

## EXTENDED ABSTRACT

### MAPK network properties determining cell fate decision in PC-12 cells

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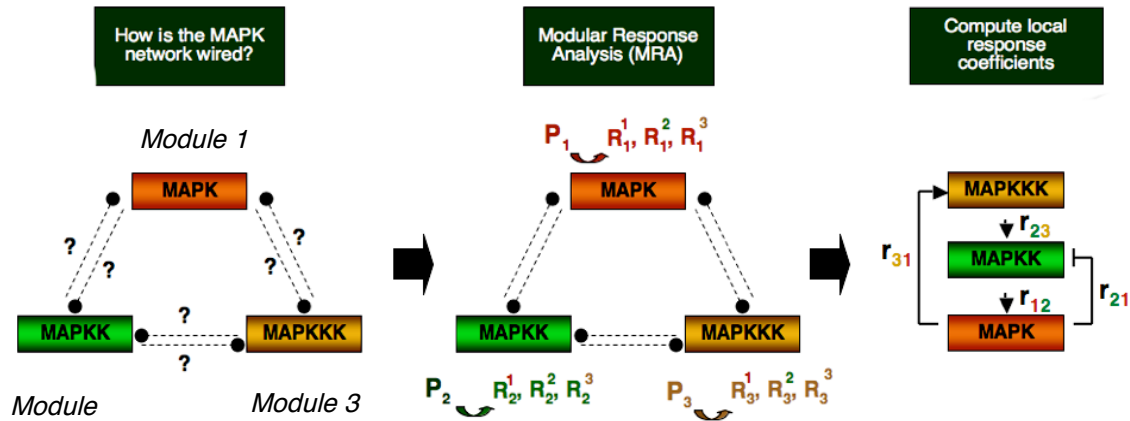
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The Mitogen-activated protein kinase (MAPK) network is a conserved signalling module that regulates cellular fate by transducing a myriad of growth factor signals. The module's ability to coordinate and process a variety of inputs from different growth factor receptors into specific biological responses is, however, still not understood. We investigated how the MAPK canonical network (Raf-Mek-Erk) brings about signal specificity in PC-12 cells, a model for neuronal differentiation. Here, the duration of Erk activation was shown to be critical for cell fate decision, in which, nerve growth factor (NGF) induces a sustained Erk activity, a condition sufficient for cell differentiation while epidermal growth factor (EGF) induces Erk transient response and proliferation of PC-12 cells (1). Recent work proposed that the duration of Erk signalling is interpreted through stabilization of immediate early genes, which occurs only when Erk activity is maintained leading to specific gene expression patterns (2). The remaining question was thus how the difference in Erk dynamics was controlled by the MAPK upstream regulators. We postulated that, by being embedded within larger regulatory networks, the MAPK core network could acquire different connectivity (i.e. *logical topology*), depending on whether the network was activated by EGF or by NGF stimulation. Therefore, reverse engineering by Modular Response Analysis (MRA), a sensitivity analysis developed by Kholodenko *et al* (3) was applied and uncovered differences in the wiring of the MAPK core network depending on whether cells were activated with EGF or NGF (Figure 1).



**Figure 1. Schematic of Modular Response Analysis.** Sensitivity analysis is performed on three functional modules: MAPK, MAPKK and MAPKKK for which sign and strength of connectivity are unknown. Systematic perturbations ( $P_n$ ) are applied to each module and the resulting change in activity of each module is determined as a global response coefficient ( $R_n$ ). The connectivity strength between two modules as if in isolation from the network is obtained from the computation of local response coefficients ( $r_n$ ).

Upon EGF stimulation the network exhibited negative feedback only, whereas a positive feedback was apparent upon NGF stimulation. The latter was confirmed by pharmacological inhibition analysis. Importantly, the positive feedback induced by NGF stimulation, not only sustained Erk activity, but also induced a switch-like bistable dynamics on the MAPK cascade. It was indeed observed that NGF generated biological memory in the MAPK network that lead to sustained Erk activity even after stimulation withdrawal. By rewiring the revealed regulatory feedbacks, by both pharmacological and genetic means, specific cell responses to EGF and NGF were reversed. The molecular mechanism underlying the newly found positive feedback after NGF stimulation was investigated and a role for PKC mediating this feedback in a synergistic model is proposed. Furthermore it was shown that this synergistic model allows for prediction on how the MAPK network responds to dual stimulation in naïve and experienced cells. Erk activation was analyzed upon both EGF and NGF sequential stimulation over time and in dose-response experiments. These confirmed a synergistic model for generating the NGF-driven positive feedback and the bistability of MAPK activation in differentiating PC12 cells. Our results demonstrate that growth factor context determines distinct topology of MAPK signaling network and that the resulting dynamics govern cell fate decisions.

## References

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3. B. N. Kholodenko *et al.*, *Proc Natl Acad Sci U S A* **99**, 12841 (Oct 1, 2002).