

Why is ERK phosphorylation transient?

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The extracellular signal-regulated kinase (ERK) signaling pathway controls gene expression governing normal and aberrant cell proliferation and differentiation responses in metazoans. Two hallmarks of ERK pathway dynamics are adaptation of MEK and ERK phosphorylation, which has been linked to ERK-dependent negative feedback, and nucleocytoplasmic shuttling, which allows active ERK to phosphorylate a host of protein substrates that reside in the nucleus and cytosol. To integrate these complex features of ERK signaling, we acquired quantitative biochemical and live-cell microscopy data to reconcile the phosphorylation, localization, and activity states of ERK. We show that while maximal growth factor stimulation elicits transient ERK phosphorylation and nuclear translocation responses, ERK activities available to phosphorylate substrates in the cytosol and nuclei show relatively little adaptation. Free ERK activity in the nucleus temporally lags the peak in nuclear translocation measured in the same cell, indicating a slow process affecting the nuclear pool of ERK. Additional experiments, guided by kinetic modeling, show that this slow process is consistent with ERK's modification of and release from nuclear substrates.

Keywords — signal transduction, MAPK cascade, kinetic modeling, receptor tyrosine kinase.

I. INTRODUCTION

The linear, sequential simplicity of the Raf \rightarrow MEK \rightarrow ERK protein kinase cascade belies a rich complexity in the regulation of ERK signaling. One important regulatory mode is negative feedback, which desensitizes upstream components. This seems to explain why ERK activation tends to be transient, as explored in recent quantitative studies [1-3]. Another complex facet of ERK1/2 regulation is its localization. Active ERK phosphorylates >150 protein substrates in the cytosol and nucleus, and thus nucleocytoplasmic shuttling is an important determinant of ERK function. In live-cell microscopy experiments, nuclear localization of ERK typically exhibits a transient peak or oscillations with time [4-6]. Does nuclear translocation of ERK simply track its phosphorylation and activity?

II. RESULTS

In this study, we combine single-cell ERK localization and activity measurements to demonstrate that this assumption is not generally justified. In fibroblasts stimulated with a high dose of PDGF, ERK1/2 nuclear translocation exhibits the typical transient kinetics, whereas neither active ERK in the cytosol nor in the nucleus show

dramatic adaptation. Strikingly, accumulation of free, active ERK in the nucleus lags its overall nuclear translocation. Together with biochemical measurements, these data are reconciled by a kinetic model accounting for interactions between ERK and its substrates in the cytosol and nucleus. The interpretation is that substrate phosphorylation by ERK, and not feedback adaptation, accounts for the dramatic overshoot of ERK phosphorylation and nuclear localization. Qualitative predictions of pathway dynamics under a range of stimulation conditions were experimentally confirmed.

III. CONCLUSION

Our analysis of ERK dynamics encompasses negative feedback, nucleocytoplasmic shuttling, and substrate interactions to more fully reconcile diverse biochemical and live-cell microscopy data addressing phosphorylation, localization, and activity states of ERK1/2. Surprisingly, we found that negative feedback is not necessary to explain the apparent transience of ERK phosphorylation and nuclear localization responses, though it is unquestionably important. Rather, considering the known competitive interactions of di-phosphorylated ERK with substrates and phosphatases [7,8], the interpretation is that transient ERK dynamics are a consequence of relatively rapid ERK activation and nuclear import followed by slower equilibration of ERK substrate phosphorylation status. With the availability of active ERK transiently buffered by substrate binding, the pools of free, active ERK in the cytosol and nucleus show little adaptation.

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