation during the practical course, quality of the work, and the laboratory notebook
- Oral examination

Friday February 26th 2016, presentation by groups of 2 pairs of students, of the results and conclusions of the different experiments obtained during the practical course.
- Slides (Powerpoint or other supported format)
- The presentation should last 20 minutes, followed by 10 minutes for questions

Written examinations (70/100)

- A report of the practical course
  This document should include:
  - The aim of each experiments
  - The analyse of your results and also those obtained by the whole group
  - An interpretation and conclusion of each experiments
  - A general conclusion

Maximum 12 pages (front side only). Times 12, spacing 1.5.

A unique paper copy of the report should be posted to:
Claire TORCHET
Equipe Métabolisme de l'ARN
UMR8226 CNRS/UPMC
Institut de Biologie Physico-Chimique
13, rue Pierre et Marie Curie
75005 Paris France

And sent by e-mail to:
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csaveanu@pasteur.fr
gbreard@pasteur.fr
yannick.andeol@snv.jussieu.fr

NO LATTER THAN March 16th 2016