Probabilistic Modeling & Analysis of Switch-Like Signal Transduction Systems

Marc R. Birtwistle[1], Boris N. Kholodenko[2], Anatoly Kiyatkin[2], Jan B. Hoek[2], and Babatunde A. Ogunnaike[1]

***Short Abstract* —In this work we investigate the switch-like behavior of Epidermal Growth Factor (EGF)-induced ERK1/2 activation in HEK293 cells using flow cytometry measurements and probability models of single-cell response distributions. Our theoretical analyses of the experimental data show that the ERK1/2 response distributions are well characterized by a convoluted Gamma-Gaussian distribution; and that the response of ERK1/2 appears analog at short times (2-5 minutes) and digital (switch-like) at longer times (10-30 minutes), implying perhaps that EGF causes dynamic remodeling of the ERK1/2 module.**

I. INTRODUCTION

Cell fate decisions are thought to rely in general on signal transduction systems exhibiting such switch-like input-output behavior as bistability/hysteresis [1] and ultrasensitivity [2], owing to their ability to transform an analog input into a "yes" or "no" output. It has indeed been shown that bistable activation of cyclin dependent kinases drives cell cycle transition decisions [3-5]. Theoretical studies have shown that these bistable and ultrasensitive systems may exist in signaling networks containing positive or double negative feedback motifs [1], saturated enzymes [6], and multi-site modification cycles [7]. However, not all networks that contain such motifs will exhibit switch-like behavior; it is the cell's precise tuning of quantitative, spatiotemporal aspects of the network that allow such digital behavior. Further, not all types of quantitative experimental techniques can capture switch-like behavior; single-cell measurements are needed [1]. In the current work, we develop probabilistic models to analyze such single-cell response data.

II. SUMMARY OF RESULTS

We stimulated HEK293 cells with steps of EGF concentration ranging from 0.01 to 10 nM and observed the dynamic response of ERK1/2 activation over 30 minutes. The cell population responses show bimodality in ERK1/2 activation, with one cell sub-population remaining at basal ERK activation levels ("ERK off") and one sub-population showing high ERK activation ("ERK on"). These results are indicative of a digital rather than analog system, since an analog system should show a single cell population whose mean increases smoothly with dose. To support these observations concretely we fitted the experimental data to fundamentally-motivated probabilistic models whose parameters provide reliable quantitative measures of system responses. In particular, we considered the probability model estimates of the mean of the "ERK on" population: for a digital system, this parameter should remain constant with increasing dose, while for an analog system it should increase with increasing dose. We found that at short times (<5 min.) the ERK response appears analog, while at long times (>5 min.), the ERK response is digital. Since the ERK module should reach a quasi-steady state on the time scale of the observed EGF-mediated effects, these results suggest that EGF mediates the dynamic remodeling of the ERK module steady-state properties from analog at short times to digital at long times.

III. CONCLUSIONS

In this work we develop models and methods to analyze single-cell measurements relevant to switch-like signal transduction systems, and show how these methods can be applied to gain understanding of signal transduction at the single cell level. In particular we find that the response of ERK1/2 to EGF in HEK293 cells appears to be analog at short times but digital at long times, implying that EGF induces remodeling of the ERK module steady-state properties.

[1]Department of Chemical Engineering, University of Delaware. Newark, DE, USA. Corresponding E-mail: ogunnaike@udel.edu

[2]Department of Anatomy, Pathology, and Cell Biology, Thomas Jefferson University. Philadelphia, PA, USA. Corresponding E-mail: boris.kholodenko@jefferson.edu

REFERENCES

[1] Ferrell, J.E., Jr. (2002) Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr Opin Cell Biol*, **14**, 140-148.

[2] Goldbeter, A. and Koshland, D.E., Jr. (1984) Ultrasensitivity in biochemical systems controlled by covalent modification. Interplay between zero-order and multistep effects. *J Biol Chem*, **259**, 14441-14447Last AB (1964)

[3] Sha, W., Moore, J., Chen, K., Lassaletta, A.D., Yi, C.S., Tyson, J.J. and Sible, J.C. (2003) Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts. *Proc Natl Acad Sci U S A*, **100**, 975-980.

[4] Tyson, J.J., Csikasz-Nagy, A. and Novak, B. (2002) The dynamics of cell cycle regulation. *Bioessays*, **24**, 1095-1109.

[5] Pomerening, J.R., Sontag, E.D. and Ferrell, J.E., Jr. (2003) Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nat Cell Biol*, **5**, 346-351.

[6] Goldbeter, A. and Koshland, D.E., Jr. (1981) An amplified sensitivity arising from covalent modification in biological systems. *Proc Natl Acad Sci U S A*, **78**, 6840-6844.

[7] Markevich, N.I., Hoek, J.B. and Kholodenko, B.N. (2004) Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J Cell Biol*, **164**, 353-359.