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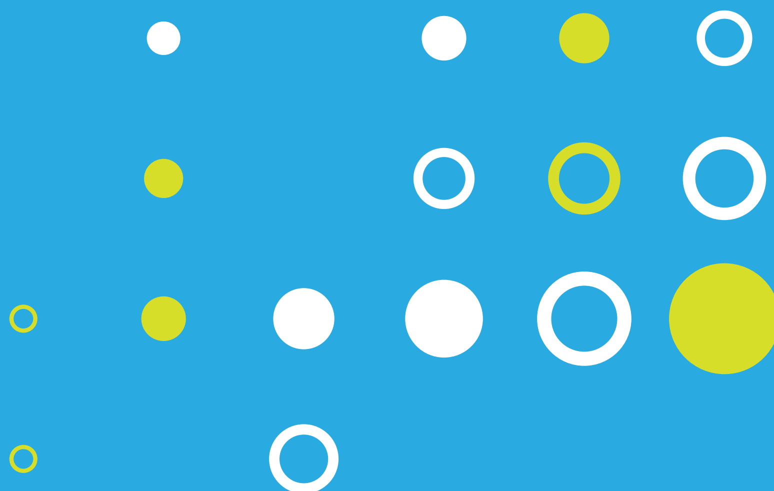

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CB-P25

ROLE OF Rab1b IN COPII DYNAMICS AND FUNCTION

García IA, Martinez HE, Slavi I, Alvarez C
Facultad Cs.Qs. Departamento de Bioquímica Clínica. U.N.C. CIBICI-CONICET. E-mail: agarcia@fcq.unc.edu.ar

In eukaryotic cells, proteins destined to be exported are translocated to the endoplasmic reticulum (ER) and are selectively sorted in specialized sites called "ER exit sites" (ERES). Selection and incorporation of the proteins in ERES are performed by Coat protein complex II (COPII). This coat is assembled by recruitment of Sec23/24 and Sec13/31 by Sar1 GTPase (activated by its GEF, Sec12). A further component, Sec16, acts as a platform for COPII assembly at ERES and it appears to stabilize Sar1-GTP. After vesicle budding, COPII is exchanged by COPI complex, a crucial step for ER-Golgi transport. Rab1b GTPase is essential to recruit COPI.
We have previously shown that Rab1b interacts with the COPII component Sec23. Furthermore, FRAP experiments co-expressing Sec13 and Rab1Q67L indicated that Rab1b activity affects Sec13 membrane association-dissociation kinetics at the ERES. Here we show that *in vivo* Rab1b also interacts with Sec24 and Sec31. In agreement, immunofluorescence assays show that Rab1b colocalizes with these COPII structures. Moreover, Rab1b inhibition delays cargo sorting at the ERES. In contrast with Sec13, Sec16 dynamics is not affected by Rab1Q67L, consistent with the fact that Sec16 acts upstream of Sar1. Our data suggest that Rab1b can interact and modulate dynamics of COPII components acting downstream of Sec16.

CB-P26

ROLE OF Rab1b GTPase IN A THYROID SECRETORY CELL LINE

Martinez HE, García IA, Romero N, Alvarez C
Facultad de Ciencias Químicas. UNC. Dpto Bioquímica Clínica. CIBICI-CONICET. E-mail: hmartinez@fcq.unc.edu.ar

Rab1b GTPase is essential for protein transport between the endoplasmic reticulum (ER) and the Golgi complex. Rab1b is ubiquitously expressed and in some tissues with high secretory activity (like thyroid, placenta and bronchial epithelial cells) Rab1b mRNA levels are significantly augmented. The impact of Rab1b increase in secretory tissues has not been analyzed. In this work we aim to analyze the role of high Rab1b levels in a thyroid secretory cell line (FRTL-5). In these cells, the thyroid-stimulating hormone (TSH or thyrotropin) stimulates synthesis and secretion of the plasma membrane protein sodium iodide symporter (NIS) as well as thyroglobulin (TG). GFP-Rab1bwt construct was transfected in FRTL5 cells incubated with and without TSH and NIS expression was analyzed by immunofluorescence and flow cytometry. Our results show that Rab1b overexpression increase NIS protein levels even in absence of TSH. This data suggest that Rab1b modulates NIS expression in absence of TSH stimulation. Finally, we analyze the impact of Rab1b level changes on regulation of NIS promoter activity by using luciferase reporter assays in FRTL5 cells. Our study indicates that Rab1b modulates NIS expression, suggesting a new Rab1b role in secretory tissues

CB-P27

MOLECULAR CHARACTERIZATION OF THE INTRACELLULAR TRAFFIC OF LRP1 AND ALPHA 2-M/LRP1 COMPLEX

Jaldín Fincati JR, Barcelona PF, Sánchez MC, Chiabrando GA
CIBICI (CONICET). Dpto. Bioq. Clín. Fac.Cs.Químicas., Univ. Nac. Córdoba. E-mail: jfincati@fcq.unc.edu.ar

The LDL receptor-related protein 1 (LRP1) is an endocytic receptor involved in the á2-Macroglubulin (á2M*) internalization. Previously we demonstrated that LRP1 mediated the á2M*-induced intracellular signaling activation. However, the molecular regulation of the LRP1 signaling and endocytosis activity are not well established. In this work we tried to characterize the LRP1 intracellular traffic with Alexa-Fluor á2M* using pull-chase experiments at 37 °C (0 to 60 min) with a previous binding step at 4 °C (30 min). The intracellular localization of Alexa-Fluor á2M* was examined by confocal microscopy using specific fluorescent antibodies against intracellular vesicles. The clatrin-mediated endocytosis of LRP1 and á2M*/LRP1 complex was compared with transferring receptor (TfR), using Alexa-Fluor Tf, and specifically blocked by a negative Eps15 mutant (EÄ95/295). Our data demonstrated that á2M* is clatrin-dependent internalized by LRP1, since it was fully blocked in cells transiently expressing EÄ95/295. Then, we show that LRP1-á2M complex is localized in early endosomes at 10 min of ligand internalization. After this time, Alexa-Fluor á2M* is localized in late endosomes and lysosomes, whereas LRP1 is in recycling endosomes. Our data suggest that the signaling activity of LRP1 induced by á2M* occur in the plasmatic membrane and/or in early endosomes.

CB-P28

CHOLESTEROL LEVELS DETERMINE THE ENDOCYTIC ROUTE FOLLOWED BY THE ACETYLCHOLINE RECEPTOR

Borroni MV, Barrantes FJ
Inst. of Biochem. UNESCO Chair Biophys. & Mol. Neurobiol., B. Blanca. E-mail: rtfjb1@criba.edu.ar

Stability of the nicotinic acetylcholine receptor (AChR) at the cell surface is critical to the correct functioning of the cholinergic synapse. Cholesterol (Chol) is an essential lipid that modulates AChR. We have studied the endocytosis of AChR in CHO-K1/A5 cells, a cell line heterologously expressing murine muscle adult-type receptor under different Chol membrane contents. Contrary to the norm, endocytosis of cell-surface AChR is accelerated by depletion of membrane Chol. This acceleration is no longer operative when membrane Chol levels are restored. We explored the possible mechanism involved in receptor loss in Chol depleted cells (Chol-). Under such conditions the AChR is internalized by a ligand-, clathrin- and dynamin independent mechanism, which does not involve the presence of the AChR-associated protein rapsyn. The small GTPase Rac1 is required: expression of a dominant negative form of Rac1, Rac1N17, abrogates receptor endocytosis. The accelerated internalization of AChR proceeds even upon disruption of the actin cytoskeleton and is furthermore found to require the activity of the small GTPase Arf6 and its effectors Rac1 and phospholipase D. Thus, membrane Chol appears to act as a key homeostatic regulator of cell-surface receptor levels, determining not only the rate but also the mechanism of AChR endocytosis.
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