Master Biologie Moléculaire et Cellulaire 'BMC',
Université Paris Cité - *UFR Sciences du Vivant*

Parcours : **Biologie et Développement Cellulaires 'BDC'**
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Fiche de Projet de Stage de M2, 2026-2027

Unité INSERM ou CNRS ou Université : **Institut Jacques Monod** **(UMR 7592 CNRS/Univ. Paris-Cité)** **Intitulé Equipe :** **Membrane trafficking, ubiquitin and signaling.** **ED d'appartenance : BioSPC** **Responsable de l'Equipe : S. Léon**	**Responsable du Stage : Sébastien LEON** **Contacts** Adresse **: Institut Jacques Monod** **15, rue Helene Brion** **75013 Paris** Email : **sebastien.leon@ijm.fr** Tel : **01 57 27 80 57**

Titre du projet : **Mechanism of AMPK inhibition by metabolism through 14-3-3 proteins**

Résumé du Projet de Stage (en 300 mots maximum, mots clés en gras)

Nutrient and energy signals are detected by proteins that bind metabolites and relay this information to signaling pathways. In mammals, the kinase **AMPK** senses energy by detecting AMP/ADP/ATP ratios. Under low energy, AMP binds the γ-subunit of AMPK to activate it, favoring catabolism over anabolism.
Yeast carries mutations in AMPK γ-subunit that render it insensitive to AMP. Instead, its activity depends on glucose availability. How glucose inhibits yeast AMPK remains unresolved. We and others found that 2-deoxyglucose (2DG), a glucose analog that is phosphorylated but not further metabolized, inhibits yeast AMPK like glucose despite a strong ATP depletion [1]. Thus, yeast AMPK senses early glycolytic events rather than energy levels.
We found that 2DG resistance is associated with high AMPK activity [2] and used this as a proxy to identify AMPK regulators. Our recent genetic screen identified many mutations in Reg1, a **PP1 phosphatase** regulatory subunit in charge of AMPK inhibition [3] that we proposed acts as a metabolite sensor. Unpublished 2DG-resistance mutations were found in **14-3-3** proteins, a class of phospho-binding proteins known to bind Reg1. These 14-3-3 mutants still bind typical partners but no longer interact with Reg1, suggesting a **non-canonical interaction** that is critical for PP1 function.
In this project, we want to obtain structural insights into how 14-3-3 proteins interact with Reg1, and how this interaction facilitates PP1 function towards AMPK in a PP1/14-3-3/AMPK megacomplex. This would be a new concept in which 14-3-3 are not only passive « readers » of phosphorylation events, but coordinate phosphatase/kinase regulation. This project will involve various experimental approaches ranging from genetic analysis, biochemistry (signaling, *in vitro* interaction studies), cell biology (subcellular localization), protein purification and structure/function studies. It will benefit from a vibrant scientific environment at the Institut Jacques Monod and dedicated collaborations for structural work.

Publications de l'équipe relatives au projet de stage (max 5)

1. Laussel C, Albanese V, Garcia-Rodriguez FJ, Ballin A, Defenouillere Q, Leon S. 2-deoxyglucose transiently inhibits yeast AMPK signaling and triggers glucose transporter endocytosis, potentiating the drug toxicity. PLoS Genet. 2022;18(8):e1010169.
2. Defenouillere Q, Verraes A, Laussel C, Friedrich A, Schacherer J, Leon S. The induction of HAD-like phosphatases by multiple signaling pathways confers resistance to the metabolic inhibitor 2-deoxyglucose. Sci Signal. 2019;12(597):aaw8000.
3. Ballin A, Albanese V, Miled S, Legros V, Chevreux G, Verraes A, et al. A genetic screen reveals a key role for Reg1 in 2-deoxyglucose sensing and yeast AMPK inhibition. PLoS Genet. 2025;21(10):e1011896.