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BC-P28
INSULIN REGULATES THE LRP1 TRANSLOCATION TO THE CELL SURFACE IN MÜLLER GLIAL CELLS

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Low-density lipoprotein (LDL) receptor-related protein-1 (LRP1) is an endocytic and signaling receptor expressed in retinal Müller glial cells (MGCs). This receptor regulates the molecular activity of different membrane proteins, included insulin receptor (IR), which is involved with the MGC motility and metabolism. Moreover, insulin increases the LRP1 expression in the cell surface of neurons and hepatic cells, although the intracellular route of this LRP1 sorting in these cells and MGCs is not well established. Hence, in the present work we investigate the insulin-induced LRP1 translocation to the plasma membrane in human retinal MGC-derived cell line, MIO-M1. By electron microscopy we observed that LRP1 is stored in small vesicles (mean diameter range of 100–120 nm), which were positive for sortilin and VAMP2, and also incorporated GLUT4 when it was transiently transfected. Next, by biotin-labeling protein assay we observed that the LRP1 translocation to the plasma membrane was promoted by insulin-regulated exocytosis through intracellular activation of the IR/PI3K/Akt axis and Rab-GTPase proteins, such as Rab8A and Rab10. Moreover, these Rab-GTPases regulated both the constitutive and insulin-induced LRP1 translocation to the plasma membrane. Finally, we found that dominant-negative Rab8A and Rab10 mutants impaired insulin-induced intracellular signaling of the IR/PI3K/Akt axis, suggesting that these GTPase proteins as well as LRP1 cell surface level are involved in insulin-induced IR activation. We propose that insulin-induced LRP1 translocation to the plasma membrane is essential for IR activity, which might be relevant for the function of MGCs during pathological disorders of the retina associated with insulin resistance.

BC-P29
Chlamydia trachomatis PERTURBS ANTIGEN CROSS PRESENTATION BY INTERFERING RAB14-CONTROLLED TRANSPORT

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Chlamydia trachomatis (*Ct*) is the most common sexually transmitted bacterium that replicates inside a vesicle called inclusion. *Ct* manipulates Rab GTPases, master controllers of vesicular transport, to ensure its survival and replication. Dendritic cells (DC) are the most powerful antigen presenting cells and an essential link between innate and adaptive immunity. We have shown that *Ct* intercepts Rab14-vesicles to acquire host lipids necessary for its growth and multiplication. In addition, DC requires Insulin-regulated aminopeptidase (IRAP)-Rab14 endosomes for efficient antigen cross presentation. We hypothesised that *Ct* recruits Rab14 not only as a strategy for nourishment, but also to interfere with antigen presentation. By confocal microscopy, we observed that Rab14 is associated with the plasma membrane at the entry site of the bacterium. Later, Rab14 is recruited to the chlamydial inclusion membrane and remains there throughout the entire bacterial life cycle. Interestingly, we distinguished two populations of chlamydial inclusions in DC. On one hand, we found small *Ct*-containing vesicles that colocalize with EEA1 or LAMP1; and on the other hand, we observed larger ones that recruit Rab14 and exclude markers from the endocytic/degradative pathway. Chlamydial infection did not modify MHC-I expression, shown by western blot. However, infection as well as Rab14 silencing caused a redistribution of MHC-I molecules and interfered with their transport towards the plasma membrane, assessed by flow cytometry. Our results suggest that Rab14 is involved in the decrease of antigen cross presentation observed in Ct-infected cells.

BC-P30
FUNCTIONAL CHARACTERIZATION OF THE HIV-1 VPU ACCESSORY PROTEIN

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The chronic and persistent viral replication that characterizes the Human Immunodeficiency Virus 1 (HIV-1) infection reflects the complex interplay between the host defenses and a number of viral factors whose primary function is to facilitate the evasion of such immune responses. In order to infect a new host, replicate on it for many years and spread to new individuals, HIV-1 should avoid not only the innate defenses, including the so-called “antiviral restriction factors”, but also the humoral and cellular adaptive defenses. To date, many restriction factors that actively act against HIV-1 have been identified, including APOBEC3G, TRIM5alpha, cyclophilin A, BST-2/tetherin, SAMHD1 and SERINC3/5. HIV-1 has evolved a variety of mechanisms to evade these factors, by either acquiring mutations in the viral proteins susceptible to their action or encoding specific proteins that neutralize them. Known as "accessory proteins" (Vif, Vpr, Vpu and Nef), those viral factors act as molecular adapters that connect specific cellular targets with proteolytic or alternative intracellular trafficking pathways. Our lab is currently focusing on Vpu, one of the more attractive HIV-1 proteins from both clinical and pharmacological points of view. Among its many functions, Vpu promotes the downregulation of its specific targets, including the viral receptor CD4 and the restriction factor BST-2/tetherin, through cellular mechanisms that are not fully understood. We sought to study them at a molecular level by creating a complete proteomic profile of cellular proteins that interact specifically with wild-type and well-characterized Vpu mutants. This information allowed us to identify and analyze 1) new Vpu substrates; 2) key cellular proteins important for its multiple actions; and 3) cellular factors that regulate the trafficking pathways followed by Vpu. On the other hand, viruses generally “hijack” specific components of "normal" cellular pathways to facilitate the progression of their infectious cycle. Our analysis could eventually also define the participation of yet unknown factors in the complex cellular mechanisms that Vpu uses to perform each of its specific actions.