Competing Positive and Negative Feedbacks Mediated by PH Domain Ligand Interactions Regulate Itk Kinase Activation Kinetics in T Cells

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***Short Abstract* — Inositol phosphate second messengers can regulate interactions between receptor signaling and lipid metabolic networks, critically affecting cell decision processes. However, the molecular mechanisms underlying such cross-regulation are poorly understood. Pairing mathematical modeling and experiments, we elucidate these mechanisms in thymocyte activation which is carefully controlled by TCR induced production of the membrane lipid PIP_3, soluble IP_4, and activation of the kinase Itk. By combining experimentally measured kinetics of Itk mediated PLCγ1 phosphorylation with a Maximum Entropy(MaxEnt) based computational approach, we show that models containing dueling IP_4 mediated feedbacks and oligomeric Itk PH domains are the most robust models.**

***Keywords* — T cell signaling, Maximum Entropy, Robustness.**

I. INTRODUCTION

Hydrolysis of plasma membrane phospholipids can generate various cellular messenger molecules[1]. Among these, inositol phosphates (IPs) can serve as important soluble small molecule mediators of interactions between receptor signaling and lipid metabolism(1-3). In order to promote our mechanistic understanding of the role of IPs in regulating cell decision processes, we used an approach combining computational modeling and experiments to investigate signaling kinetics in developing T cells or thymocytes in the thymus. T cells are key mediators of adaptive immune responses in jawed vertebrates. Recent experiments [2-3] have revealed an essential role for inositol(1,3,4,5) tetrakisphosphate (IP_4) in regulating T cell development.

However, the molecular interactions between Itk, PIP_3 and IP_4 that regulate generation of stable PIP_3 bound Itk complexes and consequently PLCγ and MAPK cascade activation are not well understood[2-3]. This is due to difficulties in probing these interactions *in vivo*. Therefore, based on published experiments, it is possible to construct multiple models in which PIP_3 and IP_4 can associate with Itk in thymocytes. Depending on the presence or absence of IP_4 mediated positive feedback interactions involving monomeric or dimeric Itk molecules[6], we constructed six different possible models. Each model investigated functional roles of the IP_4 mediated feedbacks and dimerization of Itk molecules in producing appropriate Itk activation kinetics. We studied the kinetics of binding of Itk to PIP_3 in all the models using deterministic mass-action kinetic rate equations described by ordinary differential equations (ODEs).

We used a Maximum Entropy (MaxEnt)[4-6] based approach to quantify robustness of each of the six models against variations in rate constants and protein expression levels at the single cell level. Each model was constrained to reproduce the transient PLC-γ1 activation kinetics of an entire cell population as measured in mouse $CD4^+CD8^+$ double-positive (DP) thymocytes stimulated by peptides of different affinities or different doses of CD3 antibodies.

II. CONCLUSION

We show that those models involving dimeric Itk molecules with IP_4 mediated competing positive and negative feedbacks are the most robust models. Actual signaling kinetics in thymocytes are likely to be robust against such variations. Thus, our results support a cooperative-allosteric mechanism for IP_4 control of Itk recruitment to PIP_3 in thymocytes. Our MaxEnt based method for quantifying robustness suggests a way to identify the most robust model(s) in cell signaling systems.

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