System-Level Investigations into the Functional role of RKIP in the Ras/Raf/MEK/ERK Signal Transduction Pathway

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The feedback mechanisms in the ERK pathway

The Ras/Raf/MEK/ERK pathway (or ERK pathway) is one of the most important signal transduction system involved in the control of cell proliferation, survival, differentiation, apoptosis, and metabolism (Fig. 1A) [1]. In spite of its topological simplicity, this pathway plays complicated roles in the regulation of various cellular processes. Up to now, many mathematical models and dynamical analyses of this ERK pathway have been reported [2], but the dynamics of positive and negative feedback mechanisms as well as the functional role of RKIP have been not so well understood [3, 4]. The aim of our study is to unravel the hidden dynamics of these feedback mechanisms and to identify the functional role of RKIP in the ERK pathway through combined efforts of *in vitro* experiments and *in silico* simulations based on an experimentally validated mathematical model (Fig. 1B). We consider two major feedback loops that are both triggered by activated ERK: (i) a positive feedback loop that results from the inactivation of the inhibitor RKIP; and (ii) a negative feedback loop that is generated by the inactivation of the Ras activating exchange factor complex Grb2-SOS

The functional role of feedback mechanisms and RKIP in the regulation of ERK dynamics

First, we compared the effects of transient (i.e., time-limited) versus chronic (i.e., sustained) stimulation. In both cases, we maintained a same level of stimulation. When Ras is chronically stimulated through PKC activation by TPA, the inhibition of the positive feedback loop by removal of RKIP increased the velocity (i.e. the rise time taken to reach its peak) and magnitude of the phosphorylated ERK response curve. However, the inhibition of the negative feedback loop increased phosphorylated ERK levels monotonically along with time (Fig. 1C). For the transient stimulation of Ras, the rise time was much shorter and the amplitude larger when the positive feedback loop was inhibited than when the negative feedback loop was inhibited (Fig. 1D). These results imply that the negative feedback loop plays a crucial role in stabilizing the system responses while the positive feedback loop plays an important role in inducing a delayed and switch-like response.

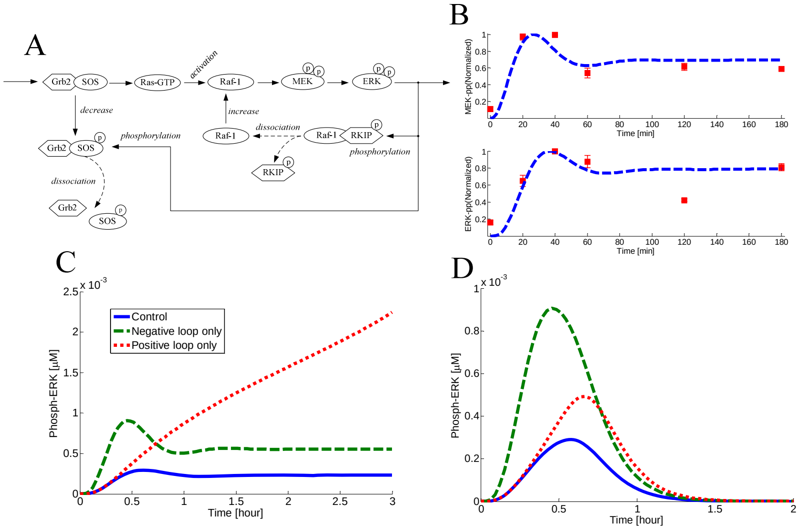

Figure 1. A schematic diagram of the ERK pathway and *in silico* simulation results. (A) A schematic diagram of the ERK signaling pathway. (B) Experimental data of the ERK pathway and *in silico* simulation for experimental validation of the mathematical model (the red squares with error bar denotes time-course data and the blue dashed line denotes *in silico* simulations). *In silico* simulation analysis of positive and negative feedback loops for chronic stimulation (C) and for transient stimulation (30 min) (D). "Negative loop only" means that the positive feedback loop has been removed, and "Positive loop only" that the negative feedback loop has been removed. "Control" means none of feedback loops have been removed.

To investigate the functional role of RKIP in COS-1 cells, we have gradually increased the concentration of RKIP (Fig. 2A) and found that the phosphorylated MEK shows a switch-like behavior (Fig. 2B), which is in well accord with the *in silico* simulation result (Fig. 2C). However, unlike the phosphorylated MEK and ERK, the phosphorylated Ras and Raf were dramatically increased by RKIP beyond a certain threshold (Fig. 2D).

To unravel the functional role of RKIP, we simulated phosphorylated ERK levels with respect to increasing TPA stimulation at three different RKIP levels. At RKIP=0μM, the phosphorylated ERK monotonically increased along with the TPA stimulation, but it increased like a sigmoid function above RKIP=1μM (Fig. 2E). The dynamics of the phosphorylated ERK over a specific range of TPA stimulation showed different characteristics depending on RKIP (Fig. 2F). For instance, it showed a sustained oscillation at RKIP=4μM while it showed damped oscillations beyond this range.

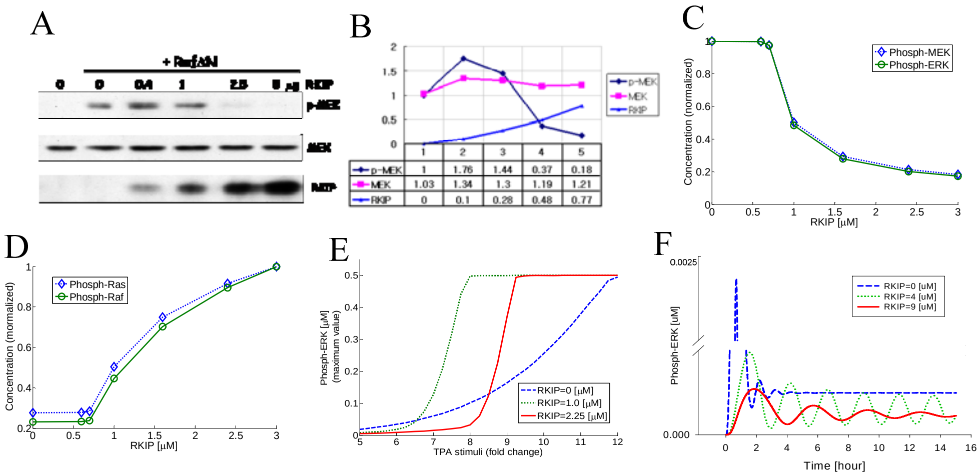

Figure 2: Identification of the functional role of RKIP in the ERK pathway. (A) The blotting of protein and its phosphorylation. (B) A switch-like behavior of the phosphorylated MEK to the increasing RKIP in COS-1 cells. (C) Decrease of the phosphorylated MEK and ERK like a sigmoid function (*in silico* simulations). (D) Increase of the phosphorylated Ras and Raf like a sigmoid function (*in silico* simulations). (E) The system response to the increasing TPA stimuli exhibits a switch-like behavior depending on RKIP (*in silico* simulations). (F) Quite different dynamics of the ERK pathway depending on RKIP (*in silico* simulations).

Conclusions

We have revealed that the negative feedback loop plays an important role in stabilizing the system response while the positive feedback loop plays a crucial role in inducing a delayed and switch-like response. We have also found that RKIP has a crucial role in the regulation of ERK dynamics.

References

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