

P003 Protein kinases involved in PIKfyve phosphorylation

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One major effect of insulin is the rapid stimulation of glucose uptake into fat and muscle by promoting the translocation of intracellular vesicles containing glucose transporter 4 (GLUT4) to the plasma membrane. It is well established that PI3-kinase is pivotal to these signalling events. Furthermore, protein kinase B (PKB/Akt) has been identified as a downstream target of PI3-kinase, which is required but not sufficient to induce the translocation of GLUT4. Therefore, the PKB substrates that ultimately result in the translocation of GLUT4 need identifying. We have recently reported that PKB phosphorylates serine-318 on the FYVE domain-containing PtdIns(3)P 5-kinase (PIKfyve) leading to stimulation of its PtdIns(3)P-5-kinase activity *in vitro*. Interestingly, even though it has been reported that PIKfyve and GLUT4 show little co-localisation we have identified numerous IRAP labelled GLUT4 vesicles that co-express GFP-PIKfyve, which appear to traffic along microtubules from the cell periphery towards the perinuclear region. Over-expression of a S318A PIKfyve mutant increases insulin-stimulated translocation of IRAP/GLUT4 vesicles suggesting a role for PIKfyve in GLUT4 trafficking. Here we report that PIKfyve is also phosphorylated by other closely related AGC kinases including MAPKAP-K2, p70S6-kinase, and Rsk1 *in vitro* at S318 and other unidentified sites signifying that PIKfyve is an integrator of signal transduction.