Published in final edited form as:

Phys Biol. ; 6(1): 16007. doi:10.1088/1478-3975/6/1/016007.

Effects of cascade length, kinetics, and feedback loops on biological signal transduction dynamics in a simplified cascade model

Zhilin Qu¹ and Thomas M. Vondriska^{1,2,3}

¹ Department of Medicine (Cardiology), David Geffen School of Medicine, University of California, Los Angeles, California 90095

² Department of Anesthesiology, David Geffen School of Medicine, University of California, Los Angeles, California 90095

³ Department of Physiology, David Geffen School of Medicine, University of California, Los Angeles, California 90095

Abstract

How intracellular signals are propagated with the appropriate strength, duration and fidelity over time is poorly understood. To address these issues, intracellular signal transduction was studied both analytically and numerically using a simplified cascade model. The main observations can be summarized as follows: when the response kinetics is the Michaelis-Menten type, the signal strength will always reach the same magnitude as the cascade length increases, regardless of the type of stimulus applied (i.e., either continuous or unitary pulse). However, when the response kinetics is the Hill type (Hill coefficient >1), there exists a stimulation threshold. If the stimulus is below the threshold, the signal decays toward zero; in contrast, if the stimulus is above the threshold, the signal amplitude reaches a non-zero steady state. The time taken for the signal to proceed through the cascade increases as the half-maximum point, or Hill coefficient, increases, whereas the duration of the output signal at the end of the cascade decreases as the half-maximum point increases. In the presence of a positive feedback, the stimulation threshold increases; under these conditions, the feedback strength necessary for bistability changes (with power-law characteristics) inversely related to the length of the cascade. In the presence of a negative feedback, oscillations are induced when the Hill coefficient is greater than one and the cascade has more than two steps. Likewise, the feedback strength required to generate oscillations changes (again with power-law characteristics) inversely with the length of the cascade.

INTRODUCTION

Molecular networks are comprised of multiple signaling motifs that, when coupled together, engender distinct responses to external stimuli [1–4]. These responses have important but poorly understood temporal properties. For example, in many systems a short stimulus (i.e., a pulse) gives rise to a fleeting immediate response and a delayed onset response of prolonged [5–11] or even permanent [12–15] duration. Specific cases are illustrative: protection against heart or brain injury can be induced by a short stimulus that engenders both an early protective period that begins immediately and is short lived, and a delayed protective period that is substantially prolonged [5–7]. During the maturation of oocytes, progesterone stimulation

causes an initial short period of maturation promoting factor expression and a second, sustained period of higher expression of the protein. This second increase in maturation promoting factor persists in a plateau phase until the cell is fertilized, at which point the factor levels begin to oscillate in a behavior that drives cell cycle progression [12–14]. Another important and interesting response is the so-called early long-term potentiation and late long-term potentiation in excitable cells in which a single stimulus causes short long-term potentiation which lasts a few hours, but multiple sequential stimuli induce long long-term potentiation which lasts more than 24 hours [16]. It has been hypothesized that post-translation modifications and gene transcription account for the immediate and delayed responses, respectively. However, the mechanisms for the length of delay, the duration of the response, and stimulation threshold are not well understood.

Signaling cascades, which are ubiquitous in biological systems, have been candidate models for the mechanisms of these biological response [17,18]. It has been shown in experiments that a cascade can result in ultrasensitive response [19,20] as well as signal amplification [21]. Many mathematical modeling studies on signal transduction through cascades have been carried out, among which the mitogen-activated protein kinases (MAPK) (Fig. 1A) are the most well studied. Modeling studies of MAPK cascades have demonstrated their utility for ultra-sensitive responses [19–22] and have revealed that they produce bistability and limit cycle oscillations [23–26] under constant stimulation. In these modeling studies, either a cascade model with a single phosphorylation/dephosphorylation cycle in each step (Fig. 1B) or a cascade model with dual phosphorylation/dephosphorylation cycles in each step (Fig. 1C), was used. However, these behaviors are not unique to MAPK responses and can be observed in any signaling process involving several molecules. Signal transduction in cascades with arbitrary steps in response to a time-dependent stimulus has been investigated either numerically or analytically in recent studies [18,21,27–30]. Heinrich et al [27] and Chaves et al [28] investigated signal amplification and attenuation in a cascade of arbitrary length with the response kinetics of Fig. 1B. Sontag and Chaves [29] studied signal amplification as a general phenomenon in a class of nonlinear signaling pathways. These studies showed that depending on the reaction kinetics, a weak stimulus may be amplified into a stable signal in the cascade, or a strong stimulus may be damped gradually in the cascade and fail to propagate the signal to the target at the end of the cascade.

In this study, we used both numerical and analytical methods to investigate the signal transduction dynamics in a simplified model of signaling cascade of arbitrary length as shown in Fig. 1D. We studied the factors affecting the signal delay, the signal duration, and the stimulation threshold; we also studied the dynamics caused by positive and negative feedback loops. Our findings provide fundamental insights into distinct types of dynamical behavior engendered by variation in each of these three parameters (cascade length, kinetics and feedback) and provide rationale for why all three are varied in a signaling network to impart interesting biological responses.

MATHEMATICAL MODELING AND METHODS

The model in Fig. 1B has been used in several studies [26–30] to investigate signal transduction dynamics in the MAPK cascades. The differential equation for a protein in the cascade, e.g., for x_p , is:

$$dx_p/dt = S(1 - x_p) - ax_p = (S+a)\left(\frac{S}{S+a} - x_p\right) \quad (1)$$

where we assume $x + x_p = 1$ and a is the inactivation rate constant. The steady state response of x_p to the stimulus S is the Michaelis-Menten type (or the Hill type with coefficient=1). The differential equation for y_p (or z_p) has the same form as Eq. 1, except S is substituted by x_p (or y_p). Models of Fig. 1C were also used, in which the proteins in the cascades are dually phosphorylated/dephosphorylated [20,23]. In this case, the differential equations, e.g., for x_{2p} and x_p , are:

$$\begin{aligned} dx_{2p}/dt &= S x_p - a x_{2p} \\ dx_p/dt &= S(1 - x_p - x_{2p}) - a x_p + a x_{2p} - S x_p \end{aligned} \quad (2)$$

where $x + x_p + x_{2p} = 1$ and a is the dephosphorylation rate constant. If one assumes that the x_p reaches its equilibrium state fast, then x_p can be approximated as a function of x_{2p} , from the

second equation of Eq. 2, i.e., $x_p = \frac{S(1 - x_{2p}) + a x_{2p}}{2S + a}$. Inserting this expression into the first equation, we have:

$$dx_{2p}/dt = \frac{S^2 + S a + a^2}{2S + a} \left(\frac{S^2}{S^2 + S a + a^2} - x_{2p} \right) \quad (3)$$

The differential equation for y_{2p} (or z_{2p}) has the same form as Eq. 3, except S is substituted by x_{2p} (or y_{2p}). As shown by Huang and Ferrell [20], the steady state response at each step of the

cascade can be well approximated by a Hill function $\frac{x^\beta}{x^\beta + K^\beta}$ with coefficient β being greater than 1. When S is a constant, the steady state response of x_{2p} is $x_{2p}^{ss} = \frac{S^2}{S^2 + S a + a^2}$, and the solution of Eq. 3 is $x_{2p}(t) = x_{2p}^{ss} + c e^{-t/\tau}$ where $\tau = \frac{2S + a}{S^2 + S a + a^2}$ is the time constant of phosphorylation (activation) or dephosphorylation (inactivation) of x_{2p} . Eq. 3 can be expressed as $dx_{2p}/dt = [f(S) - x_{2p}]/\tau$.

Although analytical treatment of Eq. 1 (or the signaling cascade model in Fig. 1B) can be carried out [27,28], it becomes difficult (or almost impossible) to analytically treat Eq. 2 or Eq. 3 (or the signaling cascade model in Fig. 1C) since the differential equations are nonlinear. Based on the fact that the steady state response of the cascade in Fig. 1C can be well approximated by a Hill function [19,20,22], and to allow analytical treatment, we used, instead, a simplified general model (shown in Fig. 1D) and also assume the response of each step follows the Hill function with a response time constant τ . Then the differential equations are simplified to:

$$dX_n(t)/dt = \{f[X_{n-1}(t)] - X_n(t)\} / \tau, \quad n=1, N \quad (4)$$

where N is the length of the cascade and $X_0 = S$ is the stimulus amplitude. S can be either a constant or a pulse with duration T_0 . Note that τ is not constant (see Eq. 3) but depends on the signal of the previous step, i.e., $X_{n-1}(t)$. For simplicity and analytical treatment, we set τ to be constant in this study. For the same reason, we assume that all steps in the cascade are identical, i.e., Eq. 4 is a homogeneous cascade model. $f(X)$ was chosen to be a Hill function:

$$f(X) = \frac{X^\beta}{a^\beta + X^\beta}. \quad (5)$$

The Hill coefficient $\beta \geq 1$ was used in this study since higher Hill coefficients can be achieved in biological systems by multi-step phosphorylation [31–33] or other mechanisms [34–36] in each step of the cascade. We also studied the effects of positive and negative feedback loops (the dashed pathway in Fig. 1D); the modifications to Eq. 4 will be detailed in the corresponding sections below. The parameters used in this paper are α , β , τ , the feedback strength η , and the stimulus strength S and its duration T_0 . A constant stimulus is equivalent to $T_0 \rightarrow \infty$. The numerical results in this study were obtained by integrating Eq. 4 using a fourth-order Runge-Kutta method with a time step 0.01.

RESULTS

Signal transduction in a singling cascade in response to an external stimulus

A. Steady state response to a constant stimulus—Under a constant stimulus S , the steady state solution of Eq. 4 satisfies [we denote X_n as the steady state of $X_n(t)$]:

$$X_n = f(X_{n-1}) = \frac{X_{n-1}^\beta}{\alpha^\beta + X_{n-1}^\beta} \quad (6)$$

which is just an iterated map with $X_0 = S$. When $\beta = 1$, an explicit solution of Eq. 6 can be obtained as:

$$X_n = \frac{(1 - \alpha)S}{(1 - \alpha)\alpha^n + (1 - \alpha^n)S}. \quad (7)$$

When $\beta > 1$, we are unable to analytically solve Eq. 6 for arbitrary stimulus strength S . However, we can still determine whether a signal is amplified or attenuated through the signaling cascade by analyzing the steady states (or fixed points) of Eq. 6 and their stabilities. In other words, a fixed point of Eq. 6 corresponds to a constant signal in the signaling cascade, i.e., $X_n = X_{n-1} = \dots = S$. The steady state solution of Eq. 6 (denoted as X_s) can be solved from

$$X_s = f(X_s) = \frac{X_s^\beta}{\alpha^\beta + X_s^\beta}, \quad (8)$$

which leads to

$$X_s = 0, \quad (9)$$

or

$$\alpha = [X_s^{\beta-1}(1 - X_s)]^{1/\beta}. \quad (10)$$

The solutions for Eq. 10 are not obvious, but since $[X_s^{\beta-1}(1 - X_s)]^{1/\beta}$ is a unimodal function when $\beta > 1$, it can have either no solution or two solutions (see Fig. 2A for a graphical illustration). The two solutions degenerate into one solution $X_c = (\beta - 1)/\beta$ at $\alpha = \alpha_c = (1 - 1/$

$\beta)(\beta - 1)^{-1/\beta}$. For example (Fig. 2A), for $\beta = 2$, the two solutions are $X_s = (1 \pm \sqrt{1 - 4\alpha^2})/2$, and these two solutions degenerate into $X_c = 0.5$ at $\alpha_c = 0.5$. Therefore, for $\beta > 1$, Eq. 8 has three solutions when $\alpha < \alpha_c$ and one solution ($X_s = 0$) when $\alpha > \alpha_c$ (Fig. 2B). A special case is when $\beta = 1$, Eq. 10 has only one solution which is $X_s = 1 - \alpha$. Therefore, for $\beta = 1$, Eq. 8 has two solutions: $X_s = 0$ and $X_s = 1 - \alpha$.

We then analyze the stability of the steady states by linearizing Eq. 6 around its fixed point, i.e.,

$$\delta X_{n+1} = \frac{\beta \alpha^\beta X_s^{\beta-1}}{(\alpha^\beta + X_s^\beta)^2} \delta X_n = \lambda \delta X_n. \quad (11)$$

The steady state solution is stable when $|\lambda| < 1$, but unstable when $|\lambda| > 1$. For $\beta = 1$, $X_s = 0$ is unstable since $\lambda = 1/\alpha > 1$, while $X_s = 1 - \alpha$ is stable since $\lambda = \alpha < 1$. For $\beta > 1$, $X_s = 0$ is then stable since $\lambda = 0 < 1$. For the other two solutions, the eigenvalue can be obtained by inserting Eq. 10 into Eq. 11, which is:

$$\lambda = \beta(1 - X_s). \quad (12)$$

Since when $X_s = X_c = (\beta - 1)/\beta$, $\lambda = 1$, then $\lambda > 1$ when $X_s < X_c$, and $\lambda < 1$ when $X_s > X_c$. Therefore, the larger of the two solutions of Eq. 10 is stable and the smaller is unstable (dashed line in Fig. 2B).

From the steady states and their stabilities, one can determine if a signal is amplified or attenuated through the cascade. For $\beta = 1$, the signal is amplified when $S < 1 - \alpha$ but attenuated when $S > 1 - \alpha$. In other words, for any stimulation strength, since $X_s = 0$ is unstable, the signal strength always approaches the stable steady state ($1 - \alpha$) through the signaling cascade. For $\beta > 1$, if S is smaller than the unstable steady state (labeled as X_{th} in Fig. 2A), the signal will decay towards zero (Fig. 2D). If S is greater than X_{th} , then the signal will approach the stable steady state (X_s in Fig. 2A). Therefore, there is a stimulation threshold X_{th} for $\beta > 1$ but not for $\beta = 1$. Note that in the case of $\beta > 1$, when $\alpha > \alpha_c$, the signal always decays, approaching zero, since there is only one steady state solution which is zero. In Fig. 2E, we plot X_n versus S , showing that X_n decreases as n increases when $S < X_{th}$, but X_n increase as n increases. In other words, as more steps are added to the cascade, the sensitivity increases, taking on more Boolean activation characteristics. This is similar to what is observed by increasing the Hill coefficient with other parameters constant; however, the latter allows for rapid activation whereas the former results in an activation delay. From a stress adaptation standpoint, this type of response provides clear benefits: small stimuli can induce responses due to high sensitivity but these responses are delayed in their onset. This allows the system to detect small stresses before they are of a deleterious magnitude and to mobilize appropriate long term response.

Response to a pulse stimulus: When the stimulus S is a pulse instead of a constant, the transduction of the signal is influenced by the duration T_0 of the stimulus and the time constant τ of the cascade, in addition to α and β . When $\beta = 1$, since there is no stimulation threshold for strength and duration, any small stimulus can elicit a large output from the cascade following a given time (Fig. 3A), while a large stimulus elicits a large initial signal but then decays to a plateau level (Fig. 3B). When $\beta > 1$ a stimulation threshold exists. Under these conditions, the output elicited by a sub-threshold stimulus decays toward zero (Fig. 3C), but a supra-threshold stimulus can give rise to a stable output from the cascade (Fig. 3D). Fig. 3E shows the critical parameter α_c versus β for a signal elicited by a large stimulus ($S=1$) to transduce without

decaying to zero in the cascade for $T_0 = 10$ and $\tau = 5$ (triangles), compared with that for a constant stimulus (line). For $\beta > 1$, α_c is smaller than that for the steady state, as expected. The critical stimulus strength S_c required for successful propagation through the cascade decays rapidly as the stimulus duration T_0 increases (Fig. 3F), but increases rapidly as τ increases. Output from the cascade fails altogether when τ reaches a critical value (Fig. 3G). This behavior is akin to capacitance in the signaling system, where a small input builds up over time to ultimately reach a critical threshold.

The delay and signal duration depend on α and β . Fig. 4A plots the delay (filled circles) and signal duration (open circles) versus β for X_{10} of the cascade, showing that, initially, as β increases, the delay time (D_{10}) increases and the signal duration (T_{10}) decreases; eventually, both saturate (interestingly, this occurs around the same point of half-maximum response, or β). Fig. 4B shows the delay and signal duration versus α for X_{10} of the cascade, the delay time increases and the signal duration decreases until signal transduction fails in the cascade. Fig. 4C shows some example traces of $X_{10}(t)$ for different combinations of α and β .

Although an analytical solution cannot be easily obtained due to the nonlinearity of $f(X)$ in Eq. 4, one can derive approximate solutions under conditions of large β and a strong stimulus, since $f(X)$ is very close to a step function when β is large. One can then solve Eq. 4 in three intervals by setting $f(X_{n-1})$ to be:

$$f(X_{n-1}) = \begin{cases} 0, & t < D_{n-1} \\ \frac{\mu_{n-1}^\beta}{\alpha^\beta + \mu_{n-1}^\beta}, & D_{n-1} < t < D_{n-1} + T_n \\ 0, & t > D_{n-1} + T_n \end{cases} \quad (13)$$

where D_{n-1} is the activation delay of X_{n-1} , T_n is the signal duration of X_n , and μ_{n-1} is the amplitude of X_{n-1} . See Fig. 5 for definitions of these quantities. The solution for $X_n(t)$ in the three intervals can then be obtained as:

$$X_n(t) = \begin{cases} 0, & t < D_{n-1}, \\ \mu_{n-1}^\beta [1 - e^{-(t-D_{n-1})/\tau}] / (\alpha^\beta + \mu_{n-1}^\beta), & D_{n-1} \leq t \leq D_{n-1} + T_{n-1}, \\ \mu_n e^{-(t-D_{n-1}-T_{n-1})/\tau}, & t > D_{n-1} + T_{n-1}, \end{cases} \quad (14)$$

where $\mu_n = \mu_{n-1}^\beta (1 - e^{-T_{n-1}/\tau}) / (\alpha^\beta + \mu_{n-1}^\beta)$. In Eq. 14, $D_0 = 0$, $\mu_0 = S$, and T_0 is the width of the stimulus. D_n and T_n are calculated by the following linear iterated equations:

$$D_n = D_{n-1} + \Delta D_n, \quad T_n = T_{n-1} + \Delta T_n, \quad (15)$$

The next task is to calculate ΔD_n and ΔT_n . We define ΔD_n as the duration in which X_n grows from zero to α , and $\Delta T_n + \Delta D_n$ as the duration in which X_n decays from μ_n to α (see Fig. 5). Using Eq. 14, one obtains:

$$\begin{aligned} \Delta D_n &= -\tau \ln[1 - \alpha(\alpha^\beta + \mu_{n-1}^\beta) / \mu_{n-1}^\beta], \\ \Delta T_n &= \tau \ln(\mu_n / \alpha) - \Delta D_n. \end{aligned} \quad (16)$$

At the limit of $\beta \rightarrow \infty$, and $\tau \rightarrow 0$, then $u_n \rightarrow 1$, and thus we obtain from Eqs. 15 and 16 the delay time in activation of X_n and its duration as:

$$\begin{aligned} D_n &\approx -n\tau \ln(1 - \alpha), \\ T_n &\approx T_0 + n\tau \ln[(1 - \alpha)/\alpha]. \end{aligned} \quad (17)$$

In Fig. 4B, we also plot D_n and T_n versus α for $\beta = 2$ (lines) using Eq. 17 which agree well with the numerical results.

Effects of feedback loops on bistability and limit cycle oscillations

A. Positive feedback—In the case of positive feedback as illustrated in Fig. 1D, the differential equation for X_1 in Eq. 4 was changed to:

$$dX_1/dt = [\frac{(S + \eta X_N)^\beta}{\alpha^\beta + (S + \eta X_N)^\beta} - X_1] / \tau, \quad (18)$$

and the other equations were kept unchanged. To understand how this positive feedback loop affects the dynamics of the system, the stability of the steady state is analyzed below. From Eqs. 4 and 18, one obtains the Jacobian for a steady state as:

$$M = \frac{1}{\tau} \begin{pmatrix} -1 & 0 & \cdots & 0 & z_{1N} \\ z_{21} & -1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & z_{NN-1} & -1 \end{pmatrix}, \quad (19)$$

where

$$z_{nn-1} = \frac{df(X_{n-1})}{dX_{n-1}} = \frac{\beta \alpha^\beta X_{n-1}^{\beta-1}}{(\alpha^\beta + X_{n-1}^\beta)^2}, \quad (20)$$

and

$$z_{1N} = \frac{\eta \beta \alpha^\beta (S + \eta X_N)^{\beta-1}}{[\alpha^\beta + (S + \eta X_N)^\beta]^2}. \quad (21)$$

The characteristic equation for the eigenvalues of the Jacobian is:

$$\left(-\frac{1}{\tau} - \lambda\right)^N + \left(\frac{1}{\tau}\right)^N (-1)^N z_{1N} \prod_{n=2}^N z_{nn-1} = 0. \quad (22)$$

The solutions of Eq. 22 are:

$$\lambda_k = -\frac{1}{\tau} \left\{ 1 + [-(-1)^N z_{1N} \prod_{n=2}^N z_{nn-1}]^{1/N} \right\} \\ = -\frac{1}{\tau} \left\{ 1 + \sqrt[N]{b} \left[\cos \frac{2k\pi + \theta}{N} + i \sin \frac{2k\pi + \theta}{N} \right] \right\}, \quad k=0, \dots, N-1, \quad (23)$$

where $b = z_{1N} \prod_{n=2}^N z_{nn-1}$ which is always positive since z_{nn-1} and z_{1N} are all positive. This indicates $\theta = \pi$ when N is odd and $\theta = 0$ when N is even. The maximum eigenvalue is always a real number since $\cos \frac{2k\pi + \theta}{N} = -1$ and $\sin \frac{2k\pi + \theta}{N} = 0$ can be always satisfied in Eq. 23, i.e.,

$$\lambda_{\max} = -\frac{1}{\tau} (1 - \sqrt[N]{b}). \quad (24)$$

Therefore, the steady state is stable when $\sqrt[N]{b} < 1$ and unstable when $\sqrt[N]{b} > 1$; the loss of stability occurs via a saddle-node bifurcation.

Michaelis-Menten response kinetics ($\beta = 1$): When $\beta = 1$, the steady state can explicitly obtained. Based on Eq. 7, the steady state relation between X_N and X_1 is:

$$X_N = \frac{(1 - \alpha)X_1}{(1 - \alpha)\alpha^{N-1} + (1 - \alpha^{N-1})X_1}. \quad (25)$$

Inserting Eq. 25 into Eq. 18, the steady state solution for X_1 satisfies:

$$\frac{S + \eta \frac{(1 - \alpha)X_1}{(1 - \alpha)\alpha^{N-1} + (1 - \alpha^{N-1})X_1}}{\alpha + S + \eta \frac{(1 - \alpha)X_1}{(1 - \alpha)\alpha^{N-1} + (1 - \alpha^{N-1})X_1}} - X_1 = 0. \quad (26)$$

The solutions of Eq. 26 are:

$$X_1 = \frac{B \pm \sqrt{B^2 + 4AC}}{2A}, \quad (27)$$

where $A = (\alpha + S)(1 - \alpha^{N-1}) + \eta(1 - \alpha)$, $B = S(1 - \alpha^{N-1}) + \eta(1 - \alpha) - (\alpha + S)(1 - \alpha)\alpha^{N-1}$, and $C = S(1 - \alpha)\alpha^{N-1}$. Since A and C are positive for $S > 0$, only one of the two solutions is positive. In the special case of $S = 0$, the system has two nonnegative solutions, which are: $X_1 = 0$ and

$X_1 = \frac{(1 - \alpha)(\eta - \alpha^N)}{\alpha(1 - \alpha^{N-1}) + \eta(1 - \alpha)}$. The nonzero solution exists only when $\eta > \alpha^N$. Since $\alpha < 1$, a weaker feedback strength is needed for a larger N (i.e., a longer cascade) to maintain a nonzero signal amplitude when there is no stimulation.

Fig. 6A shows the steady state of X_1 for $N = 2$ (gray line) and $N = 5$ (black line) versus S while Fig. 6B shows the steady state X_1 versus $\lambda_{\max}$ calculated using Eq. 23. The negatively sloped region of the steady state solutions is unstable (the $\lambda_{\max} > 0$ region in Fig. 6B). Note that increasing the length also increases the magnitude of the hysteresis. Since the smaller solution only occurs in the negative S regime except when $X_1 = 0$, it is not practically interesting since

S cannot be negative. However, since $X_1 = 0$ is unstable, any stimulation is sufficient to trigger a signal through the cascade (Fig. 6C). For obvious reasons this property is undesirable in a biological system because any external or internal noise would give rise to a sustained output.

Hill type response kinetics ($\beta > 1$): When $\beta > 1$, we can numerically solve the steady state for different stimulus strength S , two examples of which are shown in Fig. 7A. The steady state X_1 versus $\lambda_{\max}$ calculated using Eq. 23 is shown in Fig. 7B. The positively sloped regions of the steady state solutions in Fig. 7A are stable (the $\lambda_{\max} < 0$ regions in Fig. 7B) and the negatively sloped region is unstable (the $\lambda_{\max} > 0$ region in Fig. 7B). Because a stimulation threshold exists, a sub-threshold stimulus is incapable of eliciting an output from the system, whereas a supra-threshold stimulus does induce signaling (Fig. 7C). The stimulation threshold increases as β increases (Fig. 7D), and also increases as the cascade length N increases (Fig. 7E). The feedback strength η that is needed for bistability to occur decreases close to power-law manner as N increases, as shown in a log-log plot (Fig. 7F). Thus, when positive feedback is present, longer cascades lower the threshold for the system to enter a bistable regime.

B. Negative feedback—In the case of negative feedback as illustrated in Fig. 1D, the differential equation for X_1 in Eq. 4 was changed to:

$$dX_1/dt = \left[\frac{S^\beta}{(\alpha + \eta X_N)^\beta + S^\beta} - X_1 \right] / \tau, \quad (28)$$

and the other equations were kept unchanged. The Jacobian is the same as the positive feedback case in Eq. 19 except that

$$z_{1N} = \frac{-\eta \beta S^\beta (\alpha + \eta X_N)^{\beta-1}}{[(\alpha + \eta X_N)^\beta + S^\beta]^2}. \quad (29)$$

The eigenvalue spectrum is thus the same as Eq. 23, except that $b = -z_{1N} \prod_{n=2}^N z_{nn-1}$, $\theta = \pi$ when N is even, and $\theta = 0$ when N is odd. Since z_{nn-1} is positive for all n and z_{1N} is negative, then b is positive. When $\beta = 1$, no instability can occur. When $\beta > 1$, instability occurs only when

$N > 2$ since when $N = 2$ the two eigenvalues are $\lambda_{1,2} = -\frac{1}{\tau}(1 \pm \sqrt{bi})$. It is well known that a three-variable model is needed for negative feedback to cause limit cycle oscillations [37,38], which is also the case for the model in this study. Figs. 8A and B show two bifurcation diagrams for $N = 5$ and $N = 8$ which show that the system underwent a Hopf bifurcation leading to limit cycle oscillations. Fig. 8C shows traces of $X_n(t)$ during limit cycle oscillations. The minimum feedback strength needed for limit cycle oscillations to occur decreases as either the cascade length or β increases (Fig. 8D), but increases as α increases (Fig. 8E). In fact, the minimum feedback strength needed for oscillation decays as N increases, following a power-law relation (Fig. 8F). It is obvious that the period of limit cycle oscillations is longer for a longer cascade or a longer protein half-life τ . Because the feedback strength necessary for oscillations decreases as the length of the cascade increases, selection for longer cascades would decrease the overall flux through the system and fine tune the amplitude of transduced signal.

DISCUSSION

General properties governing how intracellular networks respond to external stimuli are incompletely understood. It is well established that virtually all cell types exhibit a range of responses following unitary or prolonged stimuli. That a response occurs (or does not occur) following stimulation of a cell is a fundamental property of how signaling networks support the internal functions of the cell and allow the cell to interface in a coherent manner with the extracellular environment. In this study, we used a simplified signaling cascade model to investigate the dynamics of signal transduction in response to external stimuli. Several biological implications can be drawn from this study as discussed below.

First, we showed that a stimulation threshold appeared when the response kinetics is Hill type with coefficient $\beta > 1$. In this case, a stimulus that does not reach the threshold fails to generate a propagating signal and the output of the cascade decays toward zero. On the other hand, a stimulus above the threshold induces a non-zero amplitude signal through the cascade. However, it is not the case when the response kinetics is Michaelis-Menten type, i.e., any stimulus gives rise to an output from the cascade. This threshold may be responsible for the occurrence of the late long-term potentiation [16] and has been modeled previously [17]. It is noteworthy that previous studies have shown bistability caused by positive feedback acts as the dynamical mechanism underlying biological switches in many systems [14,39–42]. The Hill type response is referred to as a graded response, however, even though the response kinetics is Hill type in each step, a switch-like (or binary) behavior can be achieved in a multi-step signaling cascade without a positive feedback as long as the Hill coefficient $\beta > 1$, as shown by our analysis. Hysteresis and binary behaviors have been used in experiments as evidence of positive feedback-induced bistability; however, caution must be taken, because hysteresis and binary responses may also be observed in experimental measurements while the actual signaling network is simply a signaling cascade without positive feedback. This observation may occur due to the existence of stimulation threshold that engenders binary response behavior and memory over time (that is, the emergence of hysteresis in the system).

Secondly, an important function of stimulation threshold is to prevent sub-threshold environmental noise from eliciting an output from the cascade. However, once the external noise becomes supra-threshold, it can be further amplified by the cascade to be a robust signal. On the other hand, based on our stability analysis, the signal amplitude saturates in the cascade; therefore, once a signal is transducing in the cascade, the internal noise may be attenuated, as also shown previously [43].

Thirdly, we demonstrated that the delay time between stimulus and response and the duration of the response were both proportional to the product of the signaling cascade length and the half-life τ of the protein level or protein function/activity (Eq. 17). In biological systems, protein half-lives may vary from minutes to several hours [44]. Therefore, to maintain a 3 to 4 day signal duration, such as in cardiac or neural protection against injury [5–7], a signaling cascade with many steps is required. It should be noted that a long cascade can result in a very long (several days) signal duration which may be treated as a permanent signal experimentally due to relative short period of observation. For example, whether the maturation promoting factor levels during oocyte maturation is due to a strong positive feedback loop or a long signaling cascade remains unknown.

CONCLUSION AND OUTLOOK

We studied how parameters in a cascade affected the dynamical instabilities of the signaling system and showed that the likelihood of bistability and limit cycle oscillations increases greatly as the length of the cascade increases. This observation is attributable to the fact that

the cascade results in a very steep response which is prone to dynamical instabilities. These studies provide novel analytical insights into how cells temporally regulate phenotype, and identify critical parameters within intracellular networks that govern rapid response and delayed onset signal transduction.

We used a homogeneous cascade model with a simplified kinetics, whereas in real systems, the behavior of the individual molecules participating in a cascade and the kinetics of the biochemical reactions may all differ. We set the time constant τ to be a fixed number, yet it changes during a chemical reaction cycle. Therefore, how these temporal and structural heterogeneities affect the dynamics of the signaling cascade needs to be further evaluated. It will be worthwhile to validate the theoretical insights in a specific biological signal transduction model that incorporates the realistic molecules and biochemical reactions. Nevertheless, the simple model used in the present study provides a basic theoretical understanding of the dynamics of biological signal transduction.

Acknowledgments

This study was supported by the NIH (HL087132, TMV) and the Laubisch Endowment at UCLA.

GLOSSARY

Signal transduction

Signal transduction in biology is a process which propagates a stimulus to enact a cellular function. For example, nutrients or hormones can stimulate signaling transduction in the cell that activates genes, causes protein production, modulates metabolism or otherwise affects cell growth or survival

Signaling cascade

A signaling cascade is a series of biochemical reactions in which the reaction products of one step catalyze, or serves as necessary reactants in, reactions in the next step

Bistability

Bistability means that a system can have two stable states of equilibrium under the same conditions. The system can reside on either one of the two states depending on the initial condition of the system. Bistability is used to explain switch behaviors in biological systems [14,40,41,45,46]

Limit cycle oscillations

Limit cycle oscillations are a temporal periodic solution of a nonlinear system. For a stable limit cycle, nearby states always approach this same oscillatory state asymptotically. Most known biochemical oscillations, such as circadian rhythms or cell division cycle, are limit cycle oscillations [47,48]

References

1. Shen-Orr SS, Milo R, Mangan S, et al. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nat Genet* 2002;31:64. [PubMed: 11967538]
2. Mangan S, Alon U. Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci U S A* 2003;100:11980. [PubMed: 14530388]
3. Ma'ayan A, Jenkins SL, Neves S, et al. Formation of regulatory patterns during signal propagation in a mammalian cellular network. *Science* 2005;309:1078. [PubMed: 16099987]

4. Behar M, Dohlman HG, Elston TC. Kinetic insulation as an effective mechanism for achieving pathway specificity in intracellular signaling networks. *Proc Natl Acad Sci U S A* 2007;104:16146. [PubMed: 17913886]
5. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 2003;83:1113. [PubMed: 14506302]
6. Papadia S, Stevenson P, Hardingham NR, et al. Nuclear Ca^{2+} and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. *J Neurosci* 2005;25:4279. [PubMed: 15858054]
7. Bordet R, Deplanque D, Maboudou P, et al. Increase in endogenous brain superoxide dismutase as a potential mechanism of lipopolysaccharide-induced brain ischemic tolerance. *J Cereb Blood Flow Metab* 2000;20:1190. [PubMed: 10950379]
8. Taylor DA, McGrath JL, O'Connor BJ, et al. Allergen-induced early and late asthmatic responses are not affected by inhibition of endogenous nitric oxide. *Am J Respir Crit Care Med* 1998;158:99. [PubMed: 9655713]
9. Dweik RA, Comhair SA, Gaston B, et al. NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response. *Proc Natl Acad Sci U S A* 2001;98:2622. [PubMed: 11226289]
10. Su M, Young AJ, He C, et al. Biphasic response of the regional lymphatics in the normal lymphocyte transfer reaction. *Transplantation* 2001;72:516. [PubMed: 11502985]
11. Hong SJ, Li H, Becker KG, et al. Identification and analysis of plasticity-induced late-response genes. *Proc Natl Acad Sci U S A* 2004;101:2145. [PubMed: 14766980]
12. Murray AW, Solomon MJ, Kirschner MW. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* 1989;339:280. [PubMed: 2566918]
13. Ferrell JE Jr. *Xenopus* oocyte maturation: new lessons from a good egg. *BioEssays* 1999;21:833. [PubMed: 10497333]
14. Xiong W, Ferrell JE Jr. A positive-feedback-based bistable 'memory module' that governs a cell fate decision. *Nature* 2003;426:460. [PubMed: 14647386]
15. Fera E, O'Neil C, Lee W, et al. Fibroblast growth factor-2 and remodeled type I collagen control membrane protrusion in human vascular smooth muscle cells: biphasic activation of Rac1. *J Biol Chem* 2004;279:35573. [PubMed: 15166228]
16. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 2001;294:1030. [PubMed: 11691980]
17. Smolen P, Baxter DA, Byrne JH. A model of the roles of essential kinases in the induction and expression of late long-term potentiation. *Biophys J* 2006;90:2760. [PubMed: 16415049]
18. Han Z, Vondriska TM, Yang L, et al. Signal transduction network motifs and biological memory. *J Theor Biol* 2007;246:755. [PubMed: 17374382]
19. Ferrell JE Jr. Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem Sci* 1996;21:460. [PubMed: 9009826]
20. Huang CYF, Ferrell JE. Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proc Natl Acad Sci USA* 1996;93:10078. [PubMed: 8816754]
21. Hooshangi S, Thiberge S, Weiss R. Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. *PNAS* 2005;102:3581. [PubMed: 15738412]
22. Ferrell JE Jr. How responses get more switch-like as you move down a protein kinase cascade. *Trends Biochem Sci* 1997;22:288. [PubMed: 9270299]
23. Kholodenko BN. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur J Biochem* 2000;267:1583. [PubMed: 10712587]
24. Markevich NI, Hoek JB, Kholodenko BN. Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J Cell Biol* 2004;164:353. [PubMed: 14744999]
25. Wang X, Hao N, Dohlman HG, et al. Bistability, stochasticity, and oscillations in the mitogen-activated protein kinase cascade. *Biophys J* 2006;90:1961. [PubMed: 16361346]
26. Behar M, Hao N, Dohlman HG, et al. Mathematical and computational analysis of adaptation via feedback inhibition in signal transduction pathways. *Biophys J* 2007;93:806. [PubMed: 17513354]

27. Heinrich R, Neel BG, Rapoport TA. Mathematical models of protein kinase signal transduction. *Mol Cell* 2002;9:957. [PubMed: 12049733]
28. Chaves M, Sontag ED, Dinerstein RJ. Optimal length and signal amplification in weakly activated signal transduction cascades. *J Phys Chem B* 2004;108:15311.
29. Sontag E, Chaves M. Exact computation of amplification for a class of nonlinear systems arising from cellular signaling pathways. *Automatica* 2006;42:1987.
30. Morishita Y, Kobayashi TJ, Aihara K. An optimal number of molecules for signal amplification and discrimination in a chemical cascade. *Biophys J* 2006;91:2072. [PubMed: 16798800]
31. Nash P, Tang XJ, Orlicky S, et al. Multisite phosphorylation of a CDK inhibitor sets a threshold for the onset of DNA replication. *Nature* 2001;414:514. [PubMed: 11734846]
32. Deshaies RJ, Ferrell JE. Multisite phosphorylation and the countdown to S phase. *Cell* 2001;107:819. [PubMed: 11779457]
33. Qu Z, Weiss JN, MacLellan WR. Regulation of the mammalian cell cycle: a model of the G1-to-S transition. *Am J Physiol Cell Physiol* 2003;284:C349. [PubMed: 12388094]
34. Goldbeter A, Koshland DE Jr. An amplified sensitivity arising from covalent modification in biological systems. *Proc Natl Acad Sci U S A* 1981;78:6840. [PubMed: 6947258]
35. Qian H. Amplifying signal transduction specificity without multiple phosphorylation. *Biophys J* 2003;84:1410. [PubMed: 12547820]
36. Qian H. Phosphorylation energy hypothesis: open chemical systems and their biological functions. *Annu Rev Phys Chem* 2007;58:113. [PubMed: 17059360]
37. Goodwin B. An entrainment model for timed enzyme synthesis in bacteria. *Nature* 1966;209:479. [PubMed: 5919577]
38. Bliss RD, Painter PR, Marr AG. Role of feedback inhibition in stabilizing the classical operon. *J Theor Biol* 1982;97:177. [PubMed: 6752586]
39. Pomerening JR, Sontag ED, Ferrell JE Jr. Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nat Cell Biol* 2003;5:346. [PubMed: 12629549]
40. Sha W, Moore J, Chen K, et al. Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc Natl Acad Sci U S A* 2003;100:975. [PubMed: 12509509]
41. Becskei A, Seraphin B, Serrano L. Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. *EMBO J* 2001;20:2528. [PubMed: 11350942]
42. Ozbudak EM, Thattai M, Lim HN, et al. Multistability in the lactose utilization network of *Escherichia coli*. *Nature* 2004;427:737. [PubMed: 14973486]
43. Thattai M, van Oudenaarden A. Attenuation of noise in ultrasensitive signaling cascades. *Biophys J* 2002;82:2943. [PubMed: 12023217]
44. Belle A, Tanay A, Bitincka L, et al. Quantification of protein half-lives in the budding yeast proteome. *Proc Natl Acad Sci U S A* 2006;103:13004. [PubMed: 16916930]
45. Gardner TS, Cantor CR, Collins JJ. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* 2000;403:339. [PubMed: 10659857]
46. Qu Z, MacLellan WR, Weiss JN. Dynamics of the cell cycle--checkpoints, sizers and timers. *Biophys J* 2003;85:3600. [PubMed: 14645053]
47. Novak B, Tyson JJ. Design principles of biochemical oscillators. *Nat Rev Mol Cell Biol* 2008;9:981. [PubMed: 18971947]
48. Tsai TY, Choi YS, Ma W, et al. Robust, tunable biological oscillations from interlinked positive and negative feedback loops. *Science* 2008;321:126. [PubMed: 18599789]

Figure 1. Schematic plots of signaling cascades. **A.** An example of MAPK signaling cascades. **B.** A linear signaling cascade model. **C.** A dual phosphorylation/dephosphorylation model of signaling cascades, respectively. **D.** A simplified scheme for a signaling cascade with arbitrary cascade length N , as used in this study. The dashed line denotes a feedback loop with can be either positive or negative.

Figure 2.

Steady state responses to a constant stimulus in a cascade. **A.** Graphical determination of steady states of the equation $\alpha = [X^{\beta-1}(1-X)]^{1/\beta}$ for different β . The horizontal solid line is $\alpha = 0.3$, which intersects with the $\beta = 2$ curve at $X_{th} = 0.1$ and $X_s = 0.9$. The dashed line is $\alpha = 0.5$, which is tangential to the $\beta = 2$ curve at $X_c = 0.5$. **B.** The two steady states of Eq. 6 versus α for $\beta = 1$. **C.** The steady states of Eq. 6 versus α when $\beta = 2$. **D.** The signal amplitude versus the cascade step number n for different S . $\alpha = 0.3$, $\beta = 2$. **E.** Steady state response to S for X_1 to X_{10} . $\alpha = 0.3$, $\beta = 2$ (the response becomes steeper from X_1 to X_{10}).

Figure 3.

Response of the cascade to a pulse stimulus and the emergence of threshold. **A.** X_n ($n=1$ to 10) versus time for a weak stimulus ($S=0.01$, the thick gray line) for $\alpha=0.5$ and $\beta=1$. **B.** Same as A but for $S=1$. **C.** Signal (X_1 to X_{10}) attenuation due to sub-threshold stimulus ($S=0.15$). $\alpha=0.3$ and $\beta=2$. **D.** Signal amplification due to supra-threshold stimulus ($S=0.16$). $\alpha=0.3$ and $\beta=2$. Note the different scales of y-axis in panels C and D. **E.** α_c vs. β . The solid line is $\alpha_c = (1 - 1/\beta)(\beta - 1)^{-1/\beta}$ which is for the constant stimulus. Symbols are from numerical simulations of Eq. 1 for pulse stimulus with duration $T_0=10$, $S=1$, and $N=10$ were used. **F.** The minimum T_0 versus S necessary for a response to be generated through the system with $\alpha=0.3$ and $\beta=2$. **G.** The maximum τ versus S to generate a response through the system with the parameters $\alpha=0.3$, $\beta=2$, and $T_0=10$. $\tau=5$ was used for A-F.

Figure 4. Regulation of signal delay and duration. **A.** Signal delay of X_{10} (D_{10}) and duration of X_{10} (T_{10}) versus β for $\alpha=0.3$. **B.** D_{10} and T_{10} versus α for $\beta=2$. Lines are the analytical results from Eq. 17. **C.** X_{10} versus time for different α and β . $T_0=10$, $\tau=5$, and $N=10$ were used.

Figure 5. Schematic plot of the relation between X_{n-1} (gray solid line), $f(X_{n-1})$ (black dashed line), and X_n (black solid line). D_n is defined as the duration between the time when the stimulus is given and the time when X_n increases to α at which $f(X_n)=1/2$. T_n is defined as the duration during which $X_n > \alpha$.

Figure 6.

Effects of positive feedback in linear signal cascade modeled with Michaelis-Menten kinetics ($\beta=1$). $\alpha=0.3$ and $\eta=0.5$. **A.** The steady state of X_1 versus stimulus strength for $N=2$ (gray) and $N=5$ (black). **B.** The steady state of X_1 versus the maximum eigenvalue $\lambda_{\max}$ for the two cases in A. **C.** X_{10} versus time for a very weak (gray) and a strong stimulus (black). $N=10$.

Figure 7.

Effects of positive feedback in nonlinear signal cascade modeled with Hill kinetics ($\beta > 1$).

A. The steady state of X_1 versus stimulus strength for $N=2$ (gray) and $N=5$ (black). **B.** The steady state of X_1 versus the maximum eigenvalue $\lambda_{\max}$ for the two cases in A. **C.** X_{10} versus time for subthreshold (gray) and suprathreshold (black) stimulus. **D.** Stimulus threshold (S_{th}) versus β for $\alpha=0.3$, $\eta=0.5$, and $N=10$. **E.** S_{th} versus N for $\alpha=0.3$, $\eta=0.5$, and $\beta=2$. **F.** Minimum η required for bistability to occur versus cascade length N for $\alpha=0.3$ and $\beta=2$.

Figure 8.

Effects of negative feedback. **A.** Bifurcation diagram versus stimulus strength S showing the stable steady state of X_1 (black circles), the unstable steady state of X_1 (dashed line), and the maximum and minimum values of X_1 during oscillations (open circles). $\alpha=0.3$, $\beta=2$, and $N=5$. **B.** Same as A except $N=8$. **C.** X_1 to X_8 versus time showing oscillations. **D.** the critical feedback strength η versus β for different cascade length for $\alpha=0.3$. **E.** The critical feedback strength η versus α for $\beta=2$ and $N=5$. **F.** Minimum η required for limit cycle oscillations to occur versus cascade length N for $\alpha=0.3$ and $\beta=2$.