Mathematical Modelling Reveals Missing Mechanism in AKT Activation

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***Short Abstract* — AKT is a central regulator of growth and is important in the transmission of the insulin signal to a range of biological processes such as protein synthesis and glucose uptake in fat and muscle cells. AKT does not phosphorylate its substrates uniformly as they exhibit different temporal profiles in terms of shapes and speeds. Additionally, the phosphorylation kinetics of AKT itself, which is commonly used as a marker for its activity, does not match the phosphorylation kinetics of its substrates. Mathematical modelling revealed that variations in substrate phosphorylation speeds are in sufficient to explain the mismatch. This suggests that there is some missing mechanism of AKT activation that requires further investigation.**

***Keywords* — Insulin Signalling, AKT, Systems Biology, Kinetic Modelling.**

I. AKT AS A KEY HUB OF SIGNALLING

AKT is a key component of insulin signaling that potentiates many downstream processes such as glucose uptake, protein synthesis, lipid synthesis as well as inhibiting glycogen metabolism [1]. After insulin triggered translocation of AKT to the plasma membrane, it is phosphorylated at its T308 residue by PDK1 and S473 residue by mTORC2 [2], [3]. These result in activation of AKT, enabling it to phosphorylate its target substrates. In this work, we explore the relationship between AKT phosphorylation and AKT activation and hypothesise that phosphorylation order and kinetics are the determining factors of activity.

II. RESULTS

Insulin signaling was explored in the 3T3-L1 cell model. Initial results suggested that T308 and S473 kinetics are very similar. At maximal doses of insulin, the AKT phosphorylation and AKT substrate phosphorylation, such as AS160 at the T642 residue and GSK3β at the S9 residue, have matching temporal patterns. However, at a submaximal dose, they are very different. This is because at 1 nM insulin stimulation, AKT phosphorylation features overshoot behavior, peaking at 2 minutes then reducing. Its substrates, however, peak at 5 minutes then sustain their response.

Through simulation of described system using our newly developed dQSSA model, we found that AKT activity cannot be directly related to T308 or S473 phosphorylation [4]. It showed that substrate phosphorylation was not slow enough for them to be insensitive to the overshoot in AKT activation.

Since AKT phosphorylation is not directly linked to substrate phosphorylation, we are now determining if AKT phosphorylation relates directly to AKT activity itself using an *in vitro* kinase assay.

Given that singly phosphorylated AKT possesses some kinase activity, we will test to see whether this contributes to the disconnect between AKT and substrate phosphorylation. We will separate singly phosphorylated AKT from doubly phosphorylation AKT by finding the perturbation in S473 and T308 phosphorylation time profiles from PDK1 and Sin1 (an mTORC2 component) knockout experiments, respectively, then analyzing them using mathematical modelling. These can then be used to determine the link between AKT activity and the pools of AKT phosphorylation.

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