PKA is a control node in a Ca^{2+}-dependent oscillatory circuit in pancreatic beta cells

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***Short Abstract* — Protein kinases play significant roles in sensing extracellular information and transducing them in a variety of signaling cascades. While protein kinases have been largely viewed as simply relaying or amplifying the input signal, they could work with other molecules in a concerted fashion to compute appropriate responses. In this study we showed that PKA activity is critical for the Ca^{2+} dependent functionality of insulin secreting pancreatic beta cells. Mathematical modeling in conjunction with experiments indicated that oscillatory acitivity of PKA in these cells can control the specificity and spatio-temporal control of regulation of integrated voltage- Ca^{2+}-PKA circuit in these cells.**

***Keywords* — PKA, oscillations, FRET, Calcium, feedback, pancreatic beta cells.**

I. Introduction

The intricate interplay between different components of a signaling cascade determines the capacity of the system to process the biological information it receives and respond in a context dependent manner. This imparts to the system the ability to effect multitudinous responses often *via* oscillatory signals or switches. A primary example of this is the occurrence of Ca^{2+} oscillations in pancreatic beta cells which is believed to be linked to the pulsatile secretion of insulin, the impairment of which has been implicated in pathologies of type II diabetes [1].

Protein kinases constitute important nodes of signaling circuits integrating multiple inputs owing to their ability to regulate and in turn be regulated by other molecules. For instance, the biphasic insulin secretion response in pancreatic beta cells is thought to be modulated by PKA mediated amplification of primary signals generated through fuel metabolism, whereas Ca^{2+} alone is unable to evoke such responses under low glucose conditions [2]. It is still unclear how PKA decodes and modulates the Ca^{2+} signals to effect such responses.

In this study, theoretical predictions based on simulations of an ODE model in conjunction with experimental validation indicate that PKA mediated feedback is critical in defining the signal properties of this circuit.

II. Results

Fura-2 imaging of Ca^{2+} was used in conjunction with genetically encoded FRET based biosensors to monitor cAMP and PKA responses in insulin-secreting MIN6 cells. Theoretical predictions were based on an ODE system comprising a core oscillatory circuit involving Ca^{2+} and membrane potential, and an auxiliary negative feedback loop consisting of Ca^{2+}, cAMP and PKA.

Co-imaging results revealed the occurrence of synchronized and interdependent oscillations of Ca^{2+}, cAMP and PKA upon depolarization of the membrane, indicating the existence of a PKA-mediated feedback.

Simulations of the model with simultaneous variation of parameters that affect PKA-mediated feedback indicated that PKA can modulate the frequency of oscillations as was also experimentally verified. Enhancement and inhibition of PKA activity directly or indirectly using pharmacological agents resulted in increase and decrease in frequency of oscillations, respectively. Complete inhibition of PKA abolished the circuit activity.

Modeling and experimental results also suggested that PKA is capable of integrating various external inputs into the system. Direct activation of PKA using cAMP analogs was observed to induce oscillations in the absence of any membrane depolarizing stimuli.

Finally, our results indicated that this oscillatory circuit is likely important for bestowing spatiotemporal properties on PKA, with increasing frequency of PKA oscillations progressively leading to switching from local PKA activity to nuclear translocation and gene regulation.

III. Conclusion

In toto, the combined theoretical and experimental results indicated that Ca^{2+}, cAMP and PKA form a tightly integrated oscillatory circuit. Ca^{2+} oscillations and hence, the functionality of the pancreatic beta cell are likely influenced by PKA mediated frequency modulation.

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