

P030 Identification of p122RhoGAP as a novel PKB substrate and insulin-stimulated phosphoprotein.
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A number of cellular processes downstream from insulin signalling are dependent upon Protein kinase B (PKB/Akt), including the stimulation of glucose uptake and glycogen synthesis. However, many downstream effectors of PKB remain unknown. We have recently identified a novel PKB substrate, p122RhoGAP, which becomes phosphorylated at Ser³²² in response to insulin stimulation. In primary adipocytes, the PI3-kinase inhibitor, wortmannin, completely blocks phosphorylation of 122RhoGAP in response to insulin stimulation, while the mTor inhibitor, rapamycin, and the MEK inhibitor, U0126, have little effect. In contrast, U0126 partially inhibits phosphorylation in CHO-T cells, while wortmannin appears to have little or no effect. Interestingly, wortmannin and U0126 in combination completely abolish p122RhoGAP phosphorylation, suggesting a potential role as a site of signal integration. Preliminary data indicates that PKB and other AGC-family kinases, including RSK1, directly phosphorylate p122RhoGAP on Ser³²² *in vitro*, further implying that p122 may be involved in signal consolidation. The data presented here indicate that p122RhoGAP is phosphorylated on Ser³²² in response to insulin stimulation, and that the signalling pathways leading to this phosphorylation vary depending on the cell type concerned