pholipase C δ1 pleckstrin homology (PH) domain inhibit Ca^{2+}-triggered catecholamines release from permeabilized PC12 cells. Furthermore, functional studies using mutant versions of syt indicate that PIP_2 steering of the cytoplasmic domain of syt toward the plasma membrane plays a role in exocytosis, perhaps by driving the close apposition of the vesicle and target membrane in response to Ca^{2+}. (Supported by NIH grants GM 56827 and MH61876, American Heart Association grant 9750326N. Jihong Bai is supported by an AHA postdoctoral Fellowship.)

44. Regulation of Phosphoinositide 3-kinase Signaling by PI-5-P 4-kinases VALERIE CARRICABURU, DEBORAH SARKES, VANESSA JUNG, and LUCIA E. RAMEH, *Boston Biomedical Research Institute, Watertown, MA*

Phosphatidylinositol-5-phosphate (PI-5-P) is a newly identified phosphoinositide with characteristics of a signaling lipid but with no known cellular function. PI-5-P levels are controlled by the type II PI-5-P 4-kinases (PIP4k II), a family of kinases that converts PI-5-P into PI-4,5-P_2. The PI-5-P pathway is an alternative route for PI-4,5-P_2 synthesis as the bulk of this lipid is generated by the canonical pathway in which phosphatidylinositol-4-phosphate (PI-4-P) is the intermediate. PI-5-P levels in cells dramatically increase following *Shigella flexneri* infection, due to the activity of the virulence factor IpgD, a bacterial phosphatase that dephosphorylates PI-4,5-P_2 to generate PI-5-P (Niebuhr et al. 2002. *EMBO J.* 21:5069–5078). Therefore, IpgD reverts the reaction catalyzed by PIP4k II. We have recently shown that overexpression of PIP4k II reduced PI-3,4,5-P_3 levels in cells stimulated with insulin or cells expressing activated phosphoinositide 3-kinase (PI3k). This reduction in PI-3,4,5-P_3 levels resulted in decreased activation of the downstream protein kinase, Akt/PKB. Conversely, expression of IpgD resulted in Akt activation and this effect was partially reversed by PIP4k II. Since PIP4k II expression did not impair insulin-dependent association of PI3k with IRS1 but abbreviated Akt activation, our data support a model in which the PI-5-P pathway controls insulin signaling that leads to Akt activation by regulating a PI-3,4,5-P_3 phosphatase (Carricaburu et al. 2003. *Proc. Natl. Acad. Sci. USA*.100:9867–9872). To further understand the role of PI-5-P and PIP4k II in growth-factor signaling and in PI-3,4,5-P_3 regulation, we suppressed the expression of PIP4k IIα, IIβ, and/or IIγ in various mammalian cell lines using RNA interference. Consistent with our previous results, we find that suppression of PIP4k II can up-regulate insulin and EGF-induced PI3k signaling that leads to Akt phosphorylation and activation. (Supported by NIH grant DK063219 and US Army, DOD, DAMD 017–01-1-0155.)

45. Class II PI3-kinase C2α Is Essential for ATP-dependent Priming of Neurosecretory Granule Exocytosis FRÉDÉRIC A. MEUNIER,[1] FRANK T. COOKE,[2] SHONA L. OSBORNE,[4] GERALD HAMMOND,[1] JAN DOMIN,[3] PETER J. PARKER,[1] and GIAMPIETRO SCHIAVO,[1] [1]*Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London WC2A 3PX, UK;* [2]*Biochemistry and Molecular Biology, University of College, London, Darwin Building, Gower Street, W1E 6BT, London, UK;* [3]*Renal Section, Faculty of Medicine, Imperial College, London W12 0NN, UK;* [4]*School of Biomedical Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia*

Phosphoinositide-3 kinase (PI3K) activity is important for several aspects of neuronal differentiation and plasticity. However, its direct involvement in regulated exocytosis is unclear, despite clear evidence for a requirement for phosphoinositides in this process. Neurotransmitter release from synaptosomes and hormonal secretion from chromaffin cells is only sensitive to high concentrations of the PI3K inhibitors wortmannin and LY294002 pointing to a possible role for the less sensitive PI3K-C2α. In neurosecretory cells PI3K-C2α was detected on a subpopulation of mature secretory granules, abutting the plasma membrane. Both PI3K-C2α antibodies and PI3K inhibitors selectively prevented ATP-dependent priming in permeabilized chromaffin cells. Transient expression of PI3K-C2α potentiated secretion, whereas its catalytically inactive mutant abolished exocytosis, suggesting a possible role of the main catalytic product of this enzyme, PtdIns-3-phosphate (PtdIns3*P*), in this process. Treatment of PC12 cells expressing the PtdIns3*P*-binding FYVE domain with a low concentration of wortmannin selectively abolished early endosomal staining and revealed a full colocalisation with PI3K-C2α on PC12 granules. Finally, sequestration of PtdIns3*P* by the overexpression of FYVE domain abolished secretion from PC12 cells. Together these data demonstrate that production of PtdIns3*P* along with PtdIns4,5P_2 is required in the acquisition of fusion competence that secretory vesicles undergo during or following docking to the plasma membrane.

46. Dolichol Phosphate Mannose Synthase Dpm1p Recruits the Lipid Phosphatase Sac1p to the Endoplasmic Reticulum FRANK FAULHAMMER, GERLINDE KONRAD, BEN BRANKATSCHK, ANDREAS KNOEDLER