Master 2 Reproduction et Développement
Stage de recherche 2024-2025

Stage proposé par
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Intitulé de l 'équipe d'accueil : BIOLOGIE DE L'OVOCYTE

Prénom et NOM du Responsable de l'équipe : Catherine JESSUS and Enrico DALDELLO

Résumé du thème de recherche de l'équipe (une dizaine de lignes maximum)
Cells proliferation is a fundamental process for development, growth and maintenance of living organisms. Despite the scientific interest in studying this process over many years, the understanding of the mechanisms driving cell division remains elusive. The entry into M-phase is achieved by the phosphorylation of hundreds of proteins that supervise the mechanics of cell division. These "mitotic substrates" are under the control of a highly conserved enzyme: the kinase Cdk1. Our research aims at understanding the mechanisms of Cdk1 activation that triggers meiotic divisions using the *Xenopus* oocyte as a model system. Oocytes are arrested in prophase of the 1st meiotic division, equivalent to a late G2 arrest. In frog oocytes, the release from this block is triggered by progesterone, which causes a rapid decrease in cAMP levels and PKA activity. This downregulation induces parallel signaling pathways that converge to activate Cdk1, driving the 1st meiotic division. Our aim is to identify the molecular players in these pathways, connecting PKA inactivation to Cdk1 activation.

Titre du projet de stage : Identifying new regulators of Cdk1 activation in *Xenopus* oocytes.

Prénom, NOM, téléphone et adresse e-mail du Responsable du stage:
Enrico Maria DALDELLO, 01 44 27 22 96, enrico.daldello@upmc.fr

Projet de stage : (une vingtaine de lignes maximum)

Despite years of research and the importance of the question, the mechanisms governing the G2-M transition remain poorly understood. The oocyte is a widely used model system for investigating these mechanisms due to its naturally occurring late G2 (prophase) arrest, which can be released by hormonal stimulation. In all vertebrates, this prophase arrest is maintained by the activity of the kinase PKA, activated by high cAMP levels. Hormonal stimulation, progesterone in our amphibian model, causes a rapid drop in cAMP, leading to PKA inactivation. This activates translation and accumulation of proteins, partly unidentified, that are necessary for Cdk1 activation. Indeed, blocking protein translation completely prevents Cdk1 activation and the resumption of meiosis. Identifying the proteins whose accumulation is required for Cdk1 activation and understanding their regulation is therefore crucial for unraveling the mechanisms controlling the cell decision to divide.
To address this issue, we used a mass spectrometry screen and identified new proteins accumulating in response to progesterone, upstream Cdk1 activation. The objective of the M2 internship is to functionally test the roles of these new candidates in meiotic maturation using gain- and loss-of-function approaches. We will suppress the accumulation of these proteins using antisense

oligonucleotides or overexpress them by microinjecting oocytes with mRNAs or recombinant proteins. We will then evaluate the ability of the oocytes to properly resume meiosis, examining the timing of meiosis resumption, Cdk1 activation through kinase assays, and several biochemical markers of Cdk1 regulation via western blot. The precise molecular functions of the candidates exhibiting the strongest effects on meiosis resumption will be further investigated by identifying their interactome using chromatography techniques and/or mutants.

Techniques mises en œuvre par le stagiaire :

During the internship, the student will acquire a diverse set of skills and techniques. These include: *(1)* molecular biology techniques: cloning and site-specific mutagenesis using Gibson assembly and overlap-extension PCR and Q-PCR; *(2)* biochemistry techniques: SDS-page, western blotting, affinity protein purification, protein chromatography, immunoprecipitations and pull-down, kinase assays; *(3)* cell biology techniques: isolation, culture, and micro-injection of *Xenopus* oocytes. Additionally, the student will actively participate in the lab's weekly meetings and receive training on oral and written presentation of scientific questions and the obtained results.

Publications du Responsable de stage au cours des 5 dernières années :

1. Multiple intersecting pathways are involved in the phosphorylation of the RNA binding protein CPEB1 and activation of translation during mouse oocyte meiosis.
   Chisato Kunitomi, Mayra Romero, **Enrico Maria Daldello**, Karen Schindler and Marco Conti.
   Accepted in Development. Pre-print available: *10.1101/2024.01.17.575938*

2. Cross-species analysis of ARPP19 phosphorylation during oocyte division charts the emergence of a new cAMP-dependent role in vertebrates.
   Ferdinand Meneau, Pascal Lapébie, **Enrico Maria Daldello**, Tran Le, Sandra Chevalier, Evelyn Houliston, Catherine Jessus, Marika Miot.
   Under revision in Development. Pre-print available: https://doi.org/10.1101/2023.07.05.547804

3. Unraveling the Interplay between PKA Inhibition and Cdk1 Activation during Oocyte Meiotic Maturation.
   Martina Santoni, Ferdiand Meneau, Nabil SekhSoukh, Sandrine Castella, Tran Le, Marika Miot, and **Enrico Maria Daldello***.
   Cell Reports 43, 113782 (2024) https://doi.org/10.1016/j.celrep.2024.113782
   (*** Corresponding author**)

4. CPEB1-dependent disruption of the mRNA translational program in oocytes during maternal aging.
   Nozomi Takahashi, Federica Franciosi, **Enrico Maria Daldello**, Xuang G. Luong, Peter Althoff, Xiaotian Wang, Marco Conti.
   Nature Communications 14, 416 (2023). https://doi.org/10.1038/s41467-023-35994-3

5. The M-phase regulatory phosphatase PP2A-B55δ opposes protein kinase A on Arpp19 to initiate meiotic division.
   Tom Lemonnier*, **Enrico Maria Daldello***, Robert Poulhe, Tran Le, Marika Miot, Catherine Jessus, and Aude Dupré.
   Nature Communications 12, 1837 (2021). https://doi.org/10.1038/s41467-021-22124-0
   (***Co-first authors**)

6. (Method article) Defining the Program of Maternal mRNA Translation During In Vitro Maturation Using a Single Oocyte Reporter Assay.
   Natasja G. J. Costermans, **Enrico Maria Daldello**, Ria J. Marathe, Marco Conti.

J. Vis. Exp. (172), e62041 (2021). doi:10.3791/62041

7. (Review article) Translational control of *Xenopus* oocyte meiosis: toward the genomic era. Ferdinand Meneau, Aude Dupré, Catherine Jessus, and **Enrico Maria Daldello** * (*** Corresponding author**)
Cells 9(6), 1502 (2020*).* https://doi.org/10.3390/cells9061502

8. Genome-wide analysis reveals a switch in the translational program upon oocyte meiotic resumption.
Xuan G. Luong*, **Enrico Maria Daldello***, Gabriel Rajkovic, Cai-Rong Yang, and Marco Conti. (***Co-first authors**)
Nucleic Acids Research 48(6):3257-3276 (2020). doi: 10.1093/nar/gkaa010.

9. The RNA binding protein DAZL functions as repressor and activator of maternal mRNA translation during oocyte maturation.
Cai-Rong Yang, Gabriel Rajkovic, **Enrico Maria Daldello**, Xuan G. Luong, Jing Chen, and Marco Conti
Nature Communications 1399 (2020). doi : 10.1038/s41467-020-15209-9

10. Cyclin B2 is required for progression through meiosis in mouse oocytes.
Enrico Maria Daldello, Xuan G. Luong, Cai-Rong Yang, Jonathan Kuhn, and Marco Conti.
Development 146, dev172734 (2019). doi: 10.1242/dev.172734

Autres informations:

Etudiants actuellement en thèse ou en M2 dans l'équipe d'accueil. Pour chaque étudiant indiquez le nom du responsable de thèse, l'année du début de la thèse et l'Ecole Doctorale de rattachement

1. Martina Santoni - Responsables : Enrico Daldello and Catherine Jessus, started in 2022, ED515: Complexité du Vivant (Sorbonne Université).
2. M2-student – Clément Barbier. Responsable : Marika Miot, stage M2 2023-2024. M2 Recherche "Reproduction et Développement" Université Paris Cité. Poursuite prévue par l'étudiant : parcours sage-femme.

Etudiants ayant préparé ou soutenu leur thèse ou leur M2 dans l'équipe d'accueil au cours des six dernières années. Pour chaque étudiant indiquez le nom du responsable de l'étudiant, l'année du début de la thèse et de fin de la thèse, l'Ecole Doctorale de rattachement et le devenir de l'étudiant.

1. Ph.D student – Ferdinand Meneau. Responsables : Marika Miot and Catherine Jessus, 2019-2023. ED515: Complexité du vivant (Sorbonne Université). Post-doctorat at University of Edinburgh (UK).
2. M2-student - Nabil Sekhsoukh. Responsable : Enrico Maria Daldello, stage M2 2021-2022. M2 Biochimie et Biologie Moléculaire, Sorbonne Université. CDD d'ingénieur France.
3. M2-student - David Perdigues. Responsable : Marika Miot, stage M2 2018-2019. M2 Recherche "Reproduction et Développement" Université Paris Cité.
4. Ph.D student - Tom Lemonnier. Responsable : Aude Dupré, 2016-2019. ED Complexité du Vivant (Sorbonne Université). Post-doctorat University of Bristol (UK) then Yale University (USA).

Cette proposition de stage s'adresse-t-elle spécifiquement à un étudiant scientifique, médecin ou vétérinaire ou bien est-il ouvert à tous les profils ?

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Étudiant scientifique

Ce sujet peut-il donner lieu à une thèse ?

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