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, 2022-2023

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 : Véronique Albanèse** **Contacts** Adresse **: Institut Jacques Monod** **15, rue Helene Brion** **75013 Paris** Email : veronique.albanese@ijm.fr Tel : **01 57 27 80 57**

Titre du projet:
Ubiquitination of the endocytic machinery during endocytosis of cargoes

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

Endocytosis is an important cellular process that regulates plasma membrane composition and allows cells to adapt quickly to environmental changes. During endocytosis, various extracellular materials and plasma-membrane- associated proteins such as transporters, receptors, and channels are packed into vesicles that are then pinched off to enter the cytosol. There, they will either be recycled, or targeted to the vacuole (lysosomes) for degradation. Ubiquitination of cargoes with **K63 ubiquitin chains** is a key step of this process and is mediated by the E3 ligase from the Nedd4 famil, Rsp5.

Clathrin-mediated endocytosis (CME) is the best characterized type of endocytosis. It requires more than 50 proteins which concentrate the cargoes in the plasma membrane and form clathrin coated-vesicles. Interestingly, several proteins of the CME have been found to be ubiquitinated by Rsp5 but so far, no role for this ubiquitination has been determined. In this project, we propose to study the function of the ubiquitination of 2 proteins of the CME machinery: the **Eps15 homolog Ede1** and the **amphiphysin Rvs167**. We will use a recently developed technique that allows the ubiquitination on demand of a protein of interest in vivo. Once the ubiquitination of the proteins is triggered, we can analyze their localization in the cell as well as their **dynamics** at the plasma membrane (Kymographs) using live cell high-resolution microscopy (AiryScan, Spinning dic and microfluidics, ImagoSeine @ IJM). We will also study at the same time the **endocytosis of cargoes** (like Can1 or Mup1) and determine if their endocytosis is affected by the ubiquitination of the endocytic machinery. Finally, their physical interactions with other components of the CME will also be assessed by immunoprecipitations (GFP-trap) and mass-spectrometry using the facility located at the institute (ProteoSeine @ IJM).

Publications de l'équipe relatives au projet de stage (max 5) (souligné : étudiants en thèse)

- Renz et al (**2024**) Ubiquiton-An inducible, linkage-specific polyubiquitylation tool, ***Molecular Cell***, doi: 10.1016/j.molcel.2023.11.016.
- Renz et al (**2020**) Ubc13-Mms2 cooperates with a family of RING E3 proteins in budding yeast membrane protein sorting. ***J.Cell Science***, doi: 10.1242/jcs.244566.
- Hovsepian et al (**2018**) The yeast arrestin-related protein Bul1 is a novel actor of glucose-induced endocytosis. ***Mol Biol Cell.*** 29:1012-20. doi: 10.1091/mbc.E17-07-0466.
- Hovsepian et al. (**2017**) Multilevel regulation of an alpha-arrestin by glucose depletion controls hexose transporter endocytosis. ***J Cell Biol*** 216(6):1811-31. doi: 10.1083/jcb.201610094.