



## SYSTEMS PROPERTIES OF THE ERK PATHWAY AS NEGATIVE FEEDBACK AMPLIFIER

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The Ras-Raf-MEK-ERK pathway regulates many fundamental cellular processes. The specificity of regulation is in part encoded by the activation and response kinetics of the pathway whose control is incompletely understood. Here, using a combination of predictive computational modelling and biochemical experimentation we have analysed whether intrinsic design features contribute to output regulation, showing that this pathway has properties of a negative feedback amplifier (NFA). This design principle is widely used in electronic circuits to convey robustness against perturbations in the amplifier, stabilise the output and smoothen non-linear signal amplification. We show that it has very similar functions in biology where the three-tiered kinase cascade Raf-MEK-ERK is perceived as amplifier module with the phosphorylation of Raf-1 by ERK as the main negative feedback. Using Raf-1 mutants resistant to NFA we demonstrate experimentally that the NFA (i) can convert intrinsic switch-like activation kinetics into a graded response; (ii) conveys robustness to internal parameter variation; and (iii) stabilises systems outputs in response to perturbations of the amplifier. Thus, the NFA properties rendered ERK activity resilient to MEK inhibition. Sensitivity could be restored by inhibiting upstream elements outside the NFA module, or by weakening the NFA through combining Raf and MEK inhibitors. These results show that the function of the ERK pathway is critically dependent on its design as NFA both in terms of regulation and fidelity of signalling. In a broader context they (i) show that proteins embedded in a NFA are not ideal drug targets; and (ii) may explain why the design feature of a three-tiered amplifier kinase cascade coupled with NF is widespread in biology.