Production and characterization of caveosomes

Caveolae are 50-100 nm flask-shaped plasma invaginations present in a range of cell types and especially abundant in endothelial and adipocytes. These cellular compartments are involved in vital cell functions such as endocytosis, signal transduction, lipid homeostasis and protection of cells against mechanical stress. These structures are maintained and stabilized by the presence of a small membrane protein, cavolin-1 (21 kDa), embedded in the cytoplasmic leaflet, and also of a cytoplasmic protein, cavin-1. Caveolin-1 (Cav-1) interacts with both proteins and lipids. Indeed, Cav-1 interacts with cytoplasmic proteins (eNOS, Src kinases Src, G proteins) and also membrane proteins (β-adrenergic receptor, Ras, adiponectin receptor…). Cav-1 also plays a role in cholesterol homoeostasis, an important lipid for the physical properties of the plasma membrane.

In that context, our goal is to describe the molecular basis of the network of interactions involving Cav-1 as these interactions are essential for the understanding of the multifunctional role of this protein.

We have recently shown that the overexpression of a fusion protein with Cav-1 in *E.coli* or insect cells induced the production of small vesicles enriched in Cav-1 and of size similar to that of caveolae. These vesicles called heterologous caveolae or caveosomes are objects of choice to study protein-lipid and protein-protein interactions involving Cav-1. From a more general point of view, results from these studies will provide key to understand the formation of membrane domains and membrane curvature involving caveolin-1.

During the M2 internship, we propose to produce caveosomes and to perform their first characterizations using biochemical and biophysical techniques. Transmission electron microscopy, dynamic light scattering, circular dichroism and infra-red spectroscopy are the main biophysical techniques for first characterizations.

References:

Role of the membrane interface on the conformation of the caveolin scaffolding domain: A CD and NMR study. Lan CL, Neumann JM and Jamin N FEBS Lett. 2006, 580(22):5301-5.

Production dans Escherichia coli de vésicules enrichies en cavéoline-1 (32-178) canine ou son fragment (76-178). Thèse N.Perrot (2015).

Gaibelet G, Tercé F, Allart S, Lebrun C, Coller X, Jamin N, Orlowski S (2017) Fluorescent probes for detecting cholesterol-rich ordered membrane microdomains: entangled relationships between structural analogies in the membrane and functional homologies in the cell. AIMS Biophysics, 4(1):121-151.

The internship will be performed at the Laboratory of Membrane Proteins and Systems at the Institute for Integrative Biology of the Cell, joint research Unit (UMR) 9198, at CEA Saclay.

Applicants are encouraged to contact us before mid-October to start the fellowship application procedure.

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