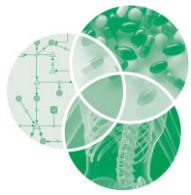
Advanced Review



Four-dimensional dynamics of MAPK information-processing systems

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Mitogen-activated protein kinase (MAPK) cascades process a myriad of stimuli received by cell-surface receptors and generate precise spatiotemporal guidance for multiple target proteins, dictating receptor-specific cellular outcomes. Computational modeling reveals that the intrinsic topology of MAPK cascades enables them to amplify signal sensitivity and amplitude, reduce noise, and display intricate dynamic properties, which include toggle switches, excitation pulses, and oscillations. Specificity of signaling responses can be brought about by signal-induced feedback and feedforward wiring imposed on the MAPK cascade backbone. Intracellular gradients of protein activities arise from the spatial separation of opposing reactions in kinase-phosphatase cycles. The membrane confinement of the initiating kinase in MAPK cascades and cytosolic localization of phosphatases can result in precipitous gradients of phosphorylated signal-transducers if they spread solely by diffusion. Endocytotic trafficking of active kinases driven by molecular motors and traveling waves of protein phosphorylation can propagate phosphorylation signals from the plasma membrane to the nucleus, especially in large cells, such as *Xenopus* eggs. *WIREs Syst Biol Med* 2009 1 28–44

Signaling through a plethora of cell-surface receptors, such as G-protein-coupled receptors (GPCRs), receptor tyrosine kinases (RTKs) and cytokine receptors, activates mitogen-activated protein kinase (MAPK) cascades, which function as central integration modules for cellular information processing.[1–4] MAPK cascades play a pivotal role in the control of fundamental cellular processes that include cell growth and division, migration, and differentiation. These pathways are evolutionarily conserved in cells from yeast to mammals (Table 1) and consist of several levels, where the activated kinase at each level phosphorylates and activates the kinase at the next level down the cascade. Phosphorylation of each kinase is reversed by phosphatases, which include serine/threonine, tyrosine, and dual-specificity phosphatases. The typical, three-tiered cascade comprises an MAPK, an MAPK kinase (MAP2K) and an MAP2K kinase (MAP3K). In some cellular systems, these kinases can be brought together by a scaffolding protein.[5,6] MAPK is activated by MAP2K-mediated phosphorylation on two conserved residues, a threonine, and tyrosine in the activation loop of the kinase domain. Active MAPK can phosphorylate a multitude of cellular targets, which include transcription factors, other enzymes, and cytoskeletal proteins.[7] In contrast, the upstream MAP2K and MAP3K are not as promiscuous as the MAPK, typically phosphorylating only the immediate downstream kinase in the cascade.

Mammalian cells can express at least four prototypical classes of MAPK cascades, extracellularly-regulated kinase (ERK1/2), ERK5, C-Jun N-terminal kinase (JNK), and p38 MAPK (Table 1), and at least three atypical MAPK cascade types, ERK3/4, ERK7/8, and nemo-like kinase (NLK), which do not follow the classical three-tiered, dual-phosphorylation-signaling structure.[11] In this review we will focus on the dynamics of information processing of the typical cascades, choosing the well-studied ERK1/2 pathway as a main

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TABLE 1 | MAPK Cascades from Yeast to Mammals

Cascade	Example Stimulus(i)	MAP3K(s)	MAP2K(s)	MAPK(s)	MAPK Phosphatase(s)	Scaffold(s)	Example MAPK Target(s)	Example Cellular Response(s)	Refs
Mammalian ERK	Growth factors, adhesion	A-Raf, B-Raf, C-Raf (Raf-1), Mos	MEK1, MEK2	ERK1, ERK2	MKP1, MKP3, VHR	KSR, MP-1, β-arrestin, Sef, IQGAP, CNK, Paxillin, MORG1	RSK, Elk1	Proliferation, migration, differentiation	8–10
Mammalian JNK (SAPK)	Heat shock, ionizing radiation	MEKK1/2/3/4, MLK1/2/3, ASK1, Tpl2	MEK4, MEK7	JNK1 (SAPKβ), JNK2 (SAPKα), JNK3 (SAPKγ)	MKP7, VH5	JIP1/2/3/4	c-Jun, c-Myc, p53	Differentiation apoptosis, inflammation	9–11
Mammalian p38	Osmotic shock, hypoxia, cytokines	MEKK4, MLK3, ASK1	MEK3, MEK6	p38α, p38β, p38γ, p38δ	MKP1, MKP5, VH5, MKP7	OSM, JIP2/4	CHOP, MNK, MSK	Stress, adaptation, cell-cycle checkpoint control, inflammation, differentiation	9–11
Mammalian ERK5	Growth factors, oxidative stress	MEKK2, MEKK3	MEK5	ERK5	Unknown	MEK5	MEF2, RSK	Differentiation	9,10, 12,13
Drosophila D-ERK (*rolled*, ERK-like)	Growth factors	D-RAF (phl)	D-MEK (dsor1)	D-ERK	D-MKP; D-MKP-3	Unknown	D-RSK	Proliferation	13–15,
Drosophila D-JNK (*basket*, JNK-like)	Oxidative and heavy metal stress, heat shock	MLK (slpr)	D-JNKK (*hep*)	D-JNK	*Puckered*, D-MKP4	Unknown	D-Jun	Stress adaptation, tissue morphogenesis	13, 15–17
Drosophila D-p38 (95*E*4 – *F*1, (p38-like)	Pathogens	D-ASK1	D-MKK3 (*lic*)	D-p38a	Unknown	Unknown	ATF2, D-Jun	Stress adaptation and Innate Immunity	13,17
C. elegans MPK-1 (ERK-like)	Growth factors	LIN-45	MEK-2	MPK-1	LIP-1	KSR1, KSR2, CNK-1	LIN-1, SUR-2, EOR-1	Vulva development, migration, meiosis	6,18

TABLE 1 | Continued

Cascade	Example Stimulus(i)	MAP3K(s)	MAP2K(s)	MAPK(s)	MAPK Phosphatase(s)	Scaffold(s)	Example MAPK Target(s)	Example Cellular Response(s)	Refs
C. elegans KGB-1 (JNK-like)	Heavy metal stress	MLK-1	MEK-1	KGB-1	VHP-1	Unknown	GLH-1	Heavy metal stress adaptation, germline development	6,19,20
C. elegans PMK (p38-like)	Pathogens, oxidative stress	NSY1	SEK1	PMK1	VHP-1	Unknown	SKN-1	Innate Immunity, neuronal asymmetry, oxidative stress adaptation	6,20,21
S. cerevisiae Fus3	α-factor	Ste11	Ste7	Fus3	Msg5, Ptp2/3	Ste5	Ste12	Mating	22
S. cerevisiae Kss1	Lack of nutrients	Ste11	Ste7	Kss1	Msg5, Ptp2/3	Unknown	Ste12, Tec1	Invasive growth	11,22
S. cerevisiae Hog1 (p38-like)	Osmotic stress	Ste11	Pbs2	Hog1	Ptp2/3, Ptc1	Pbs2	Unknown	Osmotic stress adaptation	22

MAPK cascades are conserved from yeast to mammals, respond to a myriad of stimuli, and produce diverse cellular outcomes. The biological diversity of MAP3Ks, MAP2Ks, MAPKs, MAPK phosphatases, scaffolds, and MAPK targets allows flexible yet robust information processing.

example (which is frequently referred to as ERK). MAPK cascades convert diverse inputs into different cell fate decisions, and this process is tightly regulated by feedback and feedforward loops that embrace several different MAP3Ks, MAPK phosphatases (MKP), scaffolds, and other proteins that can regulate MAPK activity. In this review we will explore only a handful of these modes of regulation. More future work has to be done, both experimental and theoretical, to explore and fully understand the signaling richness of the MAPK biology as illustrated in Table 1.

Two central biological questions have stimulated the current interest in understanding MAPK information-processing dynamics. First, given the multitude of cellular input signals that are routed through only a few conserved MAPK pathways, how can a cell convert different signals into different outcomes? A current hypothesis is that signal specificity can be achieved through complex spatiotemporal regulation of MAPK signaling. In the classical example, stimulation of PC12 cells with the epidermal growth factor (EGF) or the nerve growth factor (NGF) resulted in distinct physiological outcomes, proliferation versus differentiation, respectively. Initially, this divergent behavior was attributed to different temporal patterns of ERK1/2 activity; transient activation by EGF led to proliferation, whereas sustained activation by NGF led to differentiation.[23] Subsequent work suggested that the duration of ERK signaling is interpreted by cells through a network of immediate-early genes.[24,25] Yet, how different ERK dynamics can be robustly controlled by upstream receptors still remains unclear, although several plausible mechanisms have been proposed.[26,27] Recent discoveries show that a variety of distinct modes of the MAPK spatiotemporal dynamics emerge from differential signal-induced wiring of the cascade.[28,29]

A second key question is how MAPK cascades can transform smooth, gradual signals, such as growth factor concentration changes, into discrete (in a sense, digital) outputs and critical cell fate decisions. Initial answers came from theoretical studies that demonstrated MAPK cascades can act as analog-to-digital converters, generating bistable dynamics (where two stable 'on' and 'off' steady states coexist), abrupt, ultransensitive switches, and oscillations.[30–41] Indeed, digital outputs can correspond to 'yes-or-no' cell fate decisions; such behavior is critical for controlling cell-cycle transitions.[10,42,43]

Mathematical and computational modeling emerges as a novel and useful approach to comprehend biology of MAPK signaling. In this review, we detail how both theoretical and experimental work have synergistically increased our understanding of MAPK information-processing mechanisms. Although focus is given to the mammalian ERK1/2 pathway, we also highlight results for other MAPK systems, and show that many theoretical results from one MAPK system can apply to others. Although emphasis to theory that has already been corroborated by experimental

work is given, we do not exclude general theoretical considerations. In fact, we illustrate that some theoretical foresights preceded experimental validation. Thus, it is feasible that some theoretical predictions discussed here may receive future experimental support.

SPATIALLY COARSE-GRAINED STUDIES OF MAPK SIGNALING

Basic Characteristics of Input/Output Behavior in MAPK Cascades

The majority of experimental and theoretical MAPK signaling studies have taken spatially coarse-grained approaches where the cell is regarded as one or more well-mixed compartment(s) with no variation in spatial dimensions. Although this is a simplification of the true picture, such approaches have led to important breakthroughs in understanding of MAPK information processing. Before going into details, it is instructive to delineate different MAPK output responses to typical input signals. These signals include (1) a simple step-function, or sustained stimulation (Figure 1(a)), (2) an exponential decay function (Figure 1(b)), which approximates the activity of a receptor after stimulation by a step input, and (3) a rectangular pulse input, or pulse-chase stimulation (Figure 1(c)), which although physiologically relevant has received less attention so far. These inputs capture the temporal behavior of different upstream MAPK cascade activators, such as extracellular ligands, RTKs, GPCRs, or small G-proteins. Theoretical studies have shown that depending on the cascade architecture and kinetic parameters, different responses can arise from the same step input: a transient or adaptive response (Figure 1(d)),[44] a sustained response (Figure 1(e)),[38] damped oscillations (Figure 1(f)),[45] or sustained oscillations (Figure 1(g)).[46] Numerous experimental studies have shown that following the onset of sustained stimulation, in general, MAPK responses reach peak levels in about 3–15 min. The behavior after peak activity can be widely different. The duration of MAPK responses to a constant stimulus can range from about 15 min to several hours, depending on a cell type and external cue. Transient versus sustained ERK1/2 responses can depend on (1) the rate of receptor endocytosis,[47,48] (2) the complex regulation of the upstream cascade 'gatekeepers', small GTPases Ras and Rap1,[27,44,49] and (3) negative and positive feedback loops from ERK1/2 to SOS, GAB1, MEK, and Raf.[50–55]

Steady-State Properties

Although time-varying characteristics of MAPK signaling are critically important, so are steady-state properties of MAPK cascades. In fact, steady-state behavior presents the ultimate output of sustained MAPK signaling, and the degree of adaptation for transient signaling. Additionally, the stability of steady states is important for driving complex time-dependent behavior, such as bistability and oscillations. We here briefly review the response analysis,[56] as it forms a convenient basis to understand how the interaction topology of MAPK cascades affects steady-state properties.

Steady-state information transfer through a MAPK signaling cascade (Figure 2) can be quantified using two types of response coefficients. The *local* coefficient (r) is defined in terms of the response of a kinase at a given level to a change in the activity of a kinase at the immediately preceding level,

$$r^{i}_{i+1} \equiv \partial \ln K^{*}_{i} / \partial \ln K^{*}_{i+1} \tag{1}$$

where K^{*}_{i} is the steady-state concentration of activated MAP*i*K. The *global* (overall) response coefficient, determines the response of MAPK activation to the input signal (S)

$$R \equiv d \ln K^{*}_{1} / d \ln S \tag{2}$$

where S is the signal strength. The response coefficient is essentially equal to the % change in the target (MAP*i*K) to a 1% change in the input [MAP($i+1$) K or S], and can be thought of as the sensitivity of a target to a signal. Many of the design features common to MAPK cascades serve to modulate this sensitivity. There is a tradeoff, however, between the sensitivity and the range where MAPK is sensitive to input signals, or the working linear range. Decreasing the sensitivity leads to a broadening of the working linear range, whereas increasing the sensitivity shrinks this range, making the response more switch-like.

One might expect that the sensitivity of an MAPK cascade would be tuned for compatibility with the eventual physiological outcome. Take for example the *S. Cerevisiae* Hog1 and Fus3 cascades (Table 1). For the Hog1 cascade, which controls osmotic stress adaptation, one would expect low sensitivity with a large linear range to ensure that the cell takes appropriate action in response to a wide range of osmotic pressure changes. On the other hand, for the Fus3 cascade, which controls mating, one might expect high sensitivity so that (i) low magnitude signals, which may be confused with noise, do not inadvertently cause the mating response, and (ii) there

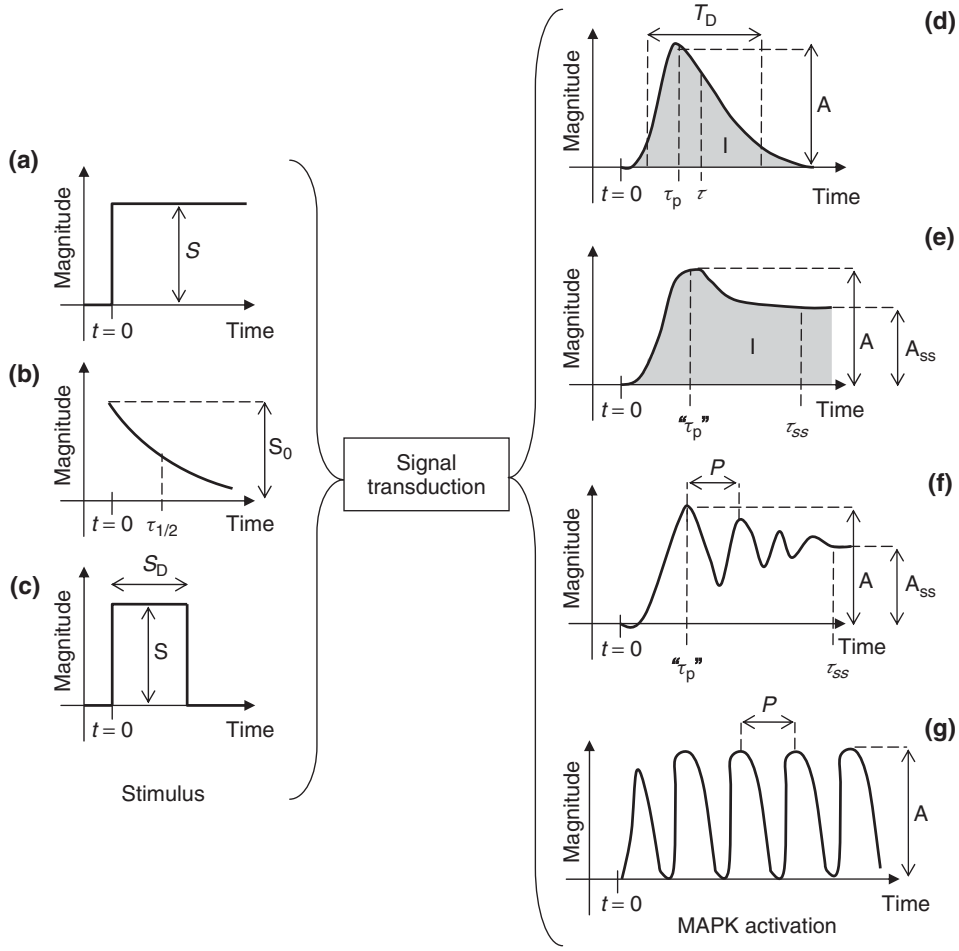

FIGURE 1 | Graphical illustration of different MAPK responses to typical stimuli. (a) A sustained, or step input, characterized by the magnitude of the stimulus S. (b) An exponential decay input, characterized by the initial strength of the stimulus S_0, and a half-life, $\tau_{1/2}$; $S(t) = S_0 \exp\left[\frac{\ln(0.5)\cdot t}{\tau_{1/2}}\right]$. (c) A rectangular pulse, or pulse-chase input, characterized by the signal magnitude S, and the signal duration S_D. (d) A transient or adaptive response. The peak of the signal is described by the amplitude A and the peak time τ_p, signal duration T_D is related to how long the response lasts, the signaling time τ is the time-averaged concentration, and the integrated signal I is the area under the curve. Different mathematical forms have been used to quantify these signaling characteristics; for example, Heinrich et al. proposed $A = \int_0^\infty K^*(t)dt2 \cdot T_D, \tau = \int_0^\infty t \cdot K^*(t)dt / \int_0^\infty K^*(t)dt$, and $T_D = \sqrt{\int_0^\infty t^2 \cdot K^*(t)dt / \int_0^\infty K^*(t)dt - \tau^2}$, where K^* is activated MAPK.[37] (e) A sustained response. The parameters A, τ_p, and I have similar meaning as for the transient response, whereas the steady-state is described by a magnitude, A_{ss}, and a time to reach 99% of the steady-state value, τ_{ss}. (f) A damped oscillatory response. The steady-state and peak are characterized similarly as to the sustained response, while the duration of the initial oscillatory period is described by P. Damped oscillations do not always show a constant period. (g) A sustained oscillatory response. After an initial transient period, oscillations are steady with a constant amplitude A and period P.

are essentially only two steady-state cascade activation states, high and low, that correspond to the 'yes-or-no' mating response.

Mechanisms Driving Input/Output Behavior of MAPK Cascades

Linear Cascades without Feedback

The simplest representation of an MAPK cascade is shown in Figure 2(a), where each level consists of a single reaction. Exploiting this simplified topology and assuming all reactions to operate far from saturation ('weakly activated pathways'), Heinrich et al. showed that for an exponential decay input, the mean signaling time and duration of transient MAPK signaling depend only on phosphatases, whereas signal amplitude is mainly determined by kinases.[57] Interestingly, these predictions were experimentally validated 3 years later.[58] It was also predicted that signaling times and durations become larger with more cascade levels, a trend that has

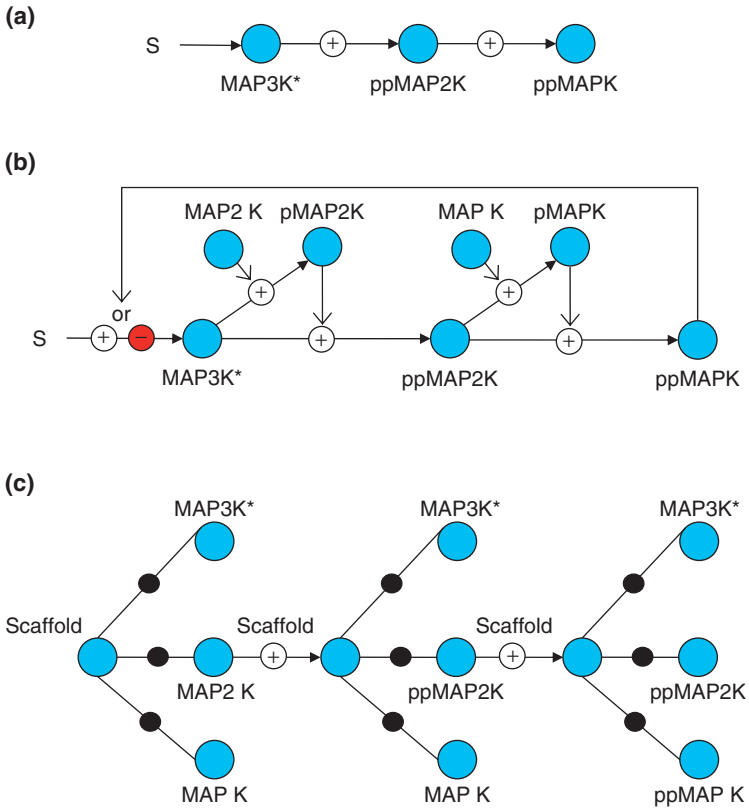

FIGURE 2 | Schematic representations of MAPK cascade signal propagation reactions. In all schemes, ATP is assumed to be in excess and rapidly binding. Throughout the literature, different, yet experimentally relevant assumptions have been made regarding rate constants, relative enzyme concentrations and saturability in diverse cell systems, and this has resulted in a variety of different rate laws being used to describe the reaction kinetics. Readers interested in these details are referred to the original papers where these different MAPK cascade models are analyzed. (a) Simple linear cascade, where double phosphorylations are lumped into a single reaction. (b) Typical cascade where MAP2K and MAPK activation consist of double phosphorylation steps. For the ERK1/2 cascade, MEK (MAP2K) activation is processive, whereas ERK (MAPK) activation is distributive. (c) The 'reactor model' of scaffold-mediated MAPK cascade activation. Each kinase in the cascade is sequentially activated without dissociating from the scaffold complex.

been observed in many studies of MAPK signaling [e.g.,[47,54]].

Although Figure 2(a) shows a simplified MAPK cascade, for a general MAPK cascade (without feedback) that incorporates double phosphorylation of MAP2K and MAPK (Figure 2(b)), Kholodenko et al. showed that the global response of ppMAPK to the signal is the product of all the local response coefficients,[56,60]

$$R = r_S^3 r_3^2 r_2^1. \tag{3}$$

If individual steps of the MAPK cascade have local response coefficients greater than 1, having more levels in the cascade will result in a higher sensitivity of the output to the input signal. This is illustrated in Figure 3 as a shift from curve *a* toward curve *c* on the plot of the steady-state input/output properties of an MAPK cascade. Such an increase in input/output sensitivity was observed experimentally in *Xenopus* oocyte extracts.[38] The Hill coefficient equals 4.9 for a three-tired cascade (the ppMAPK response to MAP3K*), much greater than 1.7 measured for a two-tired cascade (the ppMAP2K response to MAP3K*), showing that sensitivity amplification is a fundamental property of MAPK cascades.[60,61]

Theoretical analysis has revealed that multiple phosphorylation steps also lead to increased sensitivity and switch-like input/output behavior[38,39] mediating a shift in the steady-state diagram from curve *a* toward curve *c* in Figure 3. Moreover, it has recently been shown that a single cascade level with double-site phosphorylation that occurs through a nonprocessive, distributive mechanism can exhibit bistability and hysteresis,[39,62] which is illustrated in Figure 3 as a further shift of the input/output map from curve *c* to curve *d*. The necessary prerequisites for bistability include (*i*) a competitive inhibition of at least one of the two opposing enzymes by the monophosphorylated intermediate, (*ii*) saturation of that enzyme by its substrate, and (*iii*) in the first cycle the catalytic constant ratio of phosphorylation and dephosphorylation steps is less than that in the second cycle. Recent theoretical investigations based on comprehensive Monte Carlo sampling of the Huang–Ferrell MAPK cascade model parameters demonstrated that bistability is a robust system property of such cascades, with 10% of all parameter sets exhibiting bistability.[63] This analysis has also shown a 10% region of sustained oscillations,[63] which were predicted previously for an MAPK cascade with high output/input sensitivity and negative feedback.[46] Importantly, sequestration of a kinase by its substrate

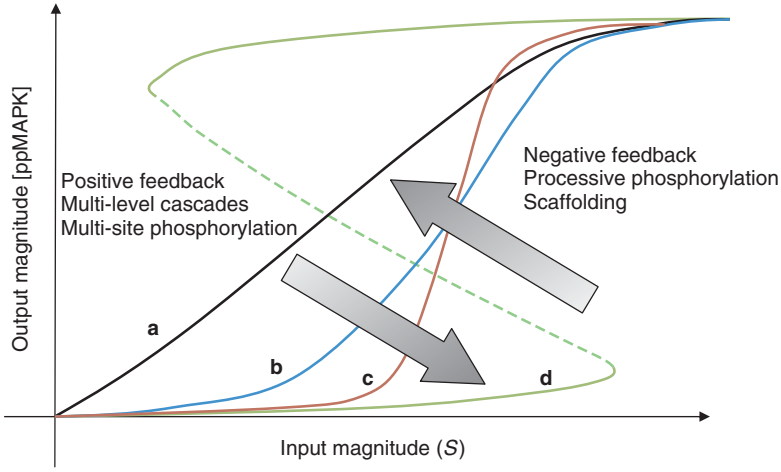

FIGURE 3 | Summary of the effects of various MAPK cascade features on the steady-state, input/output relationships. Curve a (black) is a classical Michaelian response, which is the least sensitive, but responds to the largest range of input signals. Curves b (blue) and c (red) denote progressively more sensitive input/output relationships, which can be approximated by a Hill equation [(ppMAPK) = $(S)(S_{50}^n + \text{ppMAPK})^n$], where n is the Hill coefficient and S_{50} is the half-maximal dose. Higher Hill coefficients give more sigmoidal responses, and therefore more sensitive responses. Curve *d* (green) represents a bistable response that shows hysteresis. The solid green lines denote stable steady-states, whereas the dashed green line denotes unstable steady-states. As the input magnitude is increased slowly from zero, the systems follow the lower branch of the curve *d* until the dotted line is reached, at which point the system jumps to the high branch. If the input is then decreased slowly back to zero, the upper branch of curve *d* is followed until the dotted line is reached, at which point the system jumps back to the low branch.

at the next cascade level is equivalent to negative feedback, which can lead to sustained oscillations in the presence of bistability in a cascade.[64,65] In fact, Shvartsman and coworkers showed that bistability at a single level is a necessary condition for sustained oscillations of an entire cascade with two or more levels.[63] Importantly, the potential for oscillations in MAPK cascades was recently corroborated experimentally. It was demonstrated that stimulation of human mammary epithelial cells with low EGF doses induces sustained oscillations of nuclear, active ERK1/2 (H.S. Wiley, personal communication).

Although a physiological role for MAPK activity oscillations is not known, one possibility is that these oscillations may serve as a 'persistence indicator', providing information for downstream targets that an upstream activating signal still remains. This would be similar to the physiological function of sustained oscillations in p53 expression, which are thought to indicate that DNA damage (the upstream signal) persists.[66] But why would an oscillatory signal, rather than a simpler sustained signal, would be used as a persistence indicator? We hypothesize that oscillations would be used in cases where the appropriate cellular response occurs only when the indicator is activated in short pulses. As both p53 expression and MAPK activity change the expression of a large number of genes, short pulses versus sustained p53 expression or MAPK activity would cause drastically different gene expression responses, and therefore distinct cellular outcomes.

Cascades with Feedback

Although MAPK cascades without feedback can exhibit a wide variety of behaviors, including bistability and oscillations, the role of feedback is to modulate such behavior, making it either more robust or eliminating it altogether. In the mammalian ERK1/2 pathway, ERK1/2 can phosphorylate and inactivate upstream, positive regulators, such as SOS,[50] Gab1,[67] and the EGF receptor,[9,68] creating negative feedback loops. Positive feedback has also been observed in the ERK1/2 cascade,[51] JNK cascade,[31,32] and *Xenopus* oocyte MAPK (Mos/MEK1/p42 MAPK) cascade.[69] The overall response coefficient (R_F) for MAPK cascade with feedback becomes,[56]

$$R_F = \frac{R}{1 - R \times F} \tag{4}$$

where F is the feedback strength given by $F = \mathrm{d}\ln v_1 / \mathrm{d}\ln[K^*]$, which quantifies the change in the rate v_1 of kinase activation at the first level brought about by a 1% change in the active kinase concentration at the terminal cascade level. For negative feedback $F < 0$, and for positive feedback $F > 0$.

Negative Feedback

Equation 4 shows that as the negative feedback strength is increased, overall sensitivity decreases.

Thus, we move from curve *c* toward curve *a* in Figure 3, increasing the working linear range of the cascade. An important effect of negative feedback, which is well known in engineering, is to make the output more robust to disturbances within the feedback loop. This is particularly clear at high feedback strengths ($-R \cdot F \gg 1$), where R_F depends mostly on the feedback strength ($R_F \approx 1/F$),[70] and is virtually insensitive to the properties of the individual MAPK cascade levels within the feedback loop. To illustrate how even at relatively low strengths, negative feedback attenuates disturbances, we consider a perturbation (ε to a single response coefficient,

$$r_2^1 + \varepsilon. \tag{5}$$

Substituting this into Eq. (2), we obtain

$$R = r_S^3 r_3^2 (r_2^1 + \varepsilon). \tag{6}$$

One can see that without negative feedback, the effect of the perturbation on the total response is multiplied by the rest of the local response coefficients. However, with negative feedback present, only if the perturbation is large relative to the local response coefficient will the global response be significantly affected,

$$R_F = \frac{r_S^3 r_3^2 (r_2^1 + \varepsilon)}{1 - r_S^3 r_3^2 (r_2^1 + \varepsilon) \cdot F} = \frac{R}{[1/(1 + \varepsilon/r_2^1)] - R \cdot F}. \tag{7}$$

As negative feedback decreases input/output sensitivity gives robustness to disturbances within the feedback loop, and increases the operational linear range, an MAPK cascade with negative feedback can behave as a robust linear amplifier.[70,71]

Another function of negative feedback is to create an adaptive, or transient response (Figure 1(c)) to a step input (Figure 1(a)). In fact, a transient response can be obtained when there is either negative feedback to upstream kinases or feedforward activation of MAPK phosphatases.[72] Since such feedfoward regulation has not been described previously for MAPK cascades, negative feedback is a critically important design feature for controlling transient response characteristics. Clearly, feedback must be strong to induce transient signaling. However, strong feedback requires highly active MAPK signaling, which can lead to saturation of the negative feedback and a sustained, rather than an adaptive response.[47] Thus, there is a fine balance for obtaining appreciable signal amplitude and efficient adaptation. One solution is to separate the MAPK activation time scale from the feedback timescale with multiple intermediate steps in the feedback loop.[47] However, too many intermediate steps and/or too strong of a feedback can lead to a large time delay, which can cause damped (Figure 1(e)) or sustained oscillations.[40,73,74] Indeed, ppERK1/2 oscillations in response to a step input of fibroblast growth factor have been observed experimentally.[75] Motivated by previous theoretical predictions that negative feedback can underlie oscillatory behavior,[46] Nakayama and coworkers experimentally confirmed that the negative feedback from ppERK1/2 to SOS, an ERK1/2 cascade activator upstream of the MAP3K Raf, was essential for these oscillations. Although more data are needed to distinguish completely whether the ERK-SOS negative feedback induces damped or sustained oscillations, this illustrates how theoretical and experimental work can synergize to advance our understanding of MAPK cascade behavior.

We conclude that negative feedback can have disparate effects on MAPK signaling, making the steady-state, input/output relationships more linear, but also serving as a potential source of instability for the dynamic responses. Since the linearity of the stationary, input/output characteristics mainly depends on the feedback strength, whereas the bifurcation point of the onset of oscillations depends on both the feedback strength and the feedback delay period,[46] in principle these distinct roles of negative feedback for MAPK signal processing can be regulated separately.

Positive Feedback

When feedback is positive ($F > 0$), Eq. (4) shows that signals are amplified rather than attenuated. As the strength of the positive feedback is increased, the input/output response shifts from curve *a* toward curve *c* in Figure 3, making MAPK responses more sensitive and switch-like, but decreasing the operational linear range of the cascade. Importantly, positive feedback can shift the steady-state response all the way to curve *d*, endowing a cascade with bistability.[34,76–78] Curve *d* is obtained when the denominator in Eq. (4) equals zero ($F \cdot R = 1$), which corresponds to a saddle-node bifurcation where two steady states, one unstable and one stable, are created or destroyed.[46] However, the term $F \cdot R$ cannot be more than 1 and, therefore R_F cannot be negative at any stable steady state.[46] Additionally, positive feedback combined with slow negative feedback can trigger relaxation oscillations (Figure 1(g)), where the system oscillates between the high and low branches of the hysteresis curve.[40,42,43,46,65,79]

As noted above, bistability can arise from distributive double phosphorylation,[39] and this is

a robust property of MAPK cascades.[63] Here, we see that positive feedback loops can also lead to bistability. Why would evolution incorporate positive feedback into an MAPK cascade, if bistability can already be achieved with only the cascade itself? The answer to this question relates to the robustness of the bistable behavior; although bistability can exist without positive feedback, random parameter perturbation and/or reaction rate fluctuations due to small numbers of molecules erode the bistable behavior, causing the system to become monostable and/or switch between the bistable states.[1,52,80–84] Such considerations are particularly important in extremely small cellular compartments such as neuron dendrites, where it is proposed that MAPK bistability plays a central role in long-term potentiation and memory. Without positive feedback to make the bistability robust, reaction rate fluctuations destroy the maintenance of the high ppMAPK steady-state, and thus could not serve the proposed physiological function.[80,81,84]

Scaffolding

Scaffolds are nearly ubiquitous in MAPK cascades (Table 1). They ensure signaling specificity by bringing the proper cascade components together in high local concentrations, facilitating signal transmission while preventing pathway crosstalk.[5,85,86] Levchenko et al. showed that a main effect of scaffolding is to decrease sensitivity,[87] shifting the input/output diagram from curve *d* toward curve *a* in Figure 3. This is a direct result of the scaffold changing the multi-stage, multi-step phosphorylation mechanism shown in Figure 2(b) into a processive mechanism shown in Figure 2(c).

Scaffolds have an optimal concentration for signal propagation,[88] which depends on the concentration of the MAPK cascade components, and to some extent, on their affinities for the scaffold.[57,87,89,90] Low scaffold concentration leads to formation of just a few functional scaffold complexes; alternatively, high scaffold concentration leads to formation of non-functional complexes and sequestration of MAPK cascade components. Thus, there is an intermediate concentration of scaffold protein that provides a maximum signaling benefit. The presence of an optimal scaffold concentration helps explain experimental results showing that when scaffolds are overexpressed, signaling is decreased,[91] but when scaffolds and the MAPK cascade proteins are overexpressed together, signaling is increased.[92] It was shown that the number of functional scaffold complexes decreases as $1/[\text{Scaffold}]^{(m-1)}$, where m is the scaffold occupancy, as the scaffold concentration is increased past the optimum.[87] Thus, the optimum concentration peak should be sharper for a scaffold that binds all three members of the MAPK cascade (e.g., KSR), compared with one that binds only two cascade members (e.g., MP-1). Numerical simulations of Heinrich et al. also demonstrate this behavior.[57] One situation a cell may use a two- instead of a three-member scaffold is when variations in scaffold and MAPK cascade component abundances are large; the two-member scaffold would be more robust to such variation.

The above studies only consider *intra*-scaffold signaling; however, it was shown that *inter*-scaffold interactions can also occur,[93,94] which prompted reconsideration of what a 'functional' scaffold complex is. A recent theoretical investigation considered an inter-scaffold signaling model, arguing that the traditional 'reactor model' (Figure 2(c)) imposes restrictive energetic and steric constraints.[95] A similar 'membrane recruitment' model is proposed as a preferred mechanism where partially occupied scaffolds are concentrated in the same cellular compartment, such as the plasma membrane (PM) (Figure 4).[5,85,86] Although there are a plethora of potential signaling mechanisms that can occur within this membrane recruitment, inter-scaffold signaling model, the fundamental tenet is scaffold-driven co-localization of cascade components.

SPATIOTEMPORAL MAPK SIGNALING

MAPK cascades relay extracellular stimuli from the PM to pivotal cellular targets distant from

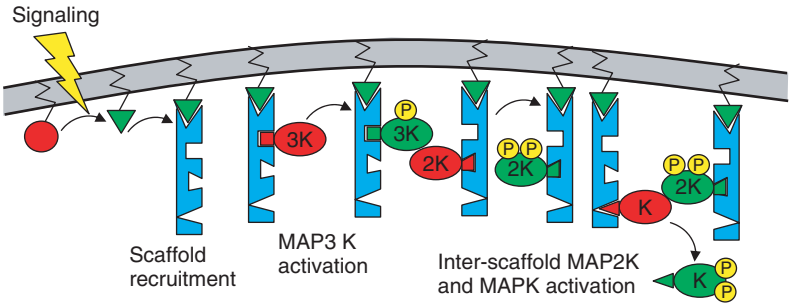

FIGURE 4 | The inter-scaffold signaling scenario. Signals cause activation of a membrane component which recruits the MAPK cascade scaffold. The scaffold can be empty, or contain any combination of MAPK cascade components, active or inactive. The role of the scaffold is to concentrate the MAPK cascade components into a small volume, where they can effectively interact and propagate the signal. Green color denotes signaling activity, while red color denotes inactivity.

the membrane in both the cytoplasm and the nucleus, for example, transcription factors. A critical feature of MAPK information processing is spatial inhomogeneity of output signaling, which cannot be captured by the coarse-grained approaches discussed above. Here we show how basic properties of spatial separation of MAPK components, diffusive movement, and endocytosis underlie processing and transmission of signals carried out by phosphorylated kinases.

Spatial Gradients of MAPK Signaling

Often, activating signals are present only on the PM where activated receptors and small G-proteins, such as Ras, reside, whereas inactivating signals (carried out by phosphatases) are distributed throughout the cytoplasm. In such scenarios, precipitous gradients of phosphorylated kinases can develop, impeding information transfer to distant cellular locations, such as the nucleus. In a model where MAP2K activation is localized to the PM, and ppMAP2K dephosphorylation occurs throughout a cell with linear kinetics, the steady-state ppMAP2K gradient is almost exponential and its depth is controlled solely by the ratio of the phosphatase rate constant to the diffusion coefficient.[96] When phosphatase activity is high compared with the diffusion coefficient, gradients are steep, and ppMAPK signals cannot propagate far from the PM. As kinases at subsequent levels of the cascade are not attached to the membrane, but are freely diffusing, the gradient becomes shallower down the cascade.[65] When a cytoplasmic cascade level is bistable, the distance the ppMAPK signal can travel is significantly increased.[97] On the basis of typical diffusion coefficients for proteins and phosphatase rate constants, MAPK activation gradients were predicted to be significant for distances ~2–5 μm and greater.[96] Subsequently, such gradients of protein active forms were reported for the small GTPase Ran,[98] phosphorylated stathmin oncoprotein 18,[99] and importantly the yeast MAPK Fus3.[100]

If spatial gradients of protein active forms are large, how can signals reach distant cellular locations? One possibility is endocytosis, which can bring signals from the PM into the cell interior.[101] Consider a spherically symmetric cell where a fraction ϕ of the total kinase activity v_{kin} is located on the PM, the remaining fraction of the kinase activity ($1-\phi$) is located on the endosomes, and the phosphatases are uniformly distributed in the cytoplasm (Figure 5(a)). The resulting signaling dynamics can be described by the so-called reaction–diffusion equations. If the phosphatases are far from saturation, then in the cytoplasm the phosphorylated signal C_p (e.g., MAP3K* or ppMAP2K) satisfies the following reaction–diffusion equation,[96]

$$\frac{\partial C_p}{\partial t} = \frac{D}{r^2}\left[\frac{\partial}{\partial r}\left(r^2\frac{\partial C_p}{\partial r}\right)\right] - k_p C_p. \qquad (8)$$

Here, D is the diffusion coefficient and k_p is the phosphatase rate constant. The cytoplasm is partitioned into two regions (see Figure 5(a)): between the PM and the PM side of the endosomes ($R_{PM} > r > R^{PM}_{endos}$, Region I) and between the nuclear membrane (NM) and the NM side of the endosomes ($R^{NM}_{endos} > r > R_{nuc}$, Region II). It is assumed that the endosomes, which move slowly compared with the characteristic time of (de)phosphorylation reactions, reside within a fixed, thin layer ($R^{PM}_{endos} > r > R^{NM}_{endos}$) of the cytoplasm (Figure 5(a)). Although for illustrative purposes we here consider only a single endosome compartment, there can be multiple endosome compartments at different radial positions (e.g., early, recycling, and late endosomes). The steady-state solution to Eq. (8) ($\partial C_p/\partial t = 0$) specifies the characteristic length of the local gradients ($L_{gradient}$) in terms of the ratio $\alpha = \sqrt{k_p/D}$ of the phosphatase rate constant and diffusivity, $L_{gradient} = 1/\alpha$.[96,102] However, the resulting spatial profile and absolute magnitudes of these gradients depend on the kinases and are specified by the boundary conditions. With a single endosome compartment, there are four boundary conditions: (1) at R_{PM}, the diffusion flux equals the kinase rate at the PM, (2) at R_{nuc} there is no diffusion flux, (3) at R^{PM}_{endos}, and (4) at R^{PM}_{endos} the flux balances include the kinase rate on endosomes, diffusion flux in the cytoplasm, and the flux from the C_p concentration difference at both sites of the endosome compartment.[65]

Calculated steady-state gradients of C_p with and without endocytosis are compared for a typical mammalian cell in Figure 5(b), and for a large cell, such as a *Xenopus oocyte* in Figure 5(c). In both cases, increasing α (decreasing diffusivity or increasing phosphatase activity) leads to deeper gradients, and for the large cell values of α must be small for signals to propagate to the nucleus. Large values of α can lead to highly localized signaling (Figure 5(c); $\alpha = 0.05\ \mu m^{-1}$), implying to obtain tight spatial control of MAPK signaling, cells may have evolved means of either decreasing the effective diffusion coefficient (e.g., localized, non-diffusible binding sites), and/or increasing soluble phosphatase activity. Interestingly, high phosphatase activity alone is sufficient for creating localized MAPK activity when activating kinases are localized. As expected, Figure 5(b, c) show that endocytosis increases the signal

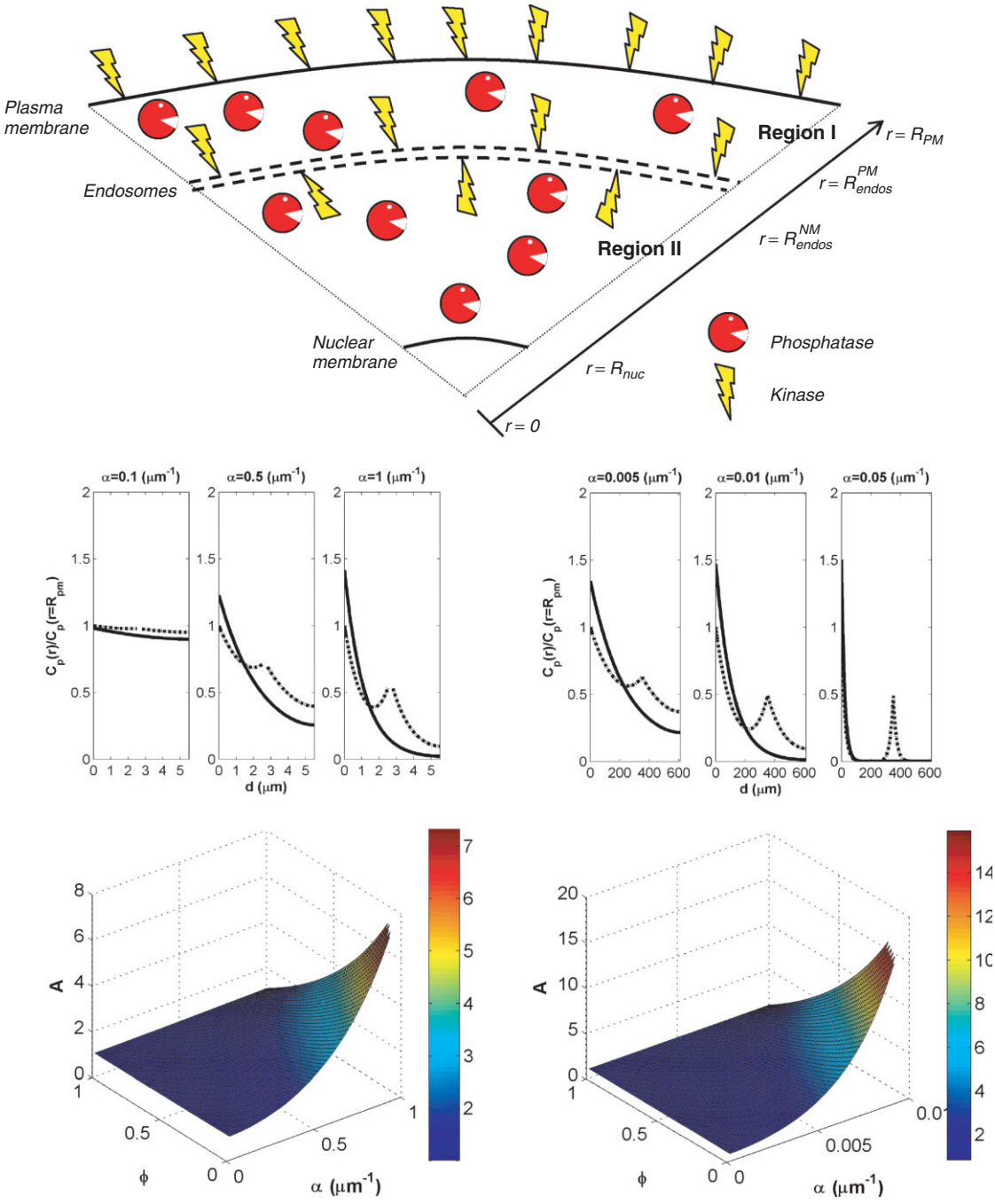

FIGURE 5 | Effect of endocytosis on spatial signal propagation. (a) Schematic of spherically symmetric, endocytosis-enhanced signal propagation. Signals are generated at the PM and in a small region of space where the endosomes reside, and are terminated by phosphatases everywhere else. Signals can diffuse throughout the region between the plasma and NMs. (b, c) Steady-state spatial profiles of C_{p} as a function of the distance from the plasma membrane $d = R_{\mathrm{PM}} - r$. Dash-dot lines correspond to cases where half the kinase activity localized at the endosomes ($\phi = 0.5$), while solid lines denote cases where all kinase activity is located at the PM. Panel (b) corresponds to a cell with dimensions $R_{\mathrm{PM}} = 9\ \mu\mathrm{m}$, $R_{\mathrm{nuc}} = 3.5\ \mu\mathrm{m}$, $R_{\mathrm{endos}}^{\mathrm{PM}} = 6.5\ \mu\mathrm{m}$, $= 6.2\ \mu\mathrm{m}$. The endosome width, $0.3\ \mu\mathrm{m} = R_{\mathrm{endos}}^{\mathrm{PM}} - R_{\mathrm{PM}}^{\mathrm{endos}}$, was taken to be three times a typical endosome diameter ($\sim$100 nm), since endosomes are not well packed. Panel (c) corresponds to a large cell, such as a *Xenopus* oocyte, with dimensions $R_{\mathrm{PM}} = 1000\ \mu\mathrm{m}$, $R_{\mathrm{nuc}} = 390\ \mu\mathrm{m}$, $= 650\ \mu\mathrm{m}$, and = 649 μm. (d, e) Endocytosis-enhanced signal amplification at the NM as a function of α and ϕ. Signal amplification is defined as $A = C_{\mathrm{p}}(R_{\mathrm{nuc}}, \phi)C_{\mathrm{p}}(R_{\mathrm{nuc}}, \phi = 1)$. Cell dimensions used for panels (d) and (e) are the same as for panels (b) and (c), respectively.

magnitude at the NM; furthermore, Figure 5(d, e) demonstrate that regardless of the values of ϕ and α, the NM signal amplification is always greater than one, implying that endocytosis should always increase the signal magnitude at the nucleus. As the fraction of kinase activity at the endosomes ($1-\phi$) or α increase, signal amplification at the NM becomes greater. We conclude that when phosphatase activity is high or diffusivity is low ($\alpha * R_{PM} \sim 10$), endocytosis may play a critical role in signal propagation from the PM to the NM.[101,103,104]

MAPK Signaling over Long Distances

Spatial gradients pose a particular problem when signals must travel over distances greater than 10–100 μm, for which diffusion is insufficient. For the transport of ppMAPK signals from the PM to the nucleus in large cells like *Xenopus* oocytes (~1 mm in diameter), it has been proposed that endocytosis can bring the signal source closer to the destination, reducing the spatial gradient and increasing information transfer.[101] Simulation results suggest that if phosphatase activity is low ($\alpha < 0.01\ \mu m^{-1}$), endocytosis combined with simple diffusion is a plausible mechanism for signal propagation to the nucleus in such cells (Figure 5(c)). However, typical diffusion coefficients ($\sim 10 \mu m^2/s$) and phosphatase activities ($\sim 1\ s^{-1}$) give $\alpha \sim 0.1$, so unless phosphatase activity is regulated to be extremely low during the initial time period of 10–20 min following stimulation, it is unlikely that endocytosis plays a significant role for signal propagation in *Xenopus* oocytes. Alternatively, cytoplasmic scaffolds and molecular-motor driven transport of signaling complexes may play a role in spatial signal propagation by protecting ppMAPK from cytoplasmic dephosphorylation.[101,105,106] For the centimeter- and even meter-scale transport of signals, such as from neuron terminals to the nucleus via the axon, especially in a large animal's extremity (e.g., from a giraffe's lower leg to its brain), present an even more challenging problem.[107] Although the retrograde transport of endosomes is an important signaling vehicle, in the NGF-TrkA system, signals can propagate through mechanisms other than endosomal transport.[108,109] Additionally, the average velocity of molecular motors (1–10 μm/s[110]) is not fast enough to account for experimentally observed signal propagation time,[111] posing the question of what mechanisms may be able to transport signals faster than retrograde transport, and over distances of meters. It has been proposed that traveling waves of protein activation can perform this task.[97] Such waves can occur when a downstream kinase positively feeds back to a cytoplasmic upstream kinase, and the stimulus duration exceeds a certain threshold. Simulation results suggest that these traveling waves transport signals at tens of μm/s, and as the strength of positive feedback is increased, the velocity increases (up to hundreds of μm/s). These traveling waves are much faster than retrograde transport, fast enough potentially to explain the experimentally observed speed of signal propagation in the NGF-TrkA system.

FUTURE DIRECTIONS

Although substantial progress has been made in understanding MAPK information processing, comparing the biological complexity listed in Table 1 and the diversity of responses shown in Figure 1 to the current level of understanding shows that there is much left to explore. Much work has been done to elucidate how MAPK cascade topology affects steady-state input/output behavior; however, equally important and less studied is how cascade topology affects the transient characteristics of MAPK activation responses, such as peak time, duration, and integral. Future work will give more focus to discovering how network topology controls these transient response characteristics. How inter-scaffold signaling affects MAPK activation is just beginning to be investigated. The intricacies of how MAPK phosphatases (MKP) can affect MAPK signaling have received little theoretical attention, although several complex mechanisms have been described, such as nuclear sequestration of MAPK by MKP,[112] stabilization of MKP by MAPK,[44] and cooperative activation of MKP activity by MAPK phosphorylation.[113] How MAPK cascades mechanistically control cellular outcomes remains an open question. It is thought that downstream targets of MAPK, such as transcription factors (e.g., c-Fos) and feedback regulators of the MAPK cascade [e.g., MKP and Receptor-Associated Late Transducer (RALT)], play a role in determining cell fate.[25,114,115] Future work will extend MAPK cascade models to include downstream MAPK targets and gene expression responses, moving closer to gaining mechanistic understanding of how MAPK cascades control physiological outputs. Application of information theoretic approaches may yield further insight into MAPK signaling. Such approaches are based on the Shannon entropy, which, analogous to the thermodynamic meaning of entropy, characterizes the 'disorder' of a probability distribution; high entropy means low information, and vice versa. Information theory has been used in the signal processing and communication fields for decades, and has recently

been applied to NF-κB signaling (A. Hoffmann, personal communication). Future work will elucidate the roles of various MAPK cascade architectural and kinetic properties in terms of information processing ability, comparing and contrasting these new features to more traditionally known function as described in this review. Spatial modeling of MAPK cascades is in its relative infancy, being mainly limited to analysis of single cascade levels and steady-state gradients. It is only recently that theoretical work has been extended to describe signal processing by multiple cascade layers and feedforward and feedback loops.[33,65,72,116,117] Future work will incorporate spatial descriptions of entire MAPK cascades, including scaffolds, and both their steady-state and transient behavior will be analyzed. As the spatial resolution of experimental imaging techniques improve, these future spatial analyses will shed new insights into how MAPK cascades can control such a variety of physiological responses.

NOTES

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