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MAPK kinase signalling dynamics regulate cell fate decisions and drug resistance

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The RAS/RAF/MEK/MAPK kinase pathway has been extensively studied for more than 25 years, yet we continue to be puzzled by its intricate dynamic control and plasticity. Different spatiotemporal MAPK dynamics bring about distinct cell fate decisions in normal vs cancer cells and developing organisms. Recent modelling and experimental studies provided novel insights in the versatile MAPK dynamics concerted by a plethora of feedforward/feedback regulations and crosstalk on multiple timescales. Multiple cancer types and various developmental disorders arise from persistent alterations of the MAPK dynamics caused by RAS/RAF/MEK mutations. While a key role of the MAPK pathway in multiple diseases made the development of novel RAF/MEK inhibitors a hot topic of drug development, these drugs have unexpected side-effects and resistance inevitably occurs. We review how RAF dimerization conveys drug resistance and recent breakthroughs to overcome this resistance.

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Introduction

The mitogen-activated protein kinase (MAPK) cascades have been in the limelight of research for over 25 years due to their involvement in cell proliferation, differentiation, survival/apoptosis, and motility. This scientific interest is also practical, because deregulation of MAPK signalling is a feature of major human diseases and developmental disorders [1,2]. The MAPK signalling cascades are activated by a plethora of external cues through a multitude of membrane receptors, including receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs). All MAPK cascades have the evolutionary conserved three-tier architecture where kinases sequentially phosphorylate and activate each other, whereas phosphatases dephosphorylate these kinases [3•]. The initiating kinases (such as RAF1/CRAF and BRAF) in the extracellular regulated kinase 1/2 (ERK1/2) cascade are commonly activated by RAS small G-proteins. The components of the RAS/RAF/MEK/ERK cascade are frequently mutated in cancer [4••] and are hot targets for an ever increasing number of anti-cancer drugs [5,6•].

Although the RAS/RAF/MEK/ERK pathway has a predominantly linear architecture, RAS and ERK are signalling hubs that have tens and hundreds of effectors and substrates, respectively. Changes in these interactions can dramatically change the ERK cascade temporal dynamics [7]. Different ERK dynamics were shown to trigger distinct cell decisions. For instance, in rat adrenal pheochromocytoma PC12 cells a transient activation of ERK induced by epidermal growth factor (EGF) results in cell proliferation, whereas sustained ERK activation by nerve growth factor (NGF) induces differentiation [8]. Subsequently, Bastiaens and colleagues showed that distinct cell decisions are explained by dynamic changes in the ERK cascade topology where EGF stimulation elicited negative feedback, whereas NGF induced positive feedback, imposed on the backbone of the same three-tier cascade structure [9]. Yet, recently Pertz and colleagues demonstrated that repeated 3 min pulses of low EGF concentration resulted in prolonged ERK activation and PC12 cell differentiation, whereas pulses of high EGF induced a more transient ERK response and PC12 cell proliferation similar to sustained EGF stimulation [10•]. The fact that the ERK cascade inputs of different frequency and amplitude rewire cell fate raised intriguing questions about the design and timescales of multiple feedforward and feedback regulations in the ERK network. Answering these questions requires a careful probing of the RTK/RAS/ERK network circuitry, uncovering timescales of major regulations, and the use of computational models. Here we present a brief overview of the current research efforts in the field, emphasizing a combined use of modelling and experiments as a tool to advance both the fundamental understanding of the control of ERK signalling and therapeutic applications.

Versatile ERK dynamics: adaptive and sustained signalling, switches and oscillations on different timescales.

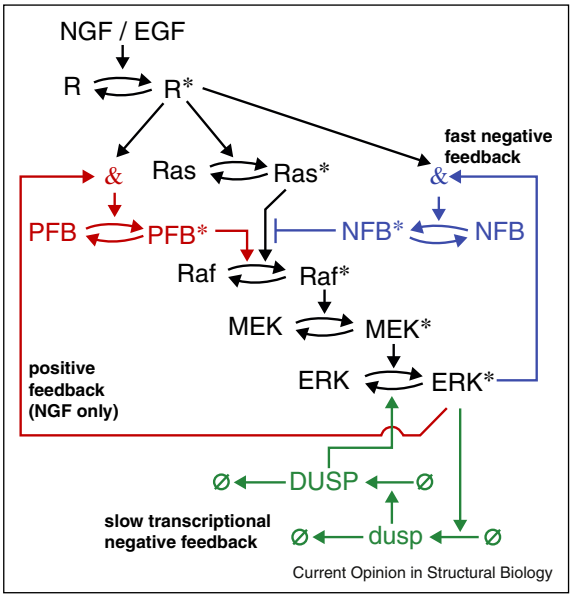
In many cells, the RAS/ERK cascade exhibits a transient, adaptive response to growth factor (GF) stimulation due to negative feedback loops from ERK to SOS and BRAF/CRAF, and the negative transcriptional feedback via ERK-induced expression of the DUSP family phosphatases on a longer timescale. However in other cells, the ERK response is sustained indicating that positive feedback and feedforward regulations reverse the effect of multiple negative feedbacks [11]. Positive feedback loops can also induce switch-like, bistable responses, where there is a threshold stimulus magnitude, and ERK is active until the stimulus decreases to much lower than threshold nearly basal values [9,12].

A main role of negative feedback is to confer resistance to perturbations of cascade components within the feedback loop. Due to several negative feedbacks, the ERK cascade can operate as a negative feedback amplifier, and this circuitry resists MEK inhibition by anti-cancer drugs [13]. When a negative feedback becomes too strong, it induces damped or sustained oscillations [14]. A combination of positive feedforward regulations by RTKs and positive and negative feedbacks can explain the ERK oscillations observed on remarkably different timescales from minutes to several hours [10•,15,16••].

Mathematical modelling helps to elucidate the intricate network dynamics [17]. Recently, the feedforward and feedback regulation of the RTK/RAS/ERK network and the dynamics of ERK responses were probed in live single PC12 cells using microfluidic devices to deliver GF input stimulation with precisely defined kinetics [10•]. Whereas sustained EGF/NGF stimulation largely confirmed previous observations (while uncovering a remarkable heterogeneity of signalling in individual cells), ERK responses to GF pulses of different frequency and amplitude showed unexpected signalling and biological outcomes. Mathematical modelling proved that known negative and positive feedback regulations could not explain the observed ERK dynamics. For instance, an EGF stimulatory regime of 3 min pulses/10 min intervals between pulses led to successive ERK pulses where the amplitude decayed much less for low than for high EGF concentrations. Since the ERK activity peaks in response to the initial EGF pulse were identical for both low and high EGF doses, ERK-induced negative feedbacks alone could not account for these observations. A dynamic model recapitulated these observation assuming that the negative ERK influence must be modulated by a feedforward signal from the EGF receptor, which was differentially activated by low and high EGF doses [10•].

A high-dosage, single NGF pulse of 10 min induced sustained ERK activity in a cell subpopulation, suggesting the presence of bistability brought about by a positive feedback from ERK, as previously reported [9]. In contrast, short 3 min NGF pulses of both high and low doses, and low dose 10 min NGF pulse elicited transient, adaptive ERK activity profiles throughout the cell population. This implies a slightly delayed and threshold activation of NGF-evoked positive feedback that occurred only if ERK activity exceeds certain levels of amplitude and duration. Since the first peak-response of NGF was similar for all pulsed NGF stimulation conditions, the data also suggested the existence of additional feedforward regulation of the ERK positive feedback strength [10•]. Figure 1 illustrates a kinetic scheme of a minimal mathematical model that was able to reproduce all these experimental observations, including heterogeneity of responses, which was more pronounced for NGF due to positive feedback from ERK.

Figure 1



A simplified diagram of feedforward and feedback regulation of the RTK/RAS/ERK network activated by EGF and NGF in PC12 cells. Modified from Ref. [10•].

Oncogenic alterations in RAS/RAF/MEK/ERK signalling

Many human cancers present hyperactivation of the MAPK pathway, most commonly through mutations in RAS, BRAF, CRAF, or MEK1/2 [4••]. Components of this pathway are therefore attractive targets for drug development [5,6•,18].

Germline mutations in genes encoding MAPK pathway components are associated with a group of developmental disorders known as RASopathies or RAS/MAPK syndromes [19,20]. Biochemical studies of these mutants as well as structural analysis and network-level data