506b Experimentation, Modeling and Parameter Sensitivity Analysis Suggest a Role of Erk in Receptor down-Modulation during T Cell Signaling

Yanan Zheng, Marietta Harrison, and Ann Rundell

The regulation of the antigen-activated T cell receptor (TCR) signaling pathways serves a critical role in the immune response. The T cell antigen-activated Erk-MAPK signaling pathway is known to possess a feedback loop from activated Erk to early signaling events through Lck; however, the nature of this feedback is uncertain and controversial. In this study, laboratory experiments, mathematical modeling and simulation efforts are integrated to determine the nature and scope of the Erk feedback modulation of the T cell antigen-activated Erk-MAPK signaling pathway. A comparison between quantitative measurements of the time-dependent phosphorylation of signaling intermediates upon TCR stimulation in the presence and absence of a kinase inhibitor confirm both positive and negative roles of the Erk feedback. This data is used to evaluate a nonlinear ordinary differential equation model reflecting the current understanding of this pathway including the known feedback from Erk to Lck. The failure of the model to capture the observed signaling dynamics indicates the presence of an additional Erk feedback. To explore potential additional Erk feedback mechanisms, different hypotheses are generated and evaluated by fitting revised models to the experimental data. A stepwise parameter identification strategy utilizes a genetic algorithm-based optimization routine from which parameter sets are preserved when a simulation exhibits a reasonable fit to experimental measurements. The distribution of these acceptable parameter values is used to quantify parameter sensitivities over the entire uncertain parameter space. The results of this study suggest that in addition to the known Erk feedback to Lck, Erk also plays a role in mediating the receptor internalization and degradation. Changes in the TCR surface expression upon T cell stimulation are experimentally quantified using flow cytometry to validate this proposed hypothesis. These experiments confirm TCR down-regulation upon stimulation and support the proposed facilitating role of Erk in this process.