

P006 An ankyrin-repeat SOCS box protein controls the signalling complex of the insulin receptor kinase

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APS is the primary adaptor protein coupling the insulin receptor (IR) to CAP and c-Cbl in the phosphatidylinositol 3-kinase-independent pathway of insulin-stimulated glucose transport. Yeast two-hybrid screening of a 3T3-L1 adipocyte library using APS as a bait identified a 418-amino acid ankyrin and SOCS (suppressor of cytokine signalling) box protein Asb6 as an interactor. Although Asb6 is an orphan member of a larger family of Asb proteins ubiquitously expressed, Asb6 appears to be restricted to adipose tissue. Asb6 is expressed in 3T3-L1 adipocytes but not in fibroblasts. In CHO-insulin receptor (CHO-IR) expressing cells myc epitope-tagged APS interacted with FLAG-tagged Asb6 in the presence or absence of insulin stimulation. In 3T3-L1 adipocytes, insulin receptor activation was accompanied by the APS-dependent recruitment of Asb6. Confocal microscopy revealed that Asb6 co-localized with APS in CHO cells and in 3T3-L1 adipocytes. Immunoprecipitation studies showed the Elongin BC complex bound to Asb6, and activation of the insulin receptor was required to facilitate Asb6 recruitment along with Elongins B/C. Prolonged insulin stimulation resulted in APS degradation when Asb6 was co-expressed but not in its absence. Therefore Asb6 functions to regulate components of the IR signalling pathway through degradation, by the APS-dependent recruitment of Asb6 and Elongins BC.