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Testing a Computational Model of Pancreatic Beta-Cell Oscillations Using Live-Cell Imaging of Islet Oscillatory Behavior

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Befitting their role as a metabolic sensor for the organism, pancreatic beta-cells adjust their metabolic output in accordance with plasma glucose concentrations, and not just their own energy requirements, in order to secrete insulin appropriately on a minute to minute basis. Glucose sensing lies in the commitment to early glycolysis, at the level of glucokinase, which is rate-limiting until phosphofructokinase-1 (PFK1) in turn fully commits substrate to allow glycolysis to proceed. As the archetypical metabolic pathway of mammalian cells, these initial steps of glycolysis have been well-characterized biochemically, which has lead to the proposal that PFK1 activity is oscillatory in beta-cells, and governs downstream oscillations in metabolism (mitochondrial NADH and O_2, ATP/ADP), electrical activity (K_{ATP} and Ca^{2+} channel activity), and finally, pulsatile insulin release [1].

Based on this hypothesis we have developed a computational model of the beta-cell, termed the 'Dual Oscillator Model' (DOM) (Fig. 1), in which slow oscillations in insulin secretion reflect slow oscillations in glycolytic PFK1 activity, which then interact with fast oscillations arising from membrane electrical activity and Ca^{2+} [2]. Here, we have compared specific glycolytic behaviors in the DOM with timelapse imaging of islet oscillatory behavior using adenovirally-delivered mutants of the glycolytic regulatory enzyme PFK2/FBPase2 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase) (Fig. 2). This bifunctional enzyme is uniquely positioned to alter flux through PFK1 in two complementary ways. First, PFK2/FBPase2 is the sole catalyst for the production and degradation of Fru2,6-BP, which allosterically activates PFK1 to a greater extent than its own product, Fru1,6-BP. Second, PFK2/FBPase2 has been proposed to bind and directly activate glucokinase, which is rate-limiting for glycolysis and which controls the flux of Fru6-P substrate to PFK1. Thus, in addition to studying the role of this glycolytic modulator as it affects the dynamic oscillatory process in living islets, our interest in the manipulation of PFK2/FBPase2 activity was to test whether the interplay between glucokinase and PFK1, the rate-limiting steps in glycolysis, affect beta-cell oscillations.

Islet Ca^{2+} oscillations induced by 11.1 mM glucose had reduced period and amplitude after PFK2 overexpression compared to controls, and increased period and amplitude after FBPase2 expression was selectively increased (Fig. 2). Measurements of islet NAD(P)H oscillations were confirmatory. These effects are consistent with our central hypothesis that slow [Ca^{2+}] oscillations are driven by PFK1-mediated oscillations in glycolysis, as shown using an appropriately modified version of the DOM. Additionally, we confirmed that PFK2/FBPase2 physically interacts with glucokinase using a live-cell FRET assay, although the level of FRET observed indicates this interaction is weak or transient. Accordingly, knockdown of endogenous PFK-2/FBPase-2 increased the oscillatory period, consistent with a reduction in Fru2,6-BP levels but inconsistent with a strong effect of PFK2/FBPase2 to activate glucokinase, since the acute activation of glucokinase with RO-0281675 increased the period of islet Ca^{2+} oscillations in 11.1 mM glucose. A parsimonious interpretation of these data is that the main effects of PFK2/FBPase2 on oscillatory activity are via

Fru2,6-BP feedback on PFK1. These results support the predictions of the DOM and the hypothesis that PFK1 mediates slow islet oscillations [3].

References

[1] K. Tornheim, *Diabetes,* 46 (1997) 1375.
[2] R. Bertram et al., *Am J Physiol Endocrinol Metabolism,* 293 (2007) 890.
[3] This work was supported by F32DK085960 (M.J.M.), NSF-DMS0917664 (R.B.), the NIH/NIDDK Intramural Research Program (A.S.), and R01DK46409 (L.S.).

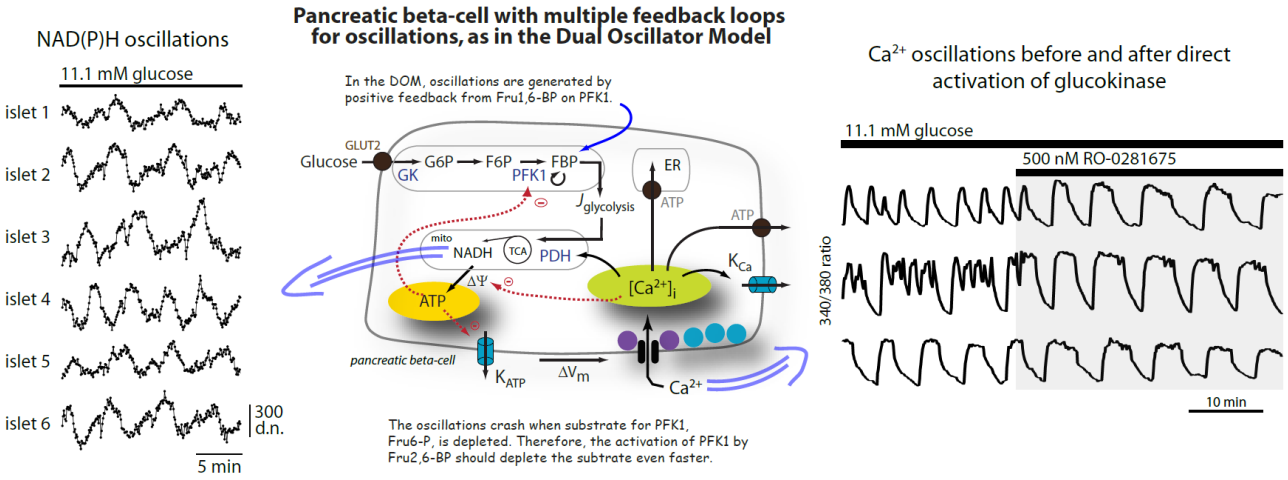

FIG. 1. Insulin release from pancreatic beta cells is pulsatile, but the source of the oscillations remains unclear. In the Dual Oscillator Model (schematic, *middle*), oscillations in NAD(P)H (*left*) and $[Ca^{2+}]_i$ (*right*) are driven by phosphofructokinase-1 (PFK1), and rate limited by glucokinase (GK).

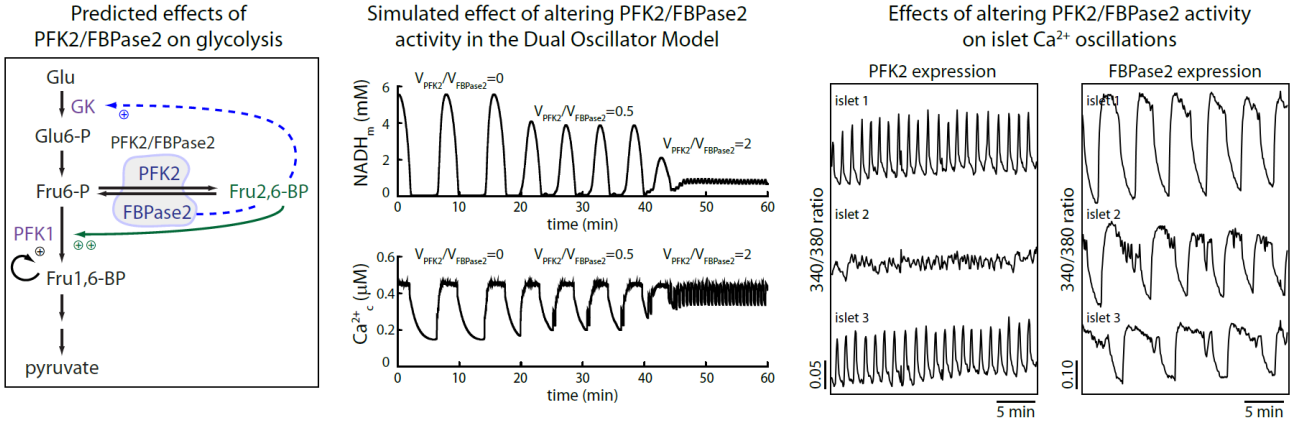

FIG. 2. PFK2/FBPase2 is predicted to increase PFK1 activity by increasing [Fru2,6-BP] and activating glucokinase (*left*). In the Dual Oscillator Model, increasing the forward rate of the PFK2/FBPase2 reaction makes oscillations in NAD(P)H and $[Ca^{2+}]$ faster and smaller, or terminates the oscillations altogether (*middle*). When PFK2 levels were increased in islets, oscillations became faster and smaller, while increasing FBPase2 levels had the opposite effect (*right*). These observations support the DOM and, indirectly, the hypothesis of PFK1-driven oscillations.

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