

P002 Role of phosphoinositide 3-kinase C2 α in insulin signalling
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We have recently identified phosphatidylinositol-3-phosphate (PtdIns-3-P) as a new important component in the TC10-dependent pathway, involved in GLUT4 translocation. This formation requires the insulin-dependent activation of TC10 and is relatively resistant to PI 3-K inhibitors. The aim of this work was to determine the enzyme responsible for generation of the insulin-dependent pool of PtdIns-3-P. The resistance to classical PI 3-K inhibitors suggested that the enzyme involved in this process might be the α isoform of class II PI 3-K (PI 3-KC2 α). Here we demonstrate that insulin translocates the endogenous enzyme PI 3-KC2 α to the plasma membrane of L6 cells. Translocation is rapid, being already visible at 1.5 min of stimulation, and transient, disappearing after 15 min of stimulation. Preservation of intact lipid rafts subdomain is necessary for this process. Overexpression of a constitutively active TC10 mutant *per se* activates PI 3-KC2 α whereas overexpression of a dominant negative mutant completely inhibits the insulin-dependent PI 3-KC2 α activation. In addition the isolated C-terminal C2 domain of PI 3-KC2 α translocates to the plasma membrane upon insulin stimulation. These data indicate that insulin generates PtdIns-3-P through a TC10-dependent activation of PI 3-KC2 α .