

P021 Identification of the APS adapter protein as a novel Protein Kinase B substrate
Katsanakis, KD; Pillay TS
*Institute of Cell Signalling, University of Nottingham
Medical School*

The APS adapter protein is recruited to the autophosphorylated kinase domain of the insulin receptor and functions to recruit CAP and c-Cbl. We have identified APS as a novel substrate for protein kinase B/Akt using a phosphospecific antibody raised against the protein kinase B substrate consensus sequence RXRXX(pS/pT). This phosphorylation of APS is observed in both 3T3-L1 adipocytes and transfected cells. The insulin-stimulated serine phosphorylation of APS was inhibited by a PI3-kinase inhibitor LY290004 and a specific PKB inhibitor, Deguelin. Serine 588 of APS is contained in a protein kinase B consensus sequence for phosphorylation that is conserved in APS across multiple species but is not found in other members of this family including SH2-B and Lnk. Mutation of Serine 588 to Alanine abolished the insulin-stimulated serine phosphorylation of APS and prevented the localisation of APS to membrane ruffles. A GST fusion protein containing amino acids 534–621 of APS was phosphorylated by purified PKB *in vitro* and mutation of Serine 588 abolished the PKB-mediated phosphorylation of APS *in vitro*. This study therefore identifies APS as a novel physiological substrate for PKB as well as the first serine phosphorylation site on APS and indicates the molecular crosstalk between the insulin-activated PI3-kinase dependent and -independent pathways which were previously thought to be distinct and divergent.