"InteRNAt" summer school: detailed program

October 6–10, 2019

Contents

1 Biogenesis and mode of action of small RNAs (1)

Date: Sunday, October 6, 3:00–7:30 pm.
Speakers: Hervé Seitz, André Verdel, Kazufumi Mochizuki.

RNA interference: an overview (H. Seitz, 3:00–3:45 pm):

1. The history of RNAi (cosuppresion in plants; the Fire and Mello paper showing the involvement of dsRNA).
2. Biochemistry of RNAi (observation of siRNAs; chemical structure of siRNAs; identification of Dicer; summary of the mechanism of RNAi: cleavage across nt 10 and 11, quality of the sequence complementarity).
3. Biotechnological application of RNAi (siRNAs instead of long dsRNAs for species with interferon response; examples of usage in the lab, drug development for the clinic).
4. Endogenous RNAi (natural functions in various eukaryotes: gene regulation, anti-pathogenic response).

microRNAs: an overview (H. Seitz, 3:45–4:30 pm):

1. Discovery of *lin-4* and its role in *lin-14* repression (conserved imperfect matches in the 3´ UTR; stem-loop precursor).
2. Discovery of *let-7*, and its deep phylogenetic conservation.
3. 2001: the three papers describing miRNA libraries and coining the name "microRNA" (all with stem-loop precursors).
4. Discovery of miRNAs in many animals, plants, *Chlamydomonas*, viruses
5. Mode of action (endonucleolytic cleavage if conditions are met; translational repression and exonucleolytic decay otherwise).
6. Location of binding sites (only 3´ UTRs ?).
7. miRNA biogenesis (involvement of Dicer, Drosha; introduce the Argonaute proteins).
8. Why have miRNAs been ignored so long?

Biochemistry of the RISC complex (H. Seitz, 4:30–5:00 pm):

1. The same RISC for siRNAs and miRNAs?
2. Who is Slicer? (present the Argonaute family, with Ago, Piwi and Wago subfamilies; identification of mammalian and *Drosophila* Slicers; crystallographic data)
3. Why only one strand of siRNAs or miRNA/miRNA* duplexes is preferentially chosen? (the asymmetry rule).
4. Molecular mechanism of the cleavage reaction; of translational repression and exonucleolytic decay.

Questions from the audience (5:00–5:15 pm).

Coffee break (5:15–5:45 pm).

Nuclear RNAi-related phenomena in fission yeast: an overview (A. Verdel, 5:45–6:30 pm):

Heterochromatinization in fission yeast: description of the initial discovery (the 2002 papers), without any mechanistic details.

Genomic rearrangements in ciliates: an overview (K. Mochizuki, 6:30–7:15 pm):

Overview of the macronuclear differentiation process, description of epigenetic phenomena pointing at a comparison between the old and new macronuclei; observation of scnRNAs.

Questions from the audience (7:15–7:30 pm).

2 Biogenesis and mode of action of small RNAs (2)

Date: Monday, October 7, 9:00 am–12:30 pm.
Speakers: Hervé Seitz, Séverine Chambeyron.

Methods for miRNA identification (H. Seitz, 9:00–9:30 am):

1. Introduction to miRBase: nomenclature issues, coverage per species.
2. Mis-annotated miRNAs.
3. Genetics: the historical examples (*lin-4*, *let-7*, *bantam*, *lsy-6*). Phenotypic effect is obvious; but this is rare (needs to be lucky).
4. miRNA-specific cloning (using the 5´-monophosphate and 3´-OH), with low- or high-throughput sequencing. Introduce experimental sources of biases.
5. Computational approach, made obsolete by the development of Small RNA-Seq.

MicroRNA biogenesis (H. Seitz, 9:30–9:45 am):

1. Transcription (Pol III controversy).
2. Drosha in animals, Dicer in plants.
3. No competition between Drosh-ing and splicing for intronic miRNAs.
4. Regulation of Dicer-mediated cleavage (*let-7* loop variants).
5. Exceptions to the rules: mirtrons, miR-451.

Questions from the audience (9:45–10:00 am).

Coffee break (10:00–10:30 am).

piRNAs (S. Chambeyron, 10:30–12:30):

1. Historical aspects (hybrid dysgenesis in *Drosophila*).
2. The 2006 papers: looking for small RNAs interacting with Piwi proteins.
3. piRNA abundance: mostly gonad-specific; seen on ethidium bromide-stained gel in mouse testis (*very* abundant).
4. Size distribution (longer than miRNAs or siRNAs), 2´-*O*-methylation on the 3´ end.

5. Homology to repeated sequences: clear in *Drosophila*, more complicated in mammals (pre-pachytene ≠ pachytene piRNAs).

6. Activity of transposable elements in the gonad (germline and infecting particles from the soma): selective pressure to transpose in the germ line's genome.

7. piRNA biogenesis: description of clusters (uni- or bi-directional), no involvement of Dicer, the ping-pong mechanism.

3 Physiological roles of small RNAs

Date: Monday, October 7, 2:00–3:45 pm.
Speakers: Jérôme Cavaillé, Hervé Seitz.

Documented roles of miRNAs in animals (J. Cavaillé, 2:00–3:00 pm):

1. Genetically-established roles for miRNAs in animals.

2. Focus on a few well-studied cases: miRNAs in neurons, miRNAs in metabolic processes, miRNAs in ES cell biology, genomically imprinted miRNAs).

Functional assignment of miRNAs and siRNAs in animals: limitations and biases (H. Seitz, 3:00–3:30 pm):

1. GO term enrichment biases.

2. Observed phenotypes vs. enriched GO terms.

3. Threshold on miRNA abundance for their biological activity.

4. Endogenous RNAi in animals (*Drosophila*, mouse oocyte-specific Dicer, vegetative RNAi in unicellular eukaryotes).

Questions from the audience (3:30–3:45 pm).

Coffee break (3:45–4:15 pm).

4 The pecularities of *C. elegans* small RNA pathways

Date: Monday, October 7, 4:15–6:00 pm.
Speaker: Germano Cecere.

1. The RNAi response (with primary and secondary, RdRP-derived, siRNAs)

2. the 21U piRNA pathway (and the few transposable elements they repress)

3. Phase-separated germ granule and small RNAs

Questions from the audience (5:45–6:00 pm).

5 Transposable element repression, transcriptional effects of small RNAs

Date: Tuesday, October 8, 9:00 am–1:00 pm.
Speakers: André Verdel, Kazufumi Mochizuki, Alain Pélisson, Marius van den Beek.

Small RNA-guided transcriptional silencing in fission yeast (A. Verdel, 9:00–10:00 am):

1. Detailed description of small RNA-guided TGS in fission yeast (genetics, biochemistry, functional consequences).
2. Give the mechanistic details, name the proteins involved, show the models.

Molecular mechanisms of scnRNA-mediated heterochromatinization in ciliates (K. Mochizuki, 10:00–11:00 am):

1. Mechanisms for the guidance of heterochromatin mark deposition.
2. Various classes of small RNAs involved in macronuclear differentiation.
3. Biased generation of scnRNAs from IES's.

Questions from the audience (11:00–11:15 am).

Coffee break (11:15–11:45 am).

piRNA-guided transcriptional silencing (A. Pélisson, M. van den Beek, 11:45 am–1:00 pm):

1. Mechanism of piRNA-guided TGS in *Drosophila*.
2. Functional consequences of the loss of the piRNA machinery in *Drosophila* (transposable element depression, activation of the DNA damage checkpoint, embryonic lethality).
3. Review of piRNA action in non-*Drosophila* model organisms (piRNA-mediated repression of transposable elements in mouse, limited repression of a few elements in nematodes).

6 Detection and quantification methods (theoretical lecture and practical class)

Date: Tuesday, October 8, 2:30–6:30 pm.
Speakers: Hervé Seitz, Marius van den Beek.

Methods for small RNA quantification (H. Seitz, 2:30–3:00 pm):

1. Overview of existing techniques (Northern blot, microarrays, RT-PCR, cloning, Small RNA-Seq).
2. For each: present their own strengths and weaknesses.

Small RNA-Seq: principle and usage (M. van den Beek, 3:00–4:00 pm):

1. Principle of Small RNA-Seq.
2. Limitations: biases, sample purity, complexity.

3. Issues with miRNA isoforms and untemplated additions.

4. Methods for the identification of differentially expressed miRNAs.

Questions from the audience (4:00–4:15 pm).

Coffee break (4:15–4:45 pm).

Small RNA-Seq analysis: practical class (M. van den Beek and H. Seitz, 4:45–6:15 pm): Using Galaxy, and pre-prepared dataset. Participants will have to bring their own laptop.

Questions from the audience (6:15–6:30 pm).

7 Target identification methods (theoretical lecture and practical class)

Date: Wednesday, October 9, 9:30–12:30 am.
Speakers: Hervé Seitz, Jérôme Cavaillé.

Experimental and computational methods for miRNA target identification (H. Seitz, 9:30–10:30 am):

1. Experimental methods: differential transcriptomics or proteomics after miRNA perturbation.

2. Experimental methods: CLIP and its derivatives.

3. Computational methods: basic rules, additional refinements (conservation, secondary structure accessibility, ...).

Questions from the audience (10:30–10:45 pm).

Coffee break (10:45–11:15 pm).

Case study: transcriptomics response to miRNA cluster knockout (J. Cavaillé and H. Seitz, 11:15–12:15 am):

1. Presentation of the biological system.

2. Technical and analytical choices influencing the results.

Questions from the audience (12:15–12:30 pm).

8 Analysis of studies from the literature

Date: Wednesday, October 9, 2:00–4:45 pm.
Speakers: Sébastien Pfeffer, André Verdel, Marius van den Beek.

This will be a "journal club" session, with 7 groups of 5 attendees each. Each group will be attributed an article, from the following list:

- ElMaghraby *et al.*, 2019: A heterochromatin-specific RNA export pathway facilitates piRNA production.

- Kowalik *et al.*, 2015: The Paf1 complex represses small-RNA-mediated epigenetic gene silencing.

- Memczak *et al.*, 2013: Circular RNAs are a large class of animal RNAs with regulatory potency.

- Tay *et al.*, 2011: Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs.
- Vrettos *et al.*, 2016: Kc167, a widely used *Drosophila* cell line, contains an active primary piRNA pathway.
- Wang *et al.*, 2019: A microRNA-inducible CRISPR-Cas9 platform serves as a microRNA sensor and cell-type-specific genome regulation tool.
- Yu *et al.*, 2018: Epigenetic inheritance mediated by coupling of RNAi and histone H3K9 methylation.

(articles selected by S. Pfeffer, A. Verdel and M. van den Beek)

Articles will have been distributed to the 7 groups on Monday, October 7. Groups will work one more hour together during this session (the organizing committee will circulate among groups to answer their questions), then each group will present their article in 15 min.

Coffee break (4:45–5:15 pm).

9 The peculiarities of plant small RNA pathways

Date: Wednesday, October 9, 5:15–6:45 pm.
Speakers: Hervé Vaucheret.

1. Classes of small RNAs (with their biogenesis and their mode of action): miRNAs, tasiRNAs, ...
2. miRNA-phased siRNA biogenesis
3. Heterochromatin guidance
4. Propagation of the silencing signal

Questions from the audience (6:45–7:00 pm).

10 Small RNAs in host-virus interactions

Date: Thursday, October 10, 9:30 am–12:30 pm.
Speakers: Sébastien Pfeffer.

RNAi as an antiviral defense system (S. Pfeffer, 9:30–10:15 am):

1. RNA silencing and viruses in plants
2. RNA silencing and viruses in insects
3. Suppression of RNA silencing, some examples
4. Role of RNAi against viruses in mammals, interplay with other defense mechanisms

Questions from the audience (10:15–10:30 pm).

Coffee break (10:30–11:00 pm).

Role of small RNAs in viral infections (S. Pfeffer, 11:00 am–12:15 pm):

1. Cellular miRNAs as negative regulators of viral infection
2. Viral response to miRNA targeting (TDMD, ...)
3. -Cellular miRNAs as positive regulators of viruses (miR-122, ...)
4. Virus-encoded miRNAs, peculiarities and functions
5. Role of piRNAs in viral infections.

Questions from the audience (12:15–12:30 pm).

11 On-site evaluation of the school, open questions

Date: Thursday, October 10, 2:00–3:30 pm.
Speakers: everyone.

First we will take some time to answer every remaining question, and engage into an interactive discussion with the attendees about their own projects, and how they envision working on small RNAs.

Then we will ask them to fill a questionnaire for the evaluation of the whole summer school (this is a requisite from CNRS), in order to identify the points that worked well and those that need to be improved for a future issue of the school.