Systems analysis of MAPK signal transduction

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Abstract

For more than a decade, the MAPK (mitogen-activated protein kinase) cascade has been studied using mathematical modelling and quantitative experimentation [1]. The MAPK cascade relays the presence of extracellular stimuli such as growth hormones to the nucleus and controls the expression of hundreds of genes. MAPKs control major cell fate decisions such as proliferation, differentiation and apoptosis, mainly by inducing alterations in gene expression. In this chapter, we discuss how systems biology analysis provides insights into the functioning of this cascade. We show how this pathway assists the cell in responding properly to extracellular cues by filtering out sub-threshold stimuli, while efficiently transmitting physiologically relevant inputs. Several different receptors signal through the MAPK pathway even though they elicit opposite biological responses, thus raising the question of how specificity is achieved in MAPK signalling. Experimental studies revealed that specific biological responses are encoded by quantitative aspects of the MAPK signal such as amplitude or duration.

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We discuss mechanisms that enable the pathway to generate quantitatively different signals, and also explain how different signals are interpreted by the downstream gene expression machinery.

Introduction

The three-tiered MAPK (mitogen-activated protein kinase) signal transduction cascades are activated upon extracellular stimulation and act as intracellular transducers of environmental signals. Active MAPKs control various physiological processes and cell fate decisions in all eukaryotic cells, mainly by regulating gene expression. The classical, most studied MAPK cascade in higher mammalian cells is the pathway comprising the serine/threonine kinases Raf, MEK [MAPK/ERK (extracellular-signal-regulated kinase) kinase] and ERK (Figure 1A). The activity of these three kinases is controlled by their phosphorylation state. Cascade activation is typically initiated by hormone binding to cognate transmembrane receptors. The resulting active receptor-ligand complexes employ various mechanisms to recruit the uppermost cascade member, Raf, to the plasma membrane, where it is phosphorylated and thereby activated. Phospho-Raf activates MEK by phosphorylating it at two residues, and phospho-MEK then in turn acts as an ERK kinase (Figure 1A). Through double-phosphorylation, ERK is activated and translocates to the nucleus, where it phosphorylates several nuclear kinases and transcription factors, which change the expression of approx. 100 genes.

The Raf-MEK-ERK cascade has received much scientific attention since it plays a central role in various physiological processes such as cell-cycle

![Figure 1. MAPK cascade](image)

(A) The MAPK cascades consist of three kinases. The classical MAPK cascade discussed in this chapter is composed of the three kinases Raf, MEK and ERK. (B) In Xenopus oocytes, ERK activation triggers maturation of the oocytes in a dose-dependent manner. Low doses of stimuli are filtered out and cause no effect, whereas high doses give rise to maturation. (C) In neuronal precursor cells (PC12), stimulation with EGF causes only brief ERK activation, whereas NGF triggers prolonged ERK signalling. The stimulus-specific ERK activation profiles (transient compared with sustained) are thought to determine the fate of PC12. Stimulation with EGF causes cellular proliferation, whereas stimulation with NGF causes differentiation.
progression, cell differentiation and cell death. Additionally, cascade activation is dysregulated in many diseases. For example, increased Raf-MEK-ERK signalling is observed in more than 30% of all cancers, and deregulation is even more frequent in some specific types of cancer, such as pancreatic cancer (90%). Finally, the system serves as the best understood paradigm for biological regulation by protein phosphorylation/dephosphorylation cycles. The human genome encodes approx. 500 kinases and 200 phosphatases, so that insights into the design principles of the MAPK cascades are expected to be applicable to cellular regulation in general.

In this chapter we depict, using a few examples, how mathematical modelling and systems biology approaches have helped to gain insights into the functioning of the mammalian Raf-MEK-ERK module. Readers interested in MAPK signalling in yeast should consult reference [2] for a review. First, we focus on how the MAPK cascade ensures reliable cell fate decisions by amplifying physiologically relevant stimuli, and filtering out background fluctuations (see Figure 1B). Then, it is discussed how stimulus-specific biological responses arise even though many different hormones signal through the ubiquitous MAPK cascade. We summarize experimental work, which reveals that biological responses are encoded in the quantitative aspects of ERK activity such as amplitude and duration (Figure 1C). Moreover, we explain how the quantitative characteristics of ERK activity are modulated in a stimulus-specific manner. Finally, it will be discussed how quantitative features in ERK activation are interpreted and decoded at the level of gene expression, and thus give rise to specific cellular responses (see Figure 1C).

**Filtering: suppression of background noise and amplification of relevant signals**

Any biochemical signalling network involved in cell fate decisions must be able to reliably discriminate between physiologically relevant signals and background fluctuations in order to avoid improper responses. Such filtering is typically realized by all-or-none, switch-like stimulus–response behaviour. These stimulus–response relationships are sigmoidal, meaning that sub-threshold stimuli fail to elicit significant responses, while strong signalling is observed in response to supra-threshold stimulation. As these sigmoidal curves are highly sensitive around the threshold, this phenomenon has been often referred to as ultrasensitivity (compare Figure 2A). Huang and Ferrell [3] observed that ERK activation in *Xenopus* oocytes is very sensitive around a threshold input concentration, with a slight increase in the stimulus having a large effect on the ERK activity leading ultimately to oocyte maturation. This observation led them to develop the first mathematical model of the MAPK pathway. Their model showed that two mechanisms might generate ultrasensitivity in this pathway, namely multistep ultrasensitivity and zero-order ultrasensitivity.

Multistep ultrasensitivity arises if an input regulates its downstream effectors at multiple points [4]. In the MAPK cascade, the kinases Raf and
MEK phosphorylate their downstream effectors, MEK and ERK respectively, at two sites, and thus establish multistep regulation. A relatively simple mathematical calculation illustrates this effect for a case where the phosphorylation sites are phosphorylated and dephosphorylated in a sequential distributed manner (i.e. if the enzymes detach after modifying the sites, and if the phosphorylation of one site always precedes the other) [3,4]. If one assumes that the enzymes are not saturated, the fraction of activated ERK, i.e. ERK\textsubscript{PP}/ERK\textsubscript{Tot}, is given by eqn (1):

\[
\frac{\text{ERK}_{\text{PP}}}{\text{ERK}_{\text{Tot}}} = \frac{k_{\text{M}} \cdot \text{MEK}}{k_{\text{Pase}} + k_{\text{MEK}}/k_{\text{Pase}}^2} + \frac{k_{\text{MEK}}}{k_{\text{Pase}}^2} \cdot \left(\frac{k_{\text{MEK}}}{k_{\text{Pase}}} \right)^2
\]  

In eqn (1), MEK and Pase denote the concentrations of MEK and the phosphatase respectively, and \(k_1\) and \(k_2\) are their respective first-order rate constants. A derivation of eqn (1) can be found in [5]. Double phosphorylation can therefore give rise to quadratic (instead of linear) kinase control over substrate phosphorylation [6]. Experimental studies revealed that ERK (de)phosphorylation indeed occurs in a sequential and distributive manner, thus suggesting that double phosphorylation is an important source for ultrasensitivity in the MAPK pathway [4,7].

Early theoretical work by Goldbeter and Koshland [8] showed that phosphorylation cycles can exhibit very strong ultrasensitivity if the catalysing enzymes (i.e. kinase and phosphatase) operate near saturation, i.e. if the Michaelis–Menten constants (\(K_m\) values) of the enzymes are much smaller than the substrate concentration. This phenomenon has been termed zero-order ultrasensitivity to reflect that the (de)phosphorylation velocities

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**Figure 2. Filtering**

(A) Sigmoidal stimulus–response relationships are often termed ultrasensitive, since they are highly sensitive to changes around a threshold. Ultrasensitive responses efficiently filter out low signals when compared with a hyperbolic response. (B) A bistable response often occurs when ultrasensitive behaviour is combined with positive feedbacks. When the response is bistable, there are two stable states in the system at a certain stimulus range, and whether the system is in the high- or low- activity state is determined by its history (compare ‘switching on’ and ‘switching off’).
are independent of the substrate concentration in the saturated regime. Recent studies however cast doubt on the physiological relevance of zero-order ultrasensitivity in mammalian MAPK signalling, as ultrasensitivity is strongly weakened if the enzyme and substrate concentrations are comparable, i.e. if the substrate is significantly sequestered on the catalysing enzymes [6,9]. Experimental studies suggest that such substrate sequestration occurs in the MAPK cascade, so that zero-order ultrasensitivity probably plays only a minor role in this system.

A third way to generate ultrasensitivity is through stoichiometric inhibition [4]. Some phosphatases of ERK seem to act as stoichiometric inhibitors for ERK and not only as catalytic deactivators, as they form stable complexes with ERK [7,10]. The mechanisms and implications of these phosphatases on ultrasensitivity are discussed elsewhere [11,12].

Switch-like stimulus–response behaviour can be further enhanced if the basic ultrasensitivity mechanisms described above are embedded into a larger biochemical network. In particular, signalling cascades such as the MAPK pathway typically show much stronger and more robust ultrasensitivity than each level in isolation [3,13,14].

Another way to amplify ultrasensitivity is positive feedback, as feedback signalling will only start to become significant once the threshold has been exceeded, thus making the near-threshold response even steeper [15]. An extreme manifestation of feedback amplification is bistability, where the system switches between two discrete states (on and off) in a true all-or-none manner. Bistable systems also display hysteresis, meaning that different stimulus–response curves are obtained depending upon whether the system began in its off or its on state (see Figure 2B). Thus, the behaviour of the system is history-dependent, as the positive feedback can act as a memory device, which maintains high activity even if stimulation strength decreases. In some cases, the on state is maintained indefinitely after the stimulus is completely removed, so that the system shows irreversible activation [16].

Bistability is an important mechanism for noise-resistant cell fate decisions, and it has been proposed that bistability arises in the mammalian MAPK cascade, because ERK activates Raf in a positive feedback loop via PKC (protein kinase C) [17]. Intriguingly, recent theoretical work indicated that implicit positive feedbacks and bistability can emerge even in the core Raf-MEK-ERK module due to enzyme sequestration effects [12,18]. Single-cell measurements strongly suggest that the Raf-MEK-ERK module is bistable in mammalian neuronal precursors (PC12 cells), because all-or-none and irreversible ERK activation was observed in response to extracellular stimulation [19]. However, this phenomenon appears to be cell-type-specific, as ERK activation at the single-cell level was gradual but ultrasensitive in fibroblasts [20]. Taken together, these results suggest that filtering due to ultrasensitivity is a general property of the mammalian Raf-MEK-ERK module, while bistability appears to be a context-dependent phenomenon.
Encoding: stimulus-specific modulation of ERK duration

Different extracellular stimuli frequently engage the same set of intracellular signalling pathways even though they elicit completely different biological responses. How can different signals be transmitted by the same pathway? It seems that stimulus-specific biological information is frequently encoded by quantitative differences in stimulus-specific activation kinetics. The Raf-MEK-ERK pathway serves as a paradigm for quantitative encoding of signalling specificity, as both the amplitude and the duration of ERK activation are critical determinants for the cell fate. The amplitude of the ERK signal seems to be important in fibroblasts, where low level of ERK signalling induces proliferation, while cell-cycle arrest is observed in response to strong ERK activation [21]. In neuronal precursors (PC12 cells), it seems to be mainly the ERK signal duration that matters, as these proliferate upon short-term ERK activation, but differentiate if ERK phosphorylation is sustained [22]. Yet the fate of PC12 cells can be induced in a stimulus-specific manner, as EGF (epidermal growth factor) stimulation elicits transient ERK activation, whereas prolonged ERK signalling is observed in response to NGF (nerve growth factor) (see Figure 1B) [22].

Various mechanisms are thought to contribute to such stimulus-specific modulation of ERK signal duration. In simple terms, transient ERK activation is observed if the MAPK cascade or upstream pathways are subject to delayed negative regulation, which ensures efficient signal termination, also referred to as signal adaptation. In contrast, ERK exhibits sustained activation if such adaptation mechanisms are absent. Three basic regulatory mechanisms, depicted in Figures 3(A)–3(C), are known to mediate signal adaptation in biochemical regulatory networks (sequestration/internalization, incoherent feed-forward regulation and negative feedback regulation). All of them seem to be involved in the modulation of MAPK signal duration. Below we discuss examples of such mechanisms.

Growth factor receptors are taken up into the cell upon ligand binding, and are deactivated and/or degraded within intracellular vesicles and therefore the signal is terminated (arrow 1 in Figure 3D). It is known that EGF receptors internalize more rapidly than NGF receptors, and it has been suggested that rapid receptor sequestration and degradation accounts for transient ERK signal duration in EGF-treated PC12 cells [23].

Glutamate induces transient ERK activation in neurons, whereas sustained signalling is observed in response to potassium chloride [24]. Strikingly, the rapid decline in ERK phosphorylation depends on the ability of glutamate to specifically activate PTP-STEP [protein tyrosine phosphatase, non-receptor type 5 (striatum-enriched)], an ERK phosphatase (arrow 2 in Figure 3D). This kind of regulation is known as an incoherent feed-forward loop, because a common upstream regulator (i.e. glutamate) both activates and inhibits a downstream effector (i.e. ERK) via two independent branches. Incoherent feed-forward loops generate transient signals if positive regulation (ERK
phosphorylation) proceeds faster than negative regulation (PTP-STEP activation), as observed in glutamate-induced neuronal MAPK signalling [24].

Early theoretical work indicated that differential feedback control in EGF compared with NGF signalling may underlie transient, compared with sustained, ERK signalling in PC12 cells [25]. A recent experimental and theoretical study confirmed that stimulus-specific feedback regulation indeed occurs in PC12 cells [19]. It was shown that EGF specifically induces negative feedback within the MAPK cascade and thereby induces early termination of ERK activity (e.g. arrow 3 in Figure 3D). NGF signalling appeared to escape signal adaptation because strong initial ERK activation (compared with EGF stimulation) specifically triggers a positive feedback that outweighs negative feedback regulation (arrow 4 in Figure 3D). In particular, this positive feedback was shown to establish bistable and irreversible ERK activation, thus ensuring prolonged signalling [19].
Both theoretical and experimental studies demonstrated that a pathway consisting of the adaptor protein FRS (fibroblast growth factor receptor substrate) and the small G-protein Rap plays a key role for sustained ERK signalling in NGF-treated PC12 cells [26–28]. Strikingly, this pathway is specifically engaged by NGF for receptor-mediated Raf activation, but not by EGF [26,28]. Moreover, sustained activation of ERK in response to NGF is abolished if Rap signalling is blocked [28]. Accordingly, the FRS-Rap pathway shows sustained activation kinetics, whereas the functionally redundant Shc (Src homology and collagen homology)-Ras pathway that mediates EGF-induced Raf activation is only transiently active [26,28]. Various explanations have been proposed why the duration of Ras and Rap signalling differs: (i) incoherent feed-forward regulation via Ras-GAP (GTPase-activating protein) limits signal duration of the Shc-Ras pathway (arrow 5 in Figure 3D), but not that of the FRS-Rap module [26]; (ii) the Shc-Ras branch is subject to negative feedback control by ERK (arrow 3 in Figure 3D), whereas no such feedback has been described for FRS-Rap signalling [25,28]; and (iii) cytosolic adaptor proteins such as Shc are only functional when recruited to active transmembrane receptors, whereas signalling via membrane-anchored adaptors like FRS can continue even without such recruitment. Thus the FRS-Rap pathway is expected to be less susceptible to signal termination by receptor down-regulation, and should therefore exhibit more sustained activation [27]. A summary of the network shaping ERK activation upon stimulation with NGF, glutamate and EGF is shown in Figure 3D.

In conclusion, it appears that stimulus-specific upstream regulatory pathways (e.g. receptors, adaptors or small G-proteins) are major determinants of ERK signal duration, because they exhibit differential susceptibility to negative regulation and thus signal termination. Additionally, the shared MAPK cascade integrates incoming inputs and generates transient or sustained output depending on their amplitude.

**Decoding: interpretation of ERK amplitude and duration at the level of gene expression**

If the biological information is encoded by quantitative differences in the ERK signal characteristics, proper biological responses require that the cell is able to accurately decode the amplitude and the duration of ERK signalling. The mechanisms of decoding are not known at present, but most probably involve those discussed in the section on filtering above. In particular, multistep regulation appears to allow for both amplitude and duration decoding as discussed below.

Single-cell measurements revealed that c-Fos, a direct target gene of ERK signalling (see Figure 4A), is induced in an all-or-none manner in response to different doses of extracellular stimulation [20]. This suggests that amplitude decoding occurs at the level of ERK-induced transcriptional induction. Although the molecular mechanism of decoding remains to be established, we will explain how ultrasensitivity could arise in ERK-induced transcription-factor regulation.
Upstream regulatory signals (such as ERK activity) usually enhance gene expression by phosphorylation of transcription factors, which in turn induces transcription factor binding to target promoter sites. Transcriptional initiation typically requires simultaneous binding of multiple transcription factors to individual promoters. This requirement for simultaneous transcription-factor binding establishes multistep regulation, because ERK needs to phosphorylate both transcription factors in order to allow for gene expression. It is relatively easy to understand why ultrasensitivity arises in this system due to multistep regulation (see also the section on filtering above). Suppose that A and B are the active pools of two ERK-regulated transcription factors both of which bind to the same promoter. For each promoter site, the probability that the site is occupied is given by eqns (2) and (3):

\[
P(A \text{ binds}) = \frac{A}{A + K_1} \quad (2)
\]

and

\[
P(B \text{ binds}) = \frac{B}{B + K_2} \quad (3)
\]

Figure 4. Duration decoding
(A) Feed-forward mechanism by which c-Fos decodes ERK duration. ERK induces c-Fos transcription, and also stabilizes c-Fos protein by phosphorylation. (B) c-Fos time series for short and long ERK activation shown in solid blue and broken black lines respectively. The model input, i.e. ERK activation pulses of different lengths, is indicated by blue and black backgrounds respectively. The maximal amount of c-Fos produced depends linearly on the input duration, and thus does not allow for duration decoding. (C) Output gene expression shown for the two stimuli. The feed-forward loop significantly changes the difference in the amount of output genes produced by the long compared with short stimulation, and thus mediates duration decoding. (Parameters for simulation: \(d_1 = 4 \text{h}^{-1}\), \(d_2 = 2 \text{h}^{-1}\), \(d_3 = 0.25 \text{h}^{-1}\) and \(k_3 = 10 \text{h}^{-1}\); all other parameters are set to 1.)
Keeping in mind that both A and B are functions of ERK activity, the joint probability that both sites are occupied can be written as eqn 4:

\[
P(A \land B \text{ bind}) = P(A \text{ binds}) \cdot P(B \text{ binds}) = \frac{A \cdot B}{(A + K_1)(B + K_2)} = \frac{f(ERK) \cdot g(ERK)}{[f(ERK) + K_1][g(ERK) + K_2]} \tag{4}
\]

Eqn (4) is in a similar form as that describing double-phosphorylation (eqn 1), and thus confirms the occurrence of ultrasensitivity with respect to ERK. Cooperativity between the sites, i.e. when the occupation of one site promotes the binding to the second site, will even enhance decoding of the ERK amplitude.

Several transcription factors remain constitutively bound to DNA, regardless of their activation state. These constitutive binders often act as transcriptional repressors in the inactive state, while being transcriptional activators in the active state. Swillen et al. [29] have theoretically analysed this case of ambivalent transcription factor action and concluded that repressor–activator switching allows for highly ultrasensitive stimulus–response behaviour. In conclusion, it seems that recurrent regulatory motifs in transcription factor networks exhibit ultrasensitive responses and thus might mediate ERK amplitude decoding.

Multistep regulation is known to allow for duration decoding in biochemical signalling networks as previously discussed for transcriptional feed-forward loops [30] and for multisite phosphorylation [31]. An example for multistep regulation that has been implicated in the decoding of ERK signal duration is the induction and stabilization of the transcription factor c-Fos (see Figure 4A) [32,33]. The transcription of c-Fos is induced by ERK. After translation, the protein is very unstable (half-life \(t_{1/2}=15\) min) unless it is stabilized by ERK-mediated phosphorylation (\(t_{1/2}=4\) h). Such a mechanism, where ERK positively regulates c-Fos at two levels (transcription and protein stability), is known as coherent feed-forward loop [30].

Coherent feed-forward regulation of c-Fos is thought to allow for selective c-Fos accumulation upon sustained ERK activation, because short ERK signals vanish before they are able to stabilize the slowly rising c-Fos protein pool. We translated this mechanism into a simple mathematical model with physiologically relevant parameter values (Figure 4). We simulated c-Fos expression for two stimuli of different lengths, 30 min and 2 h. The maximum expression level of c-Fos protein is 4-fold higher for the longer stimulus (Figure 4B, top panel). Thus the stimulus length seems to be linearly translated into maximal protein levels. Such linear translation into activated c-Fos is similar to a simpler model, where c-Fos is immediately stable without the need for feed-forward control (Figure 4B, bottom panel). This might lead to the conclusion that this feed-forward regulation of c-Fos does not allow for efficient duration decoding.

However, duration decoding at the level of c-Fos might not be necessary, because c-Fos protein solely acts as an intermediary transcription factor, which...
in turn controls the expression of physiologically relevant output genes such as those involved in cell-cycle control. Thus it might be more important that these output genes are differentially expressed depending on the duration of ERK activity. In fact, Figure 4(C) shows that the expression level of output protein increases by 6.5-fold upon a 4-fold increase in ERK signal duration. The ability to decode ERK signal duration at the level of output gene expression increases even further if one introduces a delay in transcription and translation of c-Fos (results not shown).

Why does the output gene decode the duration of ERK? The expression of a long-lived output gene integrates the time-course of c-Fos activity. For brief ERK activation, most c-Fos remains unphosphorylated (i.e. unstable), and therefore decays relatively quickly once ERK is inactivated. On the other hand, long-lasting stimuli result in most c-Fos being in the phosphorylated state, which decays less quickly. Thus the output gene expression, which integrates c-Fos protein levels over time, can differ more than 4-fold.

Taken together, our simulations suggest that the integral output (but not the amplitude) of the c-Fos feed-forward loop can efficiently decode duration if physiologically relevant parameters are assumed.

Conclusions

In this chapter, we have summarized experimental and theoretical evidence that suggests that the quantitative characteristics of ERK activation are crucial determinants for the cellular fate. Different stimuli are encoded into different lengths and amplitude of the signal by mechanisms such as sequestration, incoherent feed-forward regulation and negative feedback regulation. They are interpreted at the level of gene expression, for example through the regulated proteolysis of c-Fos. One important quantitative aspect of MAPK signalling, which deserves further systems biological analysis, is the balance of the ERK cascade with other cellular signalling pathways. For example, it was shown that single PC12 cells subjected to oxidative stress activate pro-survival ERK signalling or pro-death p53 signalling in a mutually exclusive manner, and thus perform an accurate all-or-none cell fate decision based on inhibitory signalling cross-talk [34]. Therefore we expect that in the near future mathematical modelling of the interactions and cross-talk between the major signalling pathways will boost our understanding of how cells make their decisions based on their environments.

Summary

- The MAPK signalling pathway has been subject to mathematical modelling and quantitative experimentation for more than a decade.
- The MAPK signalling pathway filters sub-threshold stimuli, with multi-site phosphorylation and positive feedback loops as major filtering mechanisms.
• Different feedback/feed-forward loops shape quantitative aspects of the signal in a stimulus-dependent manner, allowing for different signals through one pathway.
• Information can be encoded by signal duration and can be decoded by a feed-forward loop such as the induction and stabilization of the transcription factor c-Fos.

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References


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