Proposition de Stage M2, Année 2022-2023

Optogenetic control of Rab-dependent cell dynamics

Rab proteins constitute the largest family of small GTPases, represented by more than 60 members in humans. They are key regulators of intracellular trafficking pathways, controlling vesicle formation, transport, docking and fusion. Moreover, Rab proteins regulate the maturation of membrane domains. Rabs cycle between an inactive, cytosolic and an active, membrane-bound state and in their active form recruit effector proteins for vesicle trafficking and maturation. Yet, the cellular events that follow Rab attachment to membranes are not well understood.

Here we will use novel optogenetic tools to activate Rab proteins at selected membranes in order to study the dynamics of downstream events. We will focus on Rab8, a highly conserved protein found throughout all eukaryotic kingdoms. We have constructed optogenetic tools that allow to activate Rab8 at the plasma membrane at a limited, spatial controlled area (Figure 1). Preliminary results from the lab using these tools indicate that activation of Rab8 at the plasma membrane leads to local retraction of the cell.

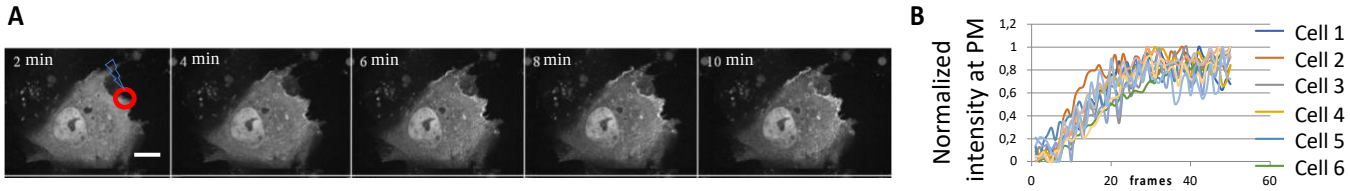

Figure 1: Recruitment of Rabin8, the GEF of Rab8, to the plasma membrane (PM) in living cells. **A**. Fluorescent image frames from a live cell experiment of a RPE1 cell transfected with CRY2-mCherry-Rabin8 (GEF domain) that is recruited to the PM which contains CIBN-CAAX. Dimerization between CRY2-CIBN is initiated by stimulation with blue light at the indicated region (red circle). Scale bar is 10 µm. **B** Quantification of the enrichment of CRY2-mCherry-Rabin8 at the PM after dimerization of CRY2 with CIBN that is found at the PM. Normaized intensity data at PM is shown for 6 independent cells.

The Master 2 project aims at better understanding the role of Rab8 in membrane retraction, testing the recruitment of selected downstream partners, on the one hand, and changes in membrane properties, on the other hand. As Rab8 has been implicated in anterograde transport during protein secretion, we will also test the effect of Rab8 recruitment on cell secretory activity. The student will learn and employ advanced technologies of optogentic activation, quantitative imaging and controlled cell culture on micropatterns. Our work is relevant to understand changes in intracellular trafficking found in cancer cells, a major interest of the Institut Gustave Roussy where the lab is located. The project, which can be extended for a PhD, will thus contribute to our understanding of the molecular mechanisms by which membrane trafficking and maturation is orchestrated by Rab proteins.

Publications

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4. Vaidžiulytė K, Coppey M, **Schauer K**. Intracellular organization in cell polarity - placing organelles into the polarity loop. J Cell Sci. 2019 Dec 13;132(24):jcs230995. Review.
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