# Dynamic Regulation of Growth Factor Signaling Networks

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***Short Abstract* — Cell responses are actuated by tightly controlled signal transduction pathways. Although the concept of an integrated signaling network replete with inter-pathway crosstalk and feedback regulation is broadly appreciated, kinetic data of the type needed to characterize such interactions in conjunction with mathematical models are lacking. In mammalian cells, the Ras/ERK pathway controls cell proliferation and other responses stimulated by growth factors, and several crosstalk and feedback mechanisms affecting its activation have been identified. In this work, we take a systematic, data-driven approach to parse the magnitudes of multiple regulatory mechanisms that attenuate ERK activation through canonical (Ras-dependent) and non-canonical (PI3K-dependent) pathways.**

The signaling pathway concept provides a useful framework for understanding information flow as an ordered series of activation steps, exemplified by the Ras → Raf → MEK → ERK pathway and other mitogen-activated protein kinase (MAPK) cascades, which control diverse cell responses. Our current understanding of signal transduction, however, encompasses the concept of signaling networks, in which the canonical pathways interact with and thus affect one another (crosstalk); the sequential pathway concept is further challenged by the regulation of signaling through negative feedback and reinforcement of signaling through positive feedback. These complexities of signaling networks have proven difficult to characterize, and most of the data that has accumulated about such mechanisms are qualitative in nature. Although kinetic models of signal transduction processes have steadily appeared over the past decade, and recently published models of the epidermal growth factor (EGF) receptor system in particular have been more tightly integrated with biochemical data to establish quantitative features of signaling networks [1, 2], a more comprehensive data acquisition effort is needed to better constrain models at the network scale of complexity.

We previously conducted a quantitative analysis of crosstalk in the platelet-derived growth factor (PDGF) receptor network [3]. The major signaling modes mediated by PDGF receptors are the phosphoinositide 3-kinase (PI3K) pathway and the aforementioned Ras/ERK pathway, which are most closely associated with chemotaxis and cell proliferation, respectively. Through measurements of PDGF-stimulated signaling in mouse fibroblasts, systematically covering a diverse array of stimulation and molecular perturbation conditions and building upon other quantitative studies [4-6], we showed that PDGF-stimulated ERK activation requires signaling through either of two, more or less independent pathways: the canonical, Ras-dependent pathway or PI3K-dependent crosstalk. Through quantitative analysis of a coarse-grained kinetic model, we estimated that the magnitudes of the Ras- and PI3K-dependent contributions to MEK/ERK activation are comparable, with a moderately higher contribution from PI3K-dependent crosstalk (~1.6:1 ratio) [3].

We have since refined our kinetic model and acquired additional data in order to quantify negative regulation of ERK signaling through three feedback loops: 1) desensitization of Ras activation, 2) desensitization of MEK phosphorylation, and 3) transcriptional up-regulation of dual specificity ERK phosphatases. Our analysis shows that the second of these is in fact the dominant layer of ERK self-regulation in our cells; the theoretical implications of such a feedback structure are well understood [7]. Support for the refined mathematical model, trained by alignment to the superset of old and new data, is demonstrated through its ability to quantitatively predict the enhancement of PDGF-stimulated MEK phosphorylation in cells with both ERK1 and ERK2 expression knocked down. A more surprising model prediction, also confirmed experimentally, is a lack of effect of MKP3/DUSP6 knockdown on ERK phosphorylation. We thus demonstrate that models of signaling networks, trained on a sufficient diversity of quantitative data, can be reasonably comprehensive, accurate, and predictive in the dynamical sense.

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