Bang-bang control of T-cell receptor signaling

Lily A. Chylek[1,2]*, Vyacheslav Akimov[3]*, Joern Dengjel[4]*, Kristoffer T.G. Rigbolt[4], Bin Hu[5], William S. Hlavacek[2,5], Blagoy Blagoev[3]

***Short Abstract* — The T-cell receptor transmits signals that can lead to T cell activation. How does signaling begin? To answer this question, we coupled quantitative, multiplexed measurements of phosphorylation dynamics to a model of chemical kinetics. Results indicate that T cell responses use bang-bang control, fast activation followed by fast inhibition, through at least two mechanisms: activation of the actin regulator WASP via a shortcut pathway, and transient positive feedbacks mediated by phosphatases, including SHP-1. Model-predicted effects of RNAi-mediated knockdowns were confirmed experimentally, revealing novel interactions and dependencies that regulate the earliest stages of T cell activation.**

***Keywords* — Cell signaling, immune signaling, T-cell receptor, proteomics, kinetic modeling, kinases, phosphatases**

I. Background & Significance

Protein phosphorylation is a driving force in cell signaling, but its dynamics are difficult to characterize with precision. It takes place rapidly, on the timescale of seconds [1], which can confound efforts to decode the order in which events occur. In addition, multiple residues in a protein may be phosphorylated, each involved in distinct regulatory mechanisms, necessitating analysis of individual sites [2]. To characterize the dynamics of site-specific phosphorylation in T-cell receptor (TCR) signaling at unprecedented time resolution, we stimulated cells for precise lengths of time using an automated quench-flow system, and quantified global changes in phosphorylation using mass spectrometry-based phosphoproteomics. We developed a computational model that reproduces experimental measurements and provides new insights into the initiation of TCR signaling.

II. Results

Regulated changes in phosphorylation occurred as early as 5 seconds after stimulation. By 60 seconds, phosphorylation and/or dephosphorylation of 238 sites were reproducibly detected in three experiments. Many of these sites are known players in TCR signaling. To investigate underlying mechanisms, we developed a model of TCR signaling using a rule-based approach, which is well-suited for modeling dynamics of individual sites [3]. Our model reproduces phosphorylation dynamics of 12 key residues and captures interactions among 16 proteins, incorporating both novel and established pathways of TCR signaling. We used our model to predict the effect of perturbations. These predictions are non-trivial, robust to variation in parameter values, and supported by experimental tests.

A. Phosphatases mediate positive feedbacks

Our data indicated that upon stimulation, multiple negative regulatory sites underwent rapid dephosphorylation as the phosphatase SHP-1 was activated. Our model predicted that loss of SHP-1 would enhance phosphorylation of these sites while attenuating phosphorylation of other specific signaling proteins, and these predictions were confirmed in knockdown experiments. These results indicate that SHP-1, a negative regulator of later signaling [4], has a positive role in early signaling.

B. WASP is recruited through a shortcut pathway

The fine time resolution of measurements allowed the order of events to be determined. One of the earliest events detected was phosphorylation of the actin regulator WASP, which occurred before other phosphorylation events that are considered prerequisite for WASP recruitment [5]. Our model predicted that a shortcut pathway activates WASP in the first minute of signaling. This prediction was supported by experiments in which a central protein in the longer, canonical pathway was knocked down. Furthermore, the shortcut appears to be progressively deactivated as components of the long pathway are assembled, representing another example of transient positive regulation. The redundancy of short and long pathways may have implications for the sensitivity of TCR signaling.

III. Conclusion

Through iterative experimentation and model-based analysis, we have uncovered mechanisms of TCR signaling that have been systematically overlooked in the past due to the rapidity with which they occur. Our results indicate that positive regulation can be quickly transformed into negative regulation, providing bang-bang control of T-cell receptor signaling.

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[1]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA

[2]Theoretical Division and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

[3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

[4]Freiburg Institute for Advanced Studies, University of Freiburg, Freiburg, Germany

[5]Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

* These authors contributed equally.