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Funded by: Life Sciences Undergraduate Research Opportunity Program

Overexpression of caveolin-1 results in increased plasma membrane targeting of phosphofructokinase: The structural basis for a membrane associated metabolic compartment

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Previous work from our laboratory has shown that glycolysis and gluconeogenesis occur in separate compartments within the vascular smooth muscle cell (VSM) and that their intermediates do not mix freely in the cytoplasm. We have previously found that the glycolytic -specific enzyme phosphofructokinase (PFK) appeared to colocalize with the fairly ubiquitous plasma membrane protein caveolin -1 (CAV - 1), consistent with a role for CAV - 1 as an anchor for glycolysis to the plasma membrane. We hypothesized that CAV - 1 serves as a scaffolding protein for PFK and may play a role for the organization of cell metabolism. To test this hypothesis , we over - expressed the CAV - 1 gene in cultured A7r5 (rat aorta VSM) cells by transfection with a CAV - 1 coding plasmid. Immunofluorescence and confocal microscopy were used to study the distribution of PFK and CAV - 1 in the transfected cells. Areas of Interest (AOI) were analyzed in a central z - plane across the cell transverse the perinuclear region. To quantify any shift in PFK localization resulting from CAV - 1 over -expression , we calculated a periphery to center (PC) index by taking the average of the two outer AOIs from each membrane region and dividing by the central one or two AOIs. We found that the PC was 2.25 ± 0.85 (mean \pm SEM, N = 5) for transfected cells and was 0.47 ± 0.16 for control cells. These results demonstrate that CAV - 1 creates binding sites for PFK that may be of higher affinity than those localized in the cytoplasm (such as microtubules and the actin cytoskeleton). We therefore conclude that CAV - 1 functions as a scaffolding protein for PFK and that this may contribute to the elucidation of the basis for carbohydrate compartmentation to the plasma membrane in VSM. Support provided by NIH 60668 (to Christopher D. Hardin) and Life Sciences Undergraduate Research Opportunity Program (LS UROP).