Oscillations in intracellular signaling cascades

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In this paper, we study the oscillatory dynamics of intracellular signaling cascades. We derive a reaction-diffusion model of the mitogen-activated protein kinase cascade, and use it to show how oscillations of the protein kinase concentrations can occur as a function of the depth of the cascade. We find that only cascades with depths of three or more layers undergo oscillatory instabilities. In addition, the oscillatory instability is spatially uniform. Thus, the oscillations synchronize the protein kinase concentrations and result in them being uniformly distributed in the cytosol, despite the presence of protein kinase diffusion. Finally, we show how the oscillations are perturbed when parallel cascades "crosstalk." We find that the protein kinases in the downstream layers of the cascade are less perturbed than those in the upstream layers. In particular, cascades of three layers are able to maintain the total power of the protein kinase activities at approximately the unperturbed level. Taken together, our results suggest that only cascades of at least three layers can synchronize the oscillations of protein kinases in the cytosol and operate in parallel in the presence of crosstalk without loss of signaling fidelity.

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I. INTRODUCTION

Within a cell, various signaling pathways convert extracellular stimuli into cellular responses by transducing signals downstream from activated cell-surface receptors into the nucleus. These signals are key to the proper regulation of the physiology and development of living organisms. In recent years, there has been an increase in the understanding of the functioning of these signaling pathways brought about by dramatic advances in experimental methodology in genomics and molecular biology. Consequently, physicists have also begun to build quantitative models of signaling pathways in order to see if there are any "laws" governing the dynamics of signaling pathways.

In this paper, we focus on a ubiquitous motif of signaling pathways, the mitogen-activated protein kinase cascade [1]. It is a highly conserved cascade of protein kinases that is found in organisms as diverse as *S. cerevisiae* (yeast), *C. elegans* (nematode worm), *D. melanogaster* (fly), and mammals. In addition, it is biologically versatile. Many cells possess several mitogen-activated protein kinase cascades acting in parallel (such as ERK, JNK, and p38). For example, in yeast, the regulation of mating from the presence of pheromones, filamentation from the lack of nutrients, glycerol production from high osmolarity, etc., are all conducted via a mitogen-activated protein kinase cascade.

Many theoretical models of the mitogen-activated protein kinase cascade have been previously developed. For example, it is now known that the cascade functions as an ultrasensitive switch [2], in which none of the terminal protein kinases are activated until a certain threshold in the stimulus is crossed, in which case almost all of the terminal protein kinases become activated. It is also known that, in the presence of positive feedback, the cascade functions like a bistable "memory module" [3], in which case a transient stimulus is converted into an irreversible response. In the presence of negative feedback, however, the cascade exhibits transient activation [4].

In this paper, we develop a model of the mitogen-activated protein kinase cascade that includes negative feedback and protein kinase diffusion. Most if not all of the existing models of signaling pathways do not consider protein kinase diffusion. Diffusion is important because the proteins are located in an intracellular environment that is inhomogeneous and whose concentrations are not governed simply by the empirical law of mass action [5]. Whether diffusion is a good approximation or not depends on the time and spatial scales involved. In the highly crowded environment within a cell, one would expect the average time for a protein to diffuse in the cytoplasm to be roughly $\sim$10 min. This is about the time scale for signal propagation along the mitogen-activated protein kinase cascade. The importance of protein diffusion in the intracellular environment has also been previously highlighted in the literature. For example, Bhalla [6] examined how diffusion and subcellular compartmentalization influences the underlying signaling processes and gives rise to a diversity of signaling outcomes which may include washout of the signals, signal amplification, and conversion of steady responses to transients. Elf and Ehrenberg [7,8] adapted Monte Carlo techniques to efficiently sample trajectories of reaction-diffusion master equations, and applied it to understand the separation of bistable biochemical systems into spatial domains of opposite phases. Metzler [9] looked at how spatial fluctuations play a non-negligible part in cellular genetic switching processes. We note that, like the other authors, we do not take into account protein kinase degradation as this happens at a time scale much longer than that required for signal propagation along the cascade.

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The main result of this paper is that our reaction-diffusion model will show that the cascade's response can be oscillatory, but only if the cascade is comprised of three or more layers. While, to the best of our knowledge, there have been no experiments that have observed oscillations in the mitogen-activated protein kinase cascade, we believe that our results will have applications in, for example, the pulsatile release of drugs targeted at protein kinases [10,11] which is postulated to improve pharmacodynamic and pharmacokinetic efficacy. In addition, there has also been a lot of recent interest in biochemical oscillations in signaling pathways. For example, there have been attempts to explain the dynamics of the mammalian circadian clock [12], the Hes1 system whose temporal oscillations are associated with the formation of spatial patterns during development [13], the p53-Mdm2 feedback loop whose number of oscillations supposedly encodes the extent of DNA damage [14], the NF-κB system whose oscillations have been postulated to regulate the transcription of target genes for apoptosis and inflammation, etc. In fact, one can venture to postulate that oscillatory phenomena in biological networks are ubiquitous.

In addition, we will also study the stability of these oscillations when there is "crosstalk" between two parallel signaling cascades. This is in line with the real situation when many cascades are activated concurrently as the cell is subject to many input signals. In particular, we study what happens when a cascade whose protein kinases are oscillating is subject to a stimulus that is itself oscillating. The source of the oscillating stimulus is presumed to be a parallel cascade whose protein kinases are oscillating and therefore modify the stimulus.

This paper is organized as follows. In Sec. II, we derive our reaction-diffusion model of the mitogen-activated protein kinase cascade. In Sec. III, we perform a linear stability analysis on this reaction-diffusion model and study how an oscillatory instability may develop as a function of the depth of the cascade and the various parameters characterizing the biochemical kinetics of the cascade. Finally, in Sec. IV, we present our conclusions.

II. METHODS

The mitogen-activated protein kinase cascade is a ubiquitous motif that is comprised of three layers whereby the protein kinases in each layer are activated sequentially. First, cell surface receptors that are activated by the extracellular ligands activate intermediate transducers such as the Ras proteins (belonging to the family of monomeric GTPases that reside on the cellular membrane). The Ras protein can be considered to be the "input," or stimulus, to the cascade. It recruits and catalyzes the phosphorylation of the protein kinase in the first layer of the cascade, called the mitogen-activated protein kinase-kinase-kinase (MAPKKK), into its active configuration (MAPKKK*). An example of the MAPKKK in the mammalian Ras signaling pathway is the Raf kinase. Simultaneously, the active MAPKKK* is also being dephosphorylated into the inactive state by the presence of phosphatases. This simultaneous phosphorylation-dephosphorylation cycle [15] is repeated for the next two layers of the protein kinase cascade. Thus, the phosphorylated MAPKKK* in turn catalyzes the phosphorylation of the protein kinase in the second layer of the cascade, the mitogen-activated protein kinase-kinase (MAPKK), into its active configuration (MAPKK*). An example of the MAPKK in the mammalian Ras signaling is the MEK protein. Simultaneously, the active MAPKK* is also being dephosphorylated into the inactive state by the presence of phosphatases. Finally, the phosphorylated MAPKK* catalyzes the phosphorylation of the mitogen-activated protein kinase (MAPK), the protein kinase in the third layer of the three-layer cascade, into its active configuration (MAPK*). An example of the MAPK in the mammalian Ras signaling pathway is the ERK protein. Simultaneously, the active MAPK* is also being dephosphorylated into the inactive state by the presence of phosphatases.

The fully activated protein kinases, namely MAPKKK*, MAPKK*, and MAPK*, dissociate from the cascade and diffuse within the cytosol. In addition, the terminal activated protein kinase MAPK* translocates into the nucleus and acts on specific target genes, leading to the transcriptional control of the expression of its target genes. While the exact physical mechanism of the movement of the terminal activated protein kinase into the nucleus is not completely known, there are evidence that the process occurs by diffusion [16]. In this paper, we assume diffusion to be the only mechanism for activated protein kinase mobility in the cytosol.

Many mitogen-activated protein kinase cascades also exhibit negative feedback [17] to serve as a signal quenching mechanism. For example, in mammalian cells, ERK (the MAPK) provides a mechanism for switching off Ras signaling (the stimulus) by acting as an allosteric inhibitor to the activation of the guanine nucleotide exchange protein SOS, which in turn catalyzes the conversion of the inactive Ras (GDP-bound) into its active state (GTP-bound) [18,19].

We thus model the mitogen-activated protein kinase cascade as a reaction-diffusion model with the diffusion of the protein kinases in the presence of a negative feedback loop. The phosphorylation and dephosphorylation of the protein kinases are assumed to be described by Michaelis-Menten kinetics [20]. Thus, the rate of change of the activated protein kinase concentration at a particular layer in the cascade is the difference between the rate of phosphorylation and the rate of dephosphorylation. The negative feedback loop is modeled as the allosteric inhibition [20] of the terminal activated protein kinase on the activation of the upstream protein kinase. Details of these derivations are provided in the Appendix.

We let a_i denote the variables of the reaction-diffusion model, namely the activity of the protein kinase of the ith layer of the cascade, i.e., the ratio of the phosphorylated protein kinase concentration to the total (both phosphorylated and unphosphorylated) protein kinase concentration in the ith layer of the cascade. We assume that the cascade has N layers, so that $i=1,\ldots,N$. We can then write down a set of N coupled equations describing the rates of change of the N protein kinase activities,

$$\frac{\partial a_i}{\partial t} = \tau_i\left(\frac{\alpha_i a_{i-1}(1-a_i)}{(\beta_i+1-a_i)(1+\delta_{i1}\rho a_N)} - \frac{a_i}{\gamma_i+a_i}\right) + \eta_i\frac{\partial^2 a_i}{\partial r^2}, \quad i=1,\ldots,N. \tag{1}$$

These equations are dimensionless. The dimensionless variables t and r denote time and space (i.e., distance away from the membrane), respectively. Space is assumed to be one dimension in this paper, with $r=0$ corresponding to the cellular membrane, and $r=1$ the nucleus.

The interpretations of the various dimensionless parameters in Eq. (1) are as follows. The τ_i's are characteristic time scales governing the chemical kinetics of the ith layer. In this paper, we will assume that the τ_i's are identical for all layers and, without loss of generality, set them all equal to unity.

The α_i's denote the ratios of the maximum phosphorylation velocity of the ith layer to the maximum dephosphorylation velocity of the i layer. Qualitatively, large (small) values of α_i's represent high (low) ith layer protein kinase activity. The product $\alpha_i a_{i-1}$ is identical to the V_1/V_2 parameter in the analysis of Goldbeter and Koshland [15]. The parameter a_0 denotes the dimensionless concentration of the stimulus to the cascade, i.e., the ratio of the input enzyme concentration to the total protein kinase concentration in the first layer. In this paper, we set $a_0=1$ without loss of generality and let the parameter α_1 reflect variations in the cascade stimulus.

The β_i's (or γ_i's) denote the ratio of the Michaelis constant of the phosphorylation (or dephosphorylation) of the i layer to the total ith layer protein kinase concentration, i.e., the degree of saturation of the active (or inactive) protein kinases. In this paper, we will assume that all the protein kinases are always saturated, i.e., that $\beta_i=\gamma_i \ll 1$ for $i=1,\ldots,N$.

The symbol δ_{i1} is the Kronecker delta symbol such that $\delta_{ij}=1$ when $i=j$ but is zero otherwise. Its occurrence simply means that the negative feedback loop acts from the Nth layer to the first layer, but nowhere else in the other layers of the cascade. The parameter ρ quantifies the strength of the feedback; it is inversely proportional to the equilibrium constant of the inhibitor-enzyme binding, such as the binding between the ERK and Ras kinases. Thus, the larger the value of ρ, the "stronger" the "strength" of the negative feedback loop. A value of $\rho=0$ corresponds to a cascade with no negative feedback.

Finally, the η_i's are the dimensionless diffusion coefficients of the protein kinases. Typically, the protein kinases of the various layers have approximately the same mass and therefore approximately the same diffusion coefficients, which works out to be on the order of unity in our dimensionless units.

The boundaries $r=0$ and $r=1$ are assumed to be Neumann boundaries, i.e., $\partial a_i/\partial r|_{r=0,1}=0$ for $i=1,\ldots,N$. These boundary conditions are chosen for illustrative purposes for this paper. In practical models, the boundaries may be Dirichlet or more general Robin boundaries.

Thus, given the boundary and initial conditions, $a_1(r,t=0)=\cdots=a_N(r,t=0)=0$, where $0\leqslant r\leqslant 1$, we numerically integrate Eq. (1) for given values of the parameters to obtain $a_N(r,t)$ for all space r and time $t>0$.

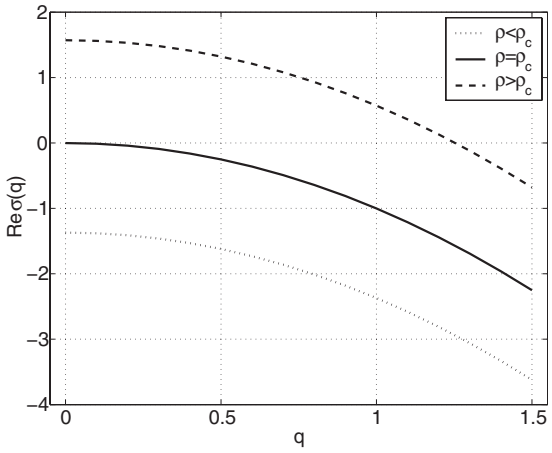

FIG. 1. The real part of the growth rate Re $\sigma(q)$ vs the wave number q for three values of the control parameter $\rho=10,15,20$, with the other parameters set at $\alpha=10$, $\beta=0.01$, and $\eta=1$. The critical value ρ_c for which the growth rate first becomes positive is approximately 15. The maximal growth rate occurs at $q=0$. Although not shown, Im $\sigma(q)>0$ when $\rho>\rho_c$ and is independent of q.

III. RESULTS

A. Oscillatory dynamics as a function of cascade depth

In this section, we show that oscillations in the protein kinase activities, the a_i's, can only occur in cascades that comprise three or more layers. We perform a linear stability analysis on the reaction-diffusion model of Eq. (1), and show how the occurrence of an oscillatory instability depends on the cascade depth. Here, we assume that the velocities and Michaelis constants of every phosphorylation and dephosphorylation reactions in the cascade are identical. Thus, we set $\alpha_1=\cdots=\alpha_N\equiv\alpha$, and $\beta_1=\cdots=\beta_N=\gamma_1=\cdots=\gamma_N\equiv\beta$.

To carry out the linear stability analysis, we first solve for the spatially uniform steady-state solutions a_i^0 and then linearize Eq. (1) about these solutions, $a_i=a_i^0+\delta a_i$. Because all the parameters that appear in Eq. (1) are constant in space, we can use the single Fourier mode ansatz

$$\delta a_i = \delta a_{i,q}(t)\exp(iqr), \quad i=1,\ldots,N, \tag{2}$$

where q is a wave number. Here, for simplicity of analysis, we assume that the spatial domain is infinite so q will take continuous values. Thus, the analysis of realistic models on a spatially finite domain will yield slightly different results. We can further write the time dependence as

$$\delta a_{i,q}(t) = \delta a_{i,q}^0 \exp(\sigma(q)t), \quad i=1,\ldots,N \tag{3}$$

with $\sigma(q)$ the growth rate, possibly complex, of the Fourier mode $\delta a_{i,q}^0$ at wave number q. We then examine $\sigma(q)$ as a function of q. An oscillatory instability is present when Re $\sigma(q)>0$ and Im $\sigma(q)\neq 0$ for some range of q.

In Fig. 1, we plot the real part of the growth rate Re $\sigma(q)$ vs the wave number q when $N=3$. We have chosen $\alpha=10 \gg 1$ to denote a highly active cascade, i.e., one that will yield protein kinase activities that are very close to unity, and $\beta=0.01 \ll 1$ to denote that the protein kinases are saturated. These values are chosen to approximately match the values

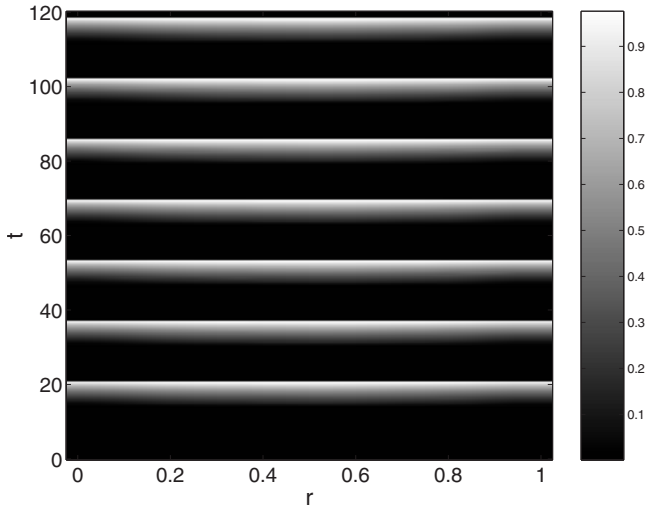

FIG. 2. Space-time plot of the terminal protein kinase activity $a_3(r,t)$ in a three-layer cascade. Space runs from $r=0$ (corresponding to the membrane, say) to $r=1$ (corresponding to the nucleus, say). Time runs from $t=0$ to $t=120$. The parameters are set at $\alpha=10$, $\beta=0.01$, $\rho=100$, and $\eta=1$.

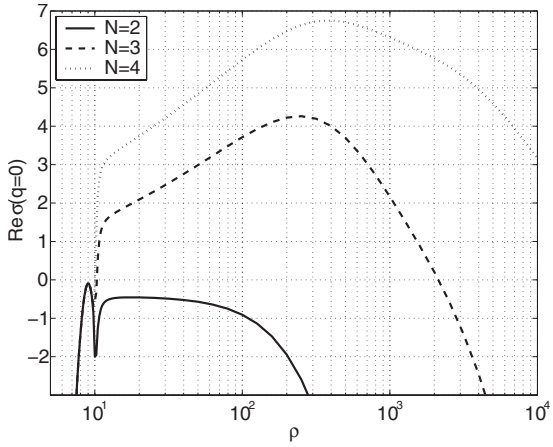

FIG. 3. The maximal growth rate, Re $\sigma(q=0)$ vs the negative feedback parameter ρ for cascades of $N=2,3$, and 4 layers. For $N=2$, there are no values of ρ for which the maximal growth rate is positive.

observed in experiments on the p42 cascade of *Xenopus* oocytes [2]. We will also set the diffusion coefficients to $\eta_i=1$ for $i=1,\ldots,N$. We then vary the negative feedback strength, ρ, as the control parameter, and observe that there exists a critical value of $\rho_c \approx 15$ for which Re $\sigma(q)$ approaches zero. When $\rho<\rho_c$, Re $\sigma(q)<0$ for all q. When $\rho>\rho_c$, Re $\sigma(q)>0$ for a range of q, with the maximal growth rate at $q=0$. Also, although not shown, for $\rho>\rho_c$, Im $\sigma(q)$ is also positive. Thus, the $N=3$ cascade's dynamics is unstable to an oscillatory instability if $\rho>\rho_c$, and this results in the protein kinase activities becoming periodic in time. The range $\rho_c \geqslant 15$ corresponds to the equilibrium constant of the ERK-Ras allosteric binding being less than approximately 20 nM.

Since the maximal growth rate occurs at $q=0$, the protein kinase activities are uniform in space [21]. This spatial uniformity means that there are no gradients [22] nor waves (both standing and traveling) of protein kinase activities throughout the cytosol. A question that is frequently asked in the context of biochemical oscillations is that of synchronization. In this case, one can ask how do the different spatial regions of a single cell ensure that the protein kinase concentrations in the different spatial regions oscillate in phase. Are there synchronization mechanisms for the protein kinase oscillations yet to be discovered? Here, the answer is that our reaction-diffusion model results in oscillations that are spatially uniform. Hence, no synchronization mechanisms are required. This result will not be obvious if diffusion of the protein kinases were not taken into account. A space-time plot of a protein kinase oscillation is shown in Fig. 2. The spatially uniform oscillations take the form of periodic build-ups from the low-activity state (dark colored) to the high-activity state (light colored), followed by an immediate drop to the low-activity state. In real time, the period of the oscillations is approximately 20 min. The algorithm used to obtain the space-time plot is one where time is discretized using the forward Euler scheme and space is discretized using second-order central differencing.

The spatial uniformity arising from our reaction-diffusion model is to be contrasted with other reaction-diffusion models, such as the Brusselator [21], that exhibit spatially periodic oscillatory instabilities. In these models, a necessary condition for the presence of traveling waves is that the diffusion coefficients of the various reacting species differ by large orders of magnitude. In our model, we find that changing the values of the various diffusion coefficients does not change our results qualitatively. We always arrive at a spatially uniform oscillatory instability.

We can also plot the maximal growth rate (occurring at $q=0$) as a function of the negative feedback parameter, ρ, for cascade of varying depths, $N=2,\ldots,4$. This is shown in Fig. 3. We can see from the graph that the maximal growth rate for $N=2$ is negative for all values of ρ, indicating that the two-layer cascade does not become unstable to an oscillatory instability. Thus, oscillations do not occur in the protein kinase activities of the two-layer cascade; the cascade must have a minimum depth of three layers in order for the protein kinase activities to oscillate. Furthermore, the three-layer cascade has the smallest range of ρ for which the oscillatory instability can occur. Equivalently, the three-layer cascade admits the smallest range of values for the equilibrium constant of the ERK-Ras allosteric binding in order for ERK to oscillate. This can be interpreted as saying that the allosteric inhibition of ERK on Ras is the most specific when the cascade is comprised of three layers.

B. Oscillatory dynamics as a function of cascade kinetics

We now characterize the oscillatory dynamics of the three-layer cascade as a function of the biochemical kinetics of the cascade. We will consider the frequency ω of the oscillations, which is given by the maximal value of Im $\sigma(q)$ over the range of q for which Re $\sigma(q)>0$. In this case, $\omega \equiv$ Im $\sigma(q=0)$. In Fig. 4, we plot ω vs the system parameters α, β, ρ, and η.

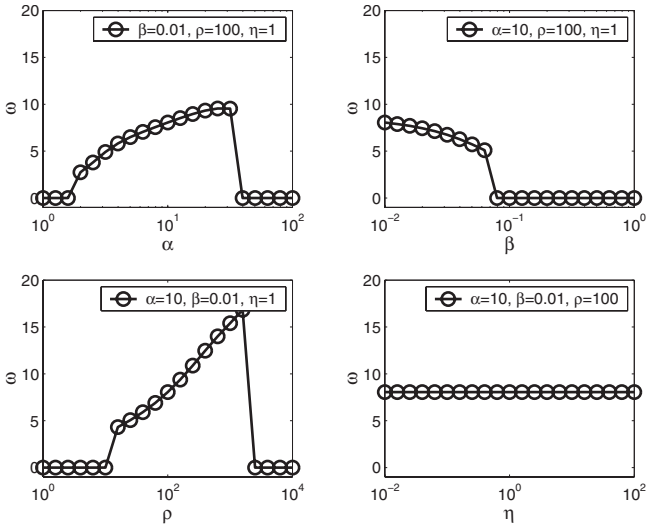

FIG. 4. Frequency ω of the oscillations of the protein kinase activities in a three-layer cascade as functions of the system parameters α, β, ρ, and η. A value of $\omega=0$ corresponds to no oscillations.

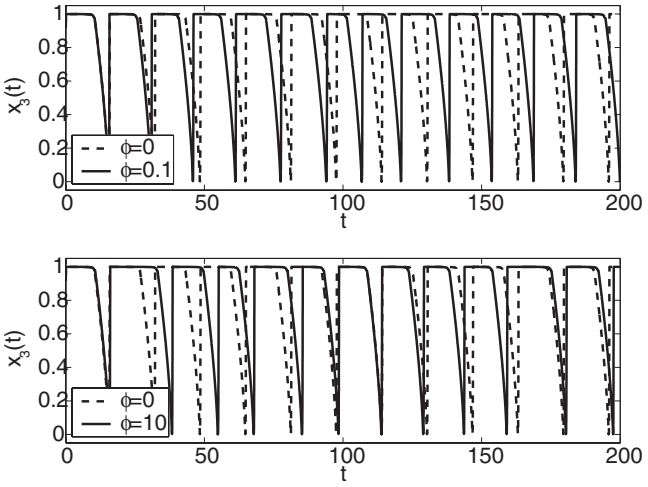

FIG. 5. Examples of perturbed oscillations for the terminal protein kinase activities $a_3(t)$ when $\phi=0.1$ (top) and $\phi=10$ (bottom) for $\xi=0.5$. The dotted lines show the unperturbed ($\phi=0$) terminal protein kinase activities.

First, from the top-left-hand graph of Fig. 4, we see that $\omega>0$ only for $1.6\leqslant\alpha\leqslant32$. Recall that α characterizes the degree of "activity" of the cascade. A value of $\alpha<1$ corresponds to the cascade being in the "off" state where the protein kinase activities are near to zero, whereas a value of $\alpha>1$ corresponds to the cascade being in the "on" state where the protein kinase activities are near to unity. Thus, we can say that protein kinase activities exhibit oscillations only when the cascade is operating in the "on" state. Interestingly, these oscillations disappear when α increases further, i.e., when the protein kinase activities become very close to unity. This suggests that the traditional view of the cascade being a biochemical switch, with either none or all of the protein kinases being active, must be modified. There are now three possible steady states for the cascade: "off," oscillatory, and "on," depending on the magnitude of α.

Second, from the top-right-hand graph of Fig. 4, we see that $\omega>0$ only for $\beta\leqslant0.1$. Recalling that a small value of β denotes the protein kinases being saturated. The protein kinases do not exhibit oscillations if they are not being saturated. Since protein kinase saturation in the cascade leads to ultrasensitivity [15], this result suggests that only ultrasensitive cascades can exhibit protein kinase oscillations [23].

Third, from the bottom-left-hand graph of Fig. 4, we see that $\omega>0$ only for $15\leqslant\rho\leqslant2500$. Thus, the cascade only exhibits oscillations when the equilibrium constant of the ERK-Ras allosteric binding is neither too large or too small. This range corresponds to the equilibrium constant being between approximately 0.1 nM and 20 nM.

Finally, from the bottom-right-hand graph of Fig. 4, we see that ω is independent of η for $10^{-2}\leqslant\eta\leqslant10^{2}$. Thus, the magnitude of the diffusion coefficients of the protein kinases does not modify the frequency of the oscillations. It is plausible that ω may vary with η for values of η outside the range considered here. However, numerical evaluations of ω are difficult to conduct for these values of η.

C. Stability of oscillatory dynamics against crosstalk

Within a cell, there are many signaling cascades operating in parallel. There is then the possibility of signaling crosstalk, i.e., the signaling activities of one cascade modifying the activities of another when both are in operation simultaneously. While the biochemical details of crosstalk have not been fully worked out, there have been theoretical suggestions on how they can be eliminated. For example, Thattai and van Oudenaarden [24] have shown that fluctuations in the stimulus to a cascade, that could arise from crosstalk, can be attenuated in ultrasensitive cascades.

In this section, we want to study the crosstalk between two cascades particularly when both are exhibiting protein kinase oscillations. We want to see how the oscillations of the protein kinases residing in one cascade perturb the oscillations of the protein kinases residing in the other. We assume that the first cascade interferes with the second cascade only via the latter's stimulus. In other words, the second cascade's stimulus now mimics the oscillations of the first cascade, i.e., we let the stimulus a_0 in the reaction-diffusion model of Eq. (1) be oscillatory,

$$a_0(t)=\tilde{a}_0-\frac{\xi}{2}[1+\sin(\omega_p t)], \tag{4}$$

where $\tilde{a}_0$ is the unperturbed magnitude of the stimulus, ξ is a parameter that quantifies the magnitude of the crosstalk, and ω_p is the frequency of the oscillation of the perturbing cascade. Thus, $\tilde{a}_0-\xi\leqslant a_0(t)\leqslant\tilde{a}_0$. If we let ω_n denote the natural frequency of the unperturbed cascade, then we want to vary ω_p over a range, say $0.1\leqslant\phi\equiv\omega_p/\omega_n\leqslant10$, and see how the perturbed protein kinase activities behave.

In Fig. 5, we show the perturbed protein kinase activities for $\phi=0.1$ and $\phi=10$ when $\xi=0.5$. We see that even when perturbed by an oscillatory stimulus whose frequency is one-tenth (10) times as small (large) as the natural frequency of the unperturbed cascade, the protein kinases still maintain

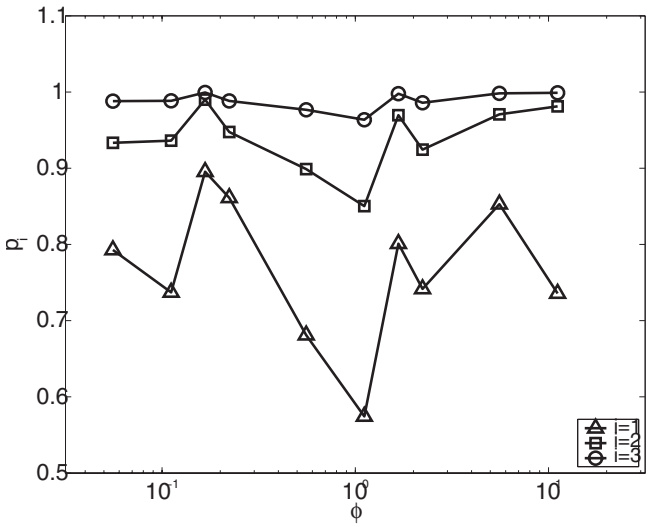

FIG. 6. Total normalized power, p_i defined in Eq. (5) vs ratio of perturbed to unperturbed frequency ϕ for $i=1,2,3$.

their oscillations. However, the oscillations are no longer periodic. In fact, they become quasiperiodic, exhibiting regular in-phase and out-of-phase behavior when compared against the unperturbed case. To quantify the effects of this crosstalk, we compute the total normalized power of the protein kinase activities, defined by

$$p_i(\phi) = \frac{\int_0^\infty |a_i(t;\phi)|^2 dt}{\int_0^\infty |a_i(t;\phi=0)|^2 dt}. \tag{5}$$

Thus, a value of $p=1$ means that, over time, the perturbed protein kinase activities still contains the same amount of total power as the unperturbed protein kinase activities. Consequently, a value of $p=1$ indicates that the protein kinase activities are not affected by the crosstalk. In Fig. 6, we plot the total normalized power for the different protein kinases for an $N=3$ cascade as a function of the ratio of perturbed to unperturbed frequency ϕ. We see that only for $i=3$ do the powers remain at approximately unity. This means that only an $N=3$ cascade is sufficiently robust against crosstalk.

Finally, it should be pointed out that it is possible for a cascade that is operating in the nonoscillating range to be perturbed into exhibiting oscillatory dynamics if the magnitude of the crosstalk is sufficiently strong. Thus, care must be taken to ensure that protein kinase oscillations observed experimentally are not induced by crosstalk.

IV. CONCLUSIONS

In this paper, we have developed a reaction-diffusion model for the mitogen-activated protein kinase cascade and have performed a linear stability analysis on this model. From this analysis, we have found that only cascades that are comprised of three or more layers undergo a spatially uniform oscillatory instability, resulting in the protein kinase activities undergoing oscillations. We have also characterized this oscillation as a function of the biochemical kinetics of the cascade. Finally, we have shown that the oscillations are stable against crosstalk, i.e., if the stimulus of the cascade is perturbed in a periodic manner, the oscillations of the protein kinases do not vary much.

Recent experiments on rat adrenal pheochromocytoma (PC-12) cells to address the network topology of the mitogen-activated protein kinase cascade [4] have been able to resolve variations in protein kinase concentrations on time scales of $\sim O(1)-O(10)$ min. Given that the period of oscillations reported in this paper is approximately 20 min, the PC-12 cell system may be a suitable system to observe protein kinase oscillations. However, these experiments may not be able to observe subcellular variations in protein concentrations, and so may not be able to distinguish if the oscillations are spatially uniform within the cell or not. Nevertheless, recent progress in imaging techniques [25,26] that allow for the measurement of diffusion coefficients of proteins in the cytosol *in vivo* may be adapted to probe if proteins in various locations in the intracellular environment are oscillating in synchrony or not.

ACKNOWLEDGMENT

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APPENDIX: DERIVATION OF MODEL

We assume that the phosphorylation and dephosphorylation of the protein kinases are described by two-step enzyme kinetics [2]. For example, the phosphorylation of the inactive MAPK (denoted by K) into its active form (denoted by K^*) is modeled as

$$K + E \underset{d_P}{\overset{a_P}{\rightleftharpoons}} KE \xrightarrow{k_P} K^* + E, \tag{A1}$$

where KE is an intermediate complex formed from the temporary capture of K by E at a rate a_P. This intermediate complex can then break up in one of two ways: the reversible release of the unmodified kinase K at a rate d_P, or the irreversible release of the modified kinase K^* at a rate k_P. Similarly, the dephosphorylation of K^* into K is modeled as a two-step reaction,

$$K^* + P \underset{d_D}{\overset{a_D}{\rightleftharpoons}} K^*P \xrightarrow{k_D} K + P. \tag{A2}$$

The Michaelis-Menten law can then be invoked to write down equations describing the rates of change of the protein kinases. For example, for Eqs. (A1) and (A2), the rate of change of the active kinase K^* can be written as the difference between the rate of phosphorylation and the rate of dephosphorylation,

$$\frac{d[K^*]}{dt} = \frac{V_P[K]}{K_P + [K]} - \frac{V_D[K^*]}{K_D + [K^*]}. \tag{A3}$$

Here, the square brackets denote species concentrations. The

various parameters are, namely, the maximum phosphorylation velocity $V_P = k_P[E]$, the maximum dephosphorylation velocity $V_D = k_D[P]$, and the Michaelis constants $K_{P,D} = (d_{P,D} + k_{P,D})/a_{P,D}$.

To model the negative feedback loop, we assume that the feedback follows the kinetics of allosteric inhibition [20]. Thus, we can replace the maximum velocity of the phosphorylation of the initial protein kinase as follows:

$$V_P \to \frac{V_P}{1 + [I]/K_I}, \tag{A4}$$

where $[I]$ is the concentration of the inhibitor (in this case, the terminal protein kinase) and K_I is an equilibrium constant of the binding between inhibitor and enzyme.

Finally, rendering these equations dimensionless results in the model of Eq. (1).

[1] H. Lodish, A. Berk, P. Matsudaira, C. A. Kaiser, M. Krieger, M. P. Scott, L. Zipursky, and J. Darnell, *Molecular Cell Biology*, 5th ed. (W. H. Freeman, San Francisco, CA, 2003).

[2] C.-Y. F. Huang and James E. Ferrell, Jr., Proc. Natl. Acad. Sci. U.S.A. **93**, 10078 (1996).

[3] W. Xiong and James E. Ferrell, Jr., Nature (London) **426**, 460 (2003).

[4] Silvia D. M. Santos, P. J. Verveer, and Philippe I. H. Bastiaens, Nat. Cell Biol. **9**, 324 (2007).

[5] K.-H. Chiam, Chee Meng Tan, V. Bhargava, and G. Rajagopal, Phys. Rev. E **74**, 051910 (2005).

[6] U. S. Bhalla, Biophys. J. **87**, 733 (2004).

[7] J. Elf, A. Dončić, and M. Ehrenberg,Proc. SPIE **5110**, 114 (2003)].

[8] J. Elf and M. Ehrenberg, System Biology **1**, 230 (2004).

[9] R. Metzler, Phys. Rev. Lett. **87**, 068103 (2001).

[10] R. A. Siegel and C. G. Pitt, J. Controlled Release **33**, 173 (1995).

[11] B. Li and R. A. Siegel, Chaos **10**, 682 (2000).

[12] J.-C. Leloup and A. Goldbeter, Proc. Natl. Acad. Sci. U.S.A. **100**, 7051 (2003).

[13] M. H. Jensen, K. Sneppen, and G. Tiana, FEBS Lett. **541**, 176 (2003).

[14] G. Lahav, N. Rosenfeld, A. Sigal, N. Geva-Zatorsky, A. J. Levine, M. B. Elowitz, and U. Alon, Nat. Genet. **36**, 147 (2004).

[15] A. Goldbeter and D. E. Koshland, Jr., Proc. Natl. Acad. Sci. U.S.A. **78**, 6840 (1981).

[16] W. R. Burack and A. S. Shaw, J. Biol. Chem. **280**, 3832 (2005).

[17] U. S. Bhalla and R. Iyengar, Science **283**, 381 (1999).

[18] W. J. Langlois, T. Sasaoka, A. R. Saltiel, and J. M. Olefsky, J. Biol. Chem. **270**, 25320 (1995).

[19] A. D. Cherniack, J. K. Klarlund, B. R. Conway, and M. P. Czech, J. Biol. Chem. **270**, 1485 (1995).

[20] J. Keener and J. Sneyd, *Mathematical Physiology* (Springer-Verlag, New York, 1998).

[21] J. D. Murray, *Mathematical Biology II: Spatial Models and Biomedical Applications*, 3rd ed. (Springer-Verlag, New York 2003).

[22] G. C. Brown and B. N. Kholodenko, FEBS Lett. **457**, 452 (1999).

[23] B. N. Kholodenko, Eur. J. Biochem. **267**, 1583 (2000).

[24] M. Thattai and A. van Oudenaarden, Biophys. J. **82**, 2943 (2002).

[25] A. S. Verkman, Trends Biochem. Sci. **27**, 27 (2002).

[26] D. Gerlich and J. Ellenberg, Nat. Cell Biol. **5**, S14 (2003).