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Multiple roles of RNAs

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► **To cite this version:**

Gwenaël Badis, Cosmin Saveanu, Claire Torchet. Multiple roles of RNAs. Master. Multiples roles of RNAs, <https://research.pasteur.fr/fr/course/multiple-roles-of-rnas-2/>, France. 2023. hal-04048211v2

HAL Id: hal-04048211

<https://hal.science/hal-04048211v2>

Submitted on 30 Mar 2023

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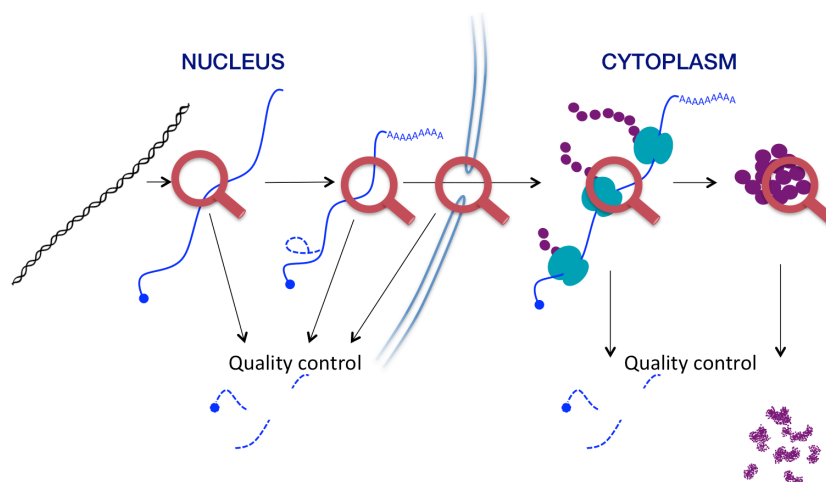


Institut Pasteur

centre d'enseignement

MULTIPLE ROLES OF RNAs

PROGRAM



2022-2023

From February 13th to 24th, 2023

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 controls. In the nucleus, the nuclear surveillance machinery degrades most of the pervasive transcription products. 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 start 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). 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, and 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

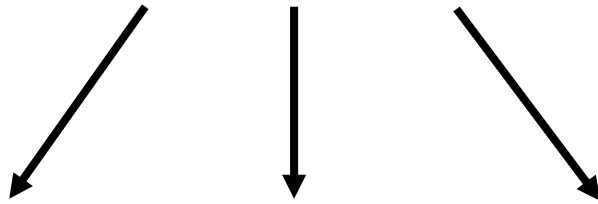
During the course, the students will:

- Extract total RNAs from WT, *Upf1* Δ , *Xrn1* Δ and *Rrp6* Δ mutant strains (**Exp 1**)
- Prepare RNA-Seq libraries, and analyse the data after sequencing* (**Exp 2**)
- Validate candidates by qRT-PCR (**Exp 3**)
- Perform Northern blot experiments (**Exp 4**)

NB:

* RNA seq runs are performed at the Platform (PF2)

Exp 1: Extraction of total RNAs WT/ mutant strains



Exp 2: RNA-seq

Exp 3: qRT-PCR

Exp 4: Northern

« MULTIPLE ROLES OF RNAs » COURSE 2022-2023
(February 13th to 24th 2023)

CONFERENCES

- 1** « RNA stability in eukaryotes: new concepts emerging from single nucleotide resolution sequencing and large-scale tests on reporter molecules » **GWENAËL BADIS-BREARD**
(Institut Pasteur)
- 2** « RNAseq general » **RACHEL LEGENDRE**
(Institut Pasteur)
- 3** « Differential analysis of RNA-Seq data: design, describe, explore and model » **STEVENN VOLANT**
(Institut Pasteur)
- 4** « Meiotic mRNA degradation and role of lncRNAs in the mitosis-meiosis transition in *Schizosaccharomyces pombe* » **MATHIEU ROUGEMAILLE**
(I2BC, Paris-Saclay)
- 5** « RNAseq : Illumina libraries preparation » **ODILE SISMEIRO**
(Institut Pasteur)
- 6** «First step with Unix » **RACHEL LEGENDRE**
(Institut Pasteur)
- 7** « NMD in host/pathogen relationship» **Vincent Mocquet**
(ENS, LYON)
- 8** «Analysis of a nucleic acid population using qRT-PCR» **MATHILDE GARCIA**
(Sorbonne Université)

PRACTICAL COURSE

- EXP. 1** Extraction of total RNA from WT and mutant strains
- EXP. 2** RNA-Seq
- EXP. 3** qRT-PCR
- EXP. 4** Northern blot

Monday 13th	Tuesday 14th	Wednesday 15th	Thursday 16th	Friday 17th	Monday 20th	Tuesday 21st	Wednesday 22th	Thursday 23th	Friday 24th
T1	T2	T3	T4	T5	T6	T7			
Exp 1: Total RNA and PolyA Extraction									
Exp 2: RNA seq library preparation and Analysis									
Exp 3: qRT-PCR									
Exp 4: Northern blot									

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

T2 Rachel Legendre « RNAseq General »

T3 Stevann Volant « Differential analysis of RNA-Seq data: design, describe, explore and model »

T4 Mathieu Rougemaille « Meiotic mRNA degradation and role of lncRNAs in the mitosis-meiosis transition in *Schizosaccharomyces pombe* »

T5 Odile Sismeiro « RNAseq : Libraries preparation »

T6 Vincent Moquet « Le NMD dans la relation hôte/pathogène »

T7 Rachel Legendre « First Steps with Unix »

T8 Mathilde Garcia « Analysis of a nucleic acid population using qRT-PCR »

* Visit of the Biomixics sequencing platform

PLANNING WEEK 1

Monday February 13th 2023

- 9:00 Welcome to students **Gwenaël BADIS-BRÉARD & Claire TORCHET** (Co-Directors of the Course)
General presentation of the course
- 10:00-10:30 General presentation of the room and safety rules **Isabelle LEQUEUTRE**
- 10:30-11:30 - **Exp.1** : Culture of yeast strains
- 11:30-13:00 **1** « RNA stability in eukaryotes: new concepts emerging from single nucleotide resolution sequencing and large-scale tests on reporter molecules »
Gwenaël BADIS-BRÉARD (Institut Pasteur)
- Lunch -
- 14:00-15:30 **2** « RNAseq General » **Rachel LEGENDRE**
(Institut Pasteur)
- 15:30-18:30 - **Exp.1** : OD measurement of yeast strains
- Extraction of total RNA and measure of the amount of total RNA

Tuesday February 14th 2023

- 9:00-11:00 **3** PART I « Differential analysis of RNA-Seq data: design, describe, explore and model »
Stevann VOLANT
(Institut Pasteur)
- 11:00-12:00 - **Exp.1** : Preparation of agarose gel and run
- Introduction to the yeast model and to the *Saccharomyces* Genome Data base (SGD)
- Lunch -
- 13:00-14:30 - **Exp.1** : Picture of agarose gel and analysis
- **Exp.1** : DNase treatment of total RNA and precipitation for the following **Exp.2** and **Exp.3**
- 14:30-16:00 **4** « Meiotic mRNA degradation and role of lncRNAs in the mitosis-meiosis transition in *Schizosaccharomyces pombe* »
Mathieu ROUGEMAILLE
(Paris-Saclay)
- 16:15-17:15 **3** PART II « Differential analysis of RNA-Seq data: design, describe, explore and model »
Stevann VOLANT
(Institut Pasteur)
- 17:30-18:00 - **Exp.2** : Results Bioanalyzer **Odile SISMEIRO**
(Institut Pasteur)

Wednesday February 15th 2023

- 9:00-12:30 **5** «Illumina libraries preparation » *Salle de TP* **Odile SISMEIRO**
(Institut Pasteur)
- **Exp.2** : PolyA purification
 - **Exp.2** : Fragmentation
 - **Exp.2** : First strand cDNA
 - **Exp.2** : Second strand cDNA
- Lunch -
- 13:30-18:00 - **Exp.2** : AMPure beads purification
- **Exp.2** : Adenylate 3' ends
 - **Exp.2** : Adapters ligation + AMPure beads purification
 - **Exp.2** : PCR amplification

Thursday February 16th 2023

- 9:00-10:00 - **Exp.2** : AMPure beads purification
- **Exp.2** : Bioanalyzer
- 10:00-12:00 **6** « First Steps with Unix » **Rachel LEGENDRE**
(Institut Pasteur)
- **Exp.2** : Quantification Qubit and sequencing preparation (Odile SISMEIRO at the platform)
- Lunch -
- 13:00-14:15 - **Exp.3** : cDNA for qRT-PCR,
- RT inactivation
- 15:00-16:30 **7** « NMD in host/pathogen relationship » **Vincent MOCQUET**
Zoom Conference (ENS, Lyon)

Friday February 17th 2023

- 9:00-13:00 *Data analysis : sequence treatment and bioinformatic* **Rachel LEGENDRE**
(Institut Pasteur)
- **Exp.2** : Quality check, aptamers trimming, mapping on the yeast genome
- Lunch -
- 14:00-16:00 - **Exp.2** : Statistics, graphics and modeling using the R programming language **Stevann VOLANT**
(Institut Pasteur)
- 16:00-18:00 - **Exp.2** : Looking for targets to test by Q-RT-PCR and Northern blot **Cosmin SAVEANU**
(Institut Pasteur)

PLANNING WEEK 2

Monday February 20th 2023

9:00-13:00 **8** - Analysis of a nucleic acid population using qRT-PCR

Mathilde GARCIA
(Sorbonne Université)

- **Exp.3** : qRT-PCR design and experiment
- **Exp.4** : Northern blot: agarose gel preparation

- Lunch -

14:00-17:00 - **Exp.4** : Northern blot: agarose gel run

- **Exp.4** : RNA probe preparation : transcription reaction

17:00-18:00 - **Exp.4** : Northern blot: transfer

Tuesday February 21st 2023

9:00-12:00 **Exp.3** : qRT-PCR data analysis

Mathilde GARCIA
(Sorbonne Université)

12:00-12:30 - **Exp.4** : Northern pre-hybridization

- Lunch -

13:30-14:00 - **Exp.4** : Northern hybridization

14:00-15:30 - **Exp.3** : qRT-PCR data analysis

15:30-18:00 - **Exp.2** : RNA seq data analysis

Wednesday February 22nd 2023

9:00-12:00 - **Exp.4** : Northern washing
- Visit of the platform (PF2)

- Lunch -

13:00-17h30 - **Exp.4** : Northern signal detection, Northern analysis with Image J

Thursday February 23rd 2023

9:30-12:30 - Data analysis and preparation of the oral presentation

- Lunch -

13:30-17:30 - Data analysis and preparation of the oral presentation

Friday February 24th 2023

9:00-12:30 - Data analysis and preparation of the oral presentation

- Lunch -

13:30-17:00 - **Oral presentation**

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.

- Oral examination

Friday February 24th 2023, presentation by groups of 4 or 3 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 20 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.

The report should be sent by e-mail to:

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NO LATER THAN March 6st 2023

CONTACTS

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