MAR05-2004-000288

Abstract for an Invited Paper
for the MAR05 Meeting of
the American Physical Society

Sub-cellular and Multi-cellular Signaling Mechanisms Revealed by Quantitative Laser Microscopies

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Newly developed instrumentation and optical probes allows us to image quantitatively dynamic processes within ever more complicated biological systems. Using methods such as fluorescence recovery after photobleaching (FRAP) and Förster resonance energy transfer (FRET) of GFPs fused to the glucose sensing enzyme glucokinase (GK), we have discovered that the location and activity of beta cell GK is acutely regulated by insulin. These findings provide a mechanism whereby the glucose sensing ability of the beta cell is tightly coupled to insulin signaling. We have also measured pancreatic β-cell metabolism during glucose stimulation by quantitative two-photon NAD(P)H imaging. We have developed methods to delineate quantitatively the NAD(P)H signals from the cytoplasm and mitochondria, and show that the metabolic response of these two compartments are differentially stimulated by glucose and other metabolites. Absolute levels of NAD(P)H were determined using two-photon excited fluorescence lifetime imaging (FLIM). These findings elucidate the relative contributions of glycolytic and citric acid cycle metabolism in normal and diabetic cells.