MASTER 2 BMC
PARCOURS GENOPATH
ANNEE 2024-2025

Titre :

Effects of post-translational modifications of Argonaute proteins on miRNA activity

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Sujet de stage :

MicroRNAs (miRNAs) are short non-coding RNAs of 20–24 nucleotides that guide Argonaute (AGO) proteins to target mRNA transcripts via Watson-Crick pairing. Once bound, Argonaute proteins serve as a platform and recruit secondary silencing factors, which trigger mRNA degradation or repress translation. MiRNAs are involved in every cellular process and are essential for animal development, cell differentiation and homeostasis. Deregulation of miRNA function is often linked to human diseases, including cancer. Determining miRNA-mRNA target interactions is the foundation for discerning miRNA biological functions and understanding how miRNA dysregulation contributes to disease, a prerequisite for the development of miRNAs as drugs or drug targets.

Argonaute proteins are subject to post-translational modifications, which may modulate the ability of miRNA-loaded Argonaute proteins to bind mRNA targets and recruit secondary silencing factors. We aim at characterizing general biochemical effects of phosphorylation events— the most common mechanism for post-translational regulation in response to signaling cascade—on miRNA activity. We will focus on 8 phosphorylation sites of AGO2 chosen because they are located on the protein surface, are within different functional domains of AGO2, and are conserved in all mammalian AGO paralogs. We will generate recombinant proteins with phosphomimetic or non-phosphorylatable substitutions and will compare their target binding by in vitro binding assays. To determine whether phosphorylation regulates the ability of miRNAs to recruit silencing effector proteins, we will

incubate recombinant AGO2 variants with cellular lysates, and will identify interacting proteins in the pull-downs. Together, this work will expand our knowledge of miRNA biology and will open a new avenue of inquiry into the mechanism of modular layer of post-transcriptional control formed by miRNAs, which enables generating complex cellular responses to environmental challenges and pathological conditions.

Modèle et techniques utilisées :
In vitro (purified RNAs and proteins) and *ex-vivo* (mouse macrophages)
Cloning, protein transient expression in *Drosophila* S2 cells, protein purification by affinity, double filter binding assay, PAGE, Western Blot

Publications d'intérêt : (‡Co-first authors, ✉Co-corresponding authors)

- Vega-Badillo J✉, Zamore PD✉, **Jouravleva K**✉. Protocol to measure protein-RNA binding using double filter-binding assays followed by phosphorimaging or high-throughput sequencing. STAR Protoc. 2023 Jun 3;4(2):102336.
- **Jouravleva K**‡, Golovenko D‡, Demo G, Dutcher RC, Tanaka Hall TM✉, Zamore PD✉, Korostelev AA✉. Structural Basis of MicroRNA Biogenesis by Dicer-1 and Its Partner Protein Loqs-PB. Mol Cell. 2022 Nov 3;82(21):4049-4063.e6.
- **Jouravleva K**✉, Vega-Badillo J, Zamore PD✉. Principles and pitfalls of high-throughput analysis of microRNA-binding thermodynamics and kinetics by RNA Bind-n-Seq. Cell Reports Methods. 2022 Mar 18;2:100185.
- Ober-Reynolds B‡, Becker WR‡, **Jouravleva K**‡, Jolly SM, Zamore PD✉, Greenleaf WJ✉. High-throughput biochemical profiling reveals functional adaptation of a bacterial Argonaute. Mol Cell. 2022 Mar 9;S1097-2765(22)00164-2
- Smith CS‡, **Jouravleva K**‡, Huisman M, Jolly SM, Zamore PD✉, Grunwald D✉. An Automated Bayesian Pipeline for Rapid Analysis of Single-Molecule Binding Data. Nat Comm. 2019 Jan 17;10(1):272.