Attenuation of Noise in Ultrasensitive Signaling Cascades

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ABSTRACT Ultrasensitive cascades often implement thresholding operations in cell signaling and gene regulatory networks, converting graded input signals into discrete all-or-none outputs. However, the biochemical and genetic reactions involved in such cascades are subject to random fluctuations, leading to noise in output signal levels. Here we prove that cascades operating near saturation have output signal fluctuations that are bounded in magnitude, even as the number of noisy cascade stages becomes large. We show that these fluctuation-bounded cascades can be used to attenuate the noise in an input signal, and we find the optimal cascade length required to achieve the best possible noise reduction. Cascades with ultrasensitive transfer functions naturally operate near saturation, and can be made to simultaneously implement thresholding and noise reduction. They are therefore ideally suited to mediate signal transfer in both natural and artificial biological networks.

INTRODUCTION

Cascades are ubiquitous in biological systems: from protein cascades such the G-protein cascade-mediating phototransduction (Lamb, 1996) and the mitogen-activated protein (MAP) kinase cascades in *Saccharomyces cerevisiae* (Gustin et al., 1998) and *Xenopus laevis* (Ferrell and Machleder, 1998), to genetic cascades such as the one that regulates timed flagellar motor development in *Escherichia coli* (Kalir et al., 2001). Cascades have long been known to possess several desirable regulatory properties (Stadtman and Chock, 1977; Chock and Stadtman, 1977), including the ability to perform thresholding operations on graded inputs (Ferrell, 1996). For example, the *Xenopus* MAP kinase cascade converts progesterone level to an all-or-none oocyte maturation response; the *E. coli* flagellar regulatory cascade is thought to activate successive operon classes in a time-dependent manner as the level of some transcription factor crosses successively higher thresholds.

Given their ubiquity, it is crucial for proper cell-wide regulation that thresholding cascades are capable of reliable signal transmission. However, regulatory signals can be corrupted by the intrinsic noise of biochemical reactions. At the low reactant concentrations of the intracellular medium, reaction rates are stochastic, so biochemical concentrations and gene expression levels will be subject to significant fluctuations (McAdams and Arkin, 1997, 1999). The implications of such fluctuations for biochemical and genetic networks have only recently come under scrutiny. There is growing interest in the effect of network structure on noise characteristics. Regulation of noise has been experimentally observed in the expression of a single gene (Ozbudak et al., in press) and in an autoregulated genetic system (Becskei and Serrano, 2000), and the role of noise in biological switches (Hasty et al., 2000; Kepler and Elston, 2001), amplifiers (Paulsson et al., 2000), and various other network structures (Thattai and van Oudenaarden, 2001) has been theoretically investigated. Here we examine the effect of cascade structural properties on noise propagation, and investigate the ability of noisy biological cascades to faithfully transmit signals.

When fluctuations become significant, the main concern is that successive stochastic cascade stages could introduce successively higher noise levels into the signal being propagated, thereby corrupting the final output. This would be especially relevant for large cascades such as the flagellar regulatory system in which the signal from the master regulator is transmitted through many intermediate regulators before finally activating transcription (Kalir et al., 2001). MAP kinase cascades also involve several stages before the final transcription signal (Gustin et al., 1998), and are similarly vulnerable to noise.

The primary purpose of this paper is to show that it is possible to limit the propagation of noise in thresholding cascades. We first examine a generic stochastic cascade and find the conditions under which the size of output fluctuations is bounded, even as successive intrinsically noisy stages are added. Such fluctuation-bounded cascades can be designed to produce an output that is less noisy than the input, essentially by piggy-backing the input signal onto a low-noise carrier. We then consider the case of ultrasensitive cascades, meaning those whose components display a sigmoidal response. Such cascades can be used to implement thresholding. In addition, as we show, they are naturally driven to a saturation regime in which the conditions for bounded fluctuations are satisfied. Noise reduction can therefore be added to the list of desirable properties of signaling cascades, and might be essential for the normal execution of their function in cell-wide signal transduction.

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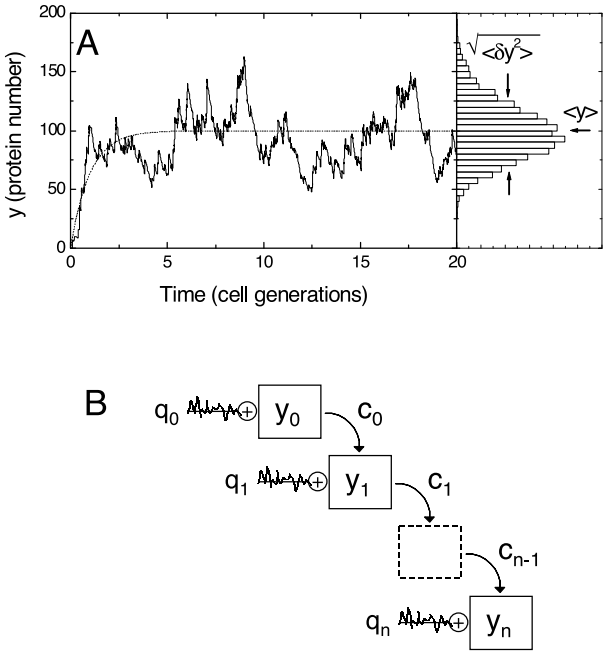

FIGURE 1 Modeling stochastic cascades. (*A*) Stochastic gene expression. Protein number is plotted as a function of time (in cell generations) according to Eq. 6, with $\gamma = 1$, $k = 20$, and $b = 5$. The dotted line shows the deterministic timecourse, and the solid line shows the result of a stochastic simulation (Thattai and van Oudenaarden, 2001). Recording the state of the system at $t = 20$ over 5000 trial runs produces a histogram that displays a mean $\langle y \rangle = 100$, and a variance $\langle \delta y^2 \rangle = 600$. For this example, the noise strength is given by $q = 2kb(1 + b) = 1200$. (*B*) A generic linearized stochastic cascade. Species concentrations y_i ($i = 0, \ldots, n$) are subject to random fluctuations of strength q_i. The differential amplification factors c_i give the response of y_{i+1} to a change in y_i. The input signal y_0 is read out at y_n.

ANALYSIS

Modeling stochastic biochemical reactions

Before we discuss noise in biological signaling cascades, we present a primer on the modeling of stochastic biochemical reactions in the general case. This discussion will also serve to introduce notation required to describe the expression of a stochastic gene. In the following sections, we will illustrate our results using examples of stochastic genetic cascades.

Traditional treatments of biochemical reactions involve the deterministic time-evolution of a vector x, whose components represent the concentrations of various chemical species, according to some equation $dx/dt = g(x, t)$. When fluctuations become significant, however, macroscopic deterministic equations are no longer sufficient to describe the system (Fig. 1 *A*). Here we use the Langevin technique to model random fluctuations (van Kampen, 1992; Kepler and Elston, 2001). This technique involves adding a time-dependent noise term, $\eta(x, t)$, to the deterministic dynamical equations so that

$$\dot{x} = g(x) + \eta(x, t), \tag{1}$$

where $\dot{x}$ represents a time derivative, and where we assume that g is time-independent. The random variable $\eta(x, t)$ is defined by its statistical properties. We assume gaussian white-noise statistics,

$$\langle \eta(x, t) \rangle = 0, \quad \langle \eta(x, t)\, \eta(x, t + \tau) \rangle = q(x)\delta(\tau), \tag{2}$$

where $\delta(\tau)$ represents the Dirac delta function, and $\langle \cdots \rangle$ represents an ensemble average. The first condition states that $\eta(x, t)$ has zero mean, and the second that the value of $\eta(x, t)$ at one time is completely uncorrelated with its value at any other time. Although these conditions only approximate the actual noise statistics, they will produce the correct values of means and variances in the limit that fluctuations are small perturbations, treated to linear order. Because we confine our analysis to the steady state, we drop the explicit state-dependence of the stochastic variable, writing $\eta(x, t)$ and $q(x)$ simply as $\eta(t)$ and q. The parameter q gives the magnitude of the fluctuations, and must be determined by considering a microscopic model of the system. We give two examples below for calculating q.

First, consider some chemical species Y that is produced at a rate r_+ and destroyed at a rate r_- in independent chemical reactions. If $y(t)$ is the number of molecules of species Y at time t, then

$$\dot{y} = r_+ - r_- + \eta(t). \tag{3}$$

In a small time interval, Δt, there will be a net mean change $\langle \delta y \rangle = n_+ - n_- = r_+ \Delta t - r_- \Delta t$ in the number of molecules y. If creation and destruction of Y are Poisson processes, the variance in the number of individual reactions will be equal to the mean, so $\langle n_+^2 \rangle = \langle n_+ \rangle$, and similarly for n_-. These variances will then add independently to produce the total variance in δy, giving

$$\langle \delta y^2 \rangle = (r_+ + r_-)\Delta t. \tag{4}$$

Now, this variance can also be calculated from Eq. 3. Fluctuations around the deterministic value are given by $\dot{\delta y}(t) = \eta(t)$ so

$$\langle \delta y^2 \rangle = \int_0^{\Delta t} \int_0^{\Delta t} \langle \eta(t)\eta(t') \rangle \, dt \, dt' = q\Delta t, \tag{5}$$

where we have applied Eq. 2 in averaging over η. For consistency between the microscopic (Eq. 4) and macroscopic (Eq. 5) descriptions, we must set $q = (r_+ + r_-)$. (The preceding argument is from Detwiler et al., 2000.)

Second, consider the production of a protein Y with decay rate γ, from a gene with transcription rate k that produces an average of b proteins per mRNA transcript (Fig. 1 *A*). If y measures protein number, then

$$\dot{y} = kb - \gamma y + \eta(t). \tag{6}$$

In steady state, it has been shown (Thattai and van Oudenaarden, 2001) that

$$\langle \delta y^2 \rangle \approx (1 + b)\langle y \rangle. \tag{7}$$

This result arises because random bursts of proteins of average size b are produced from each transcript, raising the variance above the Poisson level of $\langle y \rangle$. Expanding Eq. 6 for fluctuations around steady state, then Fourier transforming gives

$$\dot{\delta} y + \gamma \delta y = \eta(t) \Rightarrow (i\omega + \gamma)\delta y(\omega) = \eta(\omega)$$

$$\Rightarrow \langle |\delta y(\omega)|^2 \rangle = \frac{q}{\omega^2 + \gamma^2} \tag{8}$$

where the last equality is obtained by taking ensemble averages, and applying condition 2. It follows from the Wiener–Khintchine theorem that steady-state fluctuations are given by an inverse Fourier transform at $\tau = 0$,

$$\langle \delta y^2 \rangle = \int_{-\infty}^{+\infty} \frac{d\omega}{2\pi} \frac{q}{\gamma^2 + \omega^2} = \frac{q}{2\gamma}. \tag{9}$$

Because $\langle y \rangle = kb/\gamma$, consistency between Eqs. 7 and 9 requires that $q = 2kb(1 + b)$ in steady state.

Note that the formulas derived above apply only when signal levels are measured in dimensionless molecule numbers. Measuring them in concentration units or by some other normalization will require changing the value of q to preserve the form of Eq. 1. For example, scaling units by a factor a will give

$$x = a\bar{x}, \quad \eta = a\bar{\eta} \Rightarrow q = a^2\bar{q}. \tag{10}$$

We have shown here that the magnitude q of the intrinsic fluctuations of individual species depends on the microscopic features of the system. In many cases, two different systems whose time evolution is described by the same normalized macroscopic deterministic equations could nevertheless display very different noise characteristics because they differ in microscopic detail. (For example, although it is only the product kb that enters into Eq. 6, q can be changed by simply varying b.) For our purposes, the relevant microscopic properties are entirely summarized by q, which we will simply treat as an additional parameter required to describe the system.

Bounding fluctuations in a stochastic cascade

The Langevin technique described above can be used to model a generic stochastic cascade (Fig. 1 *B*). We consider a cascade of species y_i ($i = 0, \ldots, n$) in which the creation rate of y_i can only depend on y_{i-1}, and in which y_i itself undergoes first-order decay at a rate γ_i,

$$\dot{y}_i + \gamma_i y_i = f_{i-1}(y_{i-1}). \tag{11}$$

The function f_i, giving the rate of creation of a downstream species in terms of the concentration of an upstream one, is the single-stage transfer function. Linearizing Eq. 11 for fluctuations about steady state and adding a Langevin noise term, we obtain

$$\delta\dot{y}_i + \gamma_i \delta y_i = c_{i-1}\delta y_{i-1} + \eta_i,$$

$$\langle \eta_i(t)\eta_i(t + \tau) \rangle = q_i\delta(\tau). \tag{12}$$

Here, c_i is the derivative of the transfer function f, and will be referred to as the differential amplification factor. (Note that c_i and γ_i both have units of t^{-1}.)

We now make the simplifying assumption that all species decay at the same rate. This assumption is appropriate for genetic cascades, because protein concentrations typically decay by dilution (protein degradation rates are low) so γ_i will be equal to the cell growth rate. It is less clear that the assumption is valid for cascades involving covalent modification of proteins, although there are optimization arguments favoring the tuning of the decay rates in this manner (Detwiler et al., 2000). We expect that the results derived below will apply as long as all γ_i are comparable.

We can set $\gamma_i = \gamma = 1$ by our choice of time units, in which case c_i will be measured in units of γ. Fourier-transforming Eq. 12,

$$\langle \delta y_i^2(\omega) \rangle = \frac{q_i + c_{i-1}^2 \langle \delta y_{i-1}^2(\omega) \rangle}{1 + \omega^2}$$

$$\Rightarrow \zeta_i = \alpha_i + \beta_{i-1}\,\zeta_{i-1}, \tag{13}$$

where we have set $\alpha_i = q_i/(1 + \omega^2)$, $\beta_i = c_i^2/(1 + \omega^2)$, and $\zeta_i = \langle \delta y_i^2(\omega) \rangle$. Expanding the recursion relation in Eq. 13,

$$\zeta_n = \alpha_n + \beta_{n-1}\alpha_{n-1} + \beta_{n-1}\beta_{n-2}\alpha_{n-2}$$

$$+ \cdots + \beta_{n-1} \cdots \beta_0\zeta_0. \tag{14}$$

Now, we seek to place an upper bound on the magnitude of fluctuations in the output signal, even as the cascade becomes arbitrarily large; that is, we are looking for a bound on $\langle \delta y_{n\to\infty}^2 \rangle$. To this end, let $q \equiv \max(q_i)$ and $c \equiv \max(|c_i|)$ be upper bounds on noise strengths and differential amplification factors, respectively. Let $\alpha \equiv q/(1 + \omega^2)$ and $\beta \equiv c^2/(1 + \omega^2)$. Then

$$\zeta_n \le \alpha(1 + \beta + \cdots + \beta^{n-1}) + \beta^n\zeta_0 \Rightarrow \zeta_\infty \le \frac{\alpha}{1 - \beta}. \tag{15}$$

Resubstituting for α, β, and ζ, and taking an inverse Fourier transform,

$$\langle \delta y_\infty^2 \rangle \le q \int \frac{d\omega}{2\pi} \frac{1}{1 + \omega^2 - c^2}. \tag{16}$$

This gives the final fluctuation bound,

$$\langle \delta y_\infty^2 \rangle \leq \frac{q}{2\sqrt{1-c^2}}. \tag{17}$$

This result shows that fluctuations in the output signal will be bounded, as long as the differential amplification factors are below unity, i.e., for $|c_i| \leq |c| < 1$. Alternatively, note that ζ_∞ is a fixed point of the recursion relation Eq. 13. This fixed point will be stable only if $|\beta| < 1$, or equivalently, $|c| < 1$. We see that output fluctuations can be larger than intrinsic fluctuations due to any single cascade stage, because of the factor $1/\sqrt{1-c^2}$. Eq. 14 can be used to calculate the size of fluctuations in intermediate cascade stages as well. Fluctuations of the first species will be purely due to the intrinsic noise of that species, whereas propagated noise can contribute to the fluctuations of downstream species. This behavior is illustrated in Fig. 2 *A* for the example of a genetic cascade.

Noise attenuation and optimal cascade length

One possible function of a fluctuation-bounded cascade is that of noise reduction. To illustrate this property, we examine the particular case of low-noise cascades with high-noise inputs. The magnitude of output fluctuations for a cascade with $|c| < 1$ is independent of noise in the input, if the cascade is sufficiently large. The high-noise input signal is effectively transferred to a low-noise carrier, which is isolated from input fluctuations by repeated application of the factor $c^2 < 1$. A cascade with a low fluctuation bound can therefore be used to reduce the fluctuations in a noisy input signal (Figs. 2 *B* and 4 *B*).

Consider a cascade of species y_i ($i = 1, 2, \ldots, n$) with low noise strength $q_i = q$, whose input stage has a high noise strength $q_0 > q$. The inverse Fourier transform of Eq. 14 gives

$$\langle \delta y_n^2 \rangle = \sum_{j=0}^{n} q_{n-j} \int \frac{d\omega}{2\pi} \frac{c^{2j}}{(1+\omega^2)^{j+1}}$$

$$= \sum_{j=0}^{n} \frac{q_{n-j}}{2} \binom{2j}{j} \left(\frac{c}{2}\right)^{2j}. \tag{18}$$

The variance of the output signal $\langle \delta y_n^2 \rangle$ contains contributions from two distinct sources: the intrinsic noise due to the cascade, and the attenuated noise propagated from the input. After applying Stirling's approximation ($j! \approx (j/e)^j \sqrt{2\pi j}$), we rewrite Eq. 18 to explicitly show these two contributions,

$$\langle \delta y_n^2 \rangle = \underbrace{\frac{q}{2}\left(1 + \sum_{j=1}^{n-1} \frac{c^{2j}}{\sqrt{\pi j}}\right)}_{\text{cascade}} + \underbrace{\frac{q_0}{2}\left(\frac{c^{2n}}{\sqrt{\pi n}}\right)}_{\text{input}}. \tag{19}$$

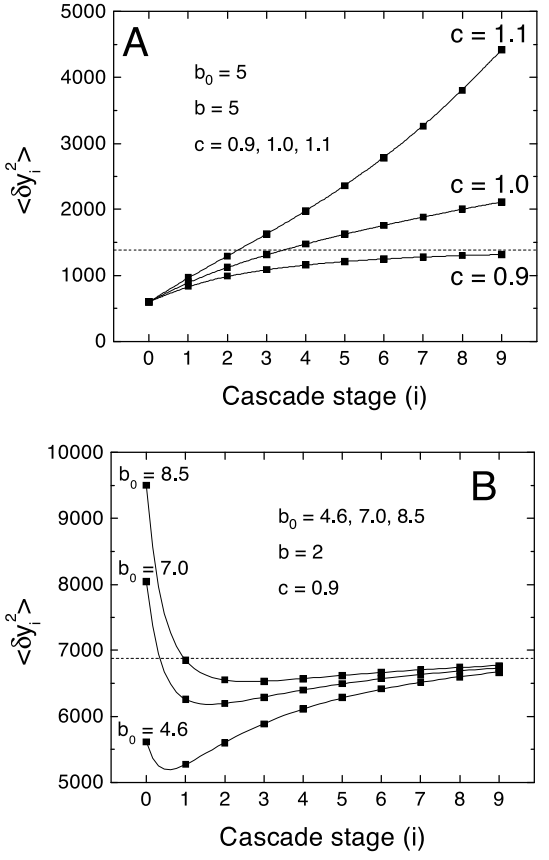

FIGURE 2 Noise propagation in a genetic cascade. The size of fluctuations is plotted versus the cascade stage. The proteins y_i obey $\dot{y} + \gamma y_i = kb(1-c) + cy_{i-1} + \eta_i$, with $y_0 = kb$ and $\gamma = 1$. This gives all cascade stages the same mean protein number $\langle y_i \rangle = kb$, so fluctuations can be directly compared. (*A*) Fluctuation-bounded and -unbounded cascades. We use $k = 20$ and $b = 5$, giving $\langle y_i \rangle = 100$. The size of fluctuations $\langle \delta y_i^2 \rangle$ depend on the value of the differential amplification factor c. Fluctuations of the first species $\langle \delta y^2 \rangle = 600$ are purely due to intrinsic noise, whereas those of downstream species are also due to propagated noise. Because $q = 2kb(1+b) = 1200$, the cascade with $c = 0.9$ has fluctuations bounded by $q/2\sqrt{1-c^2} = 1.38 \times 10^3$ (*dotted line*), but those with $c = 1$ and $c = 1.1$ have unbounded fluctuations. (*B*) Optimal cascade length for noise reduction. Parameters are chosen so that $\langle y_i \rangle = 1000$. All stages have $b = 2$ except for the noisy input stage, which has $b_0 = 4.6, 7.0, 8.5$. This gives noise minima at $n_{opt} = 1, 2, 3$, respectively. The variance at the optimal cascade length can be significantly smaller than the fluctuation bound of $\langle \delta y_\infty^2 \rangle = 6.88 \times 10^3$.

The first term in this expression increases with cascade length, whereas the second term decreases. The input noise contribution is attenuated better than exponentially,

$$\langle \delta y_n^2 \rangle_{input} \sim \frac{e^{-n/n_0}}{\sqrt{n}}, \tag{20}$$

where $n_0 = 1/\ln(1/c^2)$ sets the attenuation length scale. When $q_0 \gg q$, n_0 is a good estimate for the cascade length required to achieve the final fluctuation bound. An important consequence of better-than-exponential attenuation is that a cascade of n stages, each with differential amplifica-

tion factor c, performs better than a single cascade stage with an amplification factor c^n. Although both these systems have the same net amplification factor, the former achieves a $\sqrt{n}$ lower variance. This occurs because of the finite response time $1/\gamma$ of the cascade components. When there is a large fluctuation in the concentration of the first species, downstream components will be slow to react, and will not be able to make large excursions from the mean. This sluggish response is the price paid for an improved signal-to-noise ratio.

The interplay between the decreasing input noise contribution and the increasing cascade noise contribution creates the possibility of achieving a noise minimum, which will occur at stage n if $\langle \delta y_{n-1}^2 \rangle > \langle \delta y_n^2 \rangle < \langle \delta y_{n+1}^2 \rangle$. Using Eq. 19, this condition can be written as

$$\frac{n}{n+1} > \left(\frac{\alpha}{c^2}\right)^2 > \frac{n-1}{n}, \quad \alpha = \frac{q_0 - q}{q_0}. \qquad (21)$$

If $\alpha/c^2 < 1$, a noise minimum will therefore occur at cascade stage $n = n_{opt}$ given by

$$n_{opt} = \lfloor 1/(1 - \alpha^2/c^4) \rfloor, \qquad (22)$$

where $\lfloor x \rfloor$ represents the greatest integer less than x. This is the optimal length required for noise reduction (Fig. 2 *B*).

Ultrasensitive cascades are fluctuation bounded

We now show that both thresholding and noise reduction are naturally accomplished by ultrasensitive cascades. We first discuss how a cascade of ultrasensitive components can be used to produce a thresholded response. We then demonstrate that this architecture robustly places a limit $|c| < 1$ on the differential amplification factors, implying that the cascade is fluctuation bounded.

The ideal thresholding device produces two distinct outputs: high when the input is above threshold, and low when the input is below it. This sharp switching behavior can be approximated by a cascade of reactions with ultrasensitive single-stage transfer functions. Ultrasensitive behavior refers to a situation in which the output is more sensitive to variations in the input than is possible using hyperbolic Michaelis–Menten kinetics (Fig. 3 *A* and *B*). The sigmoidal response of an ultrasensitive reaction typically arises from positive cooperativity (Ptashne, 1992), zero-order covalent modification (e.g., phosphorylation) (Goldbeter and Koshland, 1981), or the occurrence of multiple activation sites (Ferrell, 1996).

The net transfer function of a multi-stage cascade can display an enhanced sensitivity over that of its component single-stage transfer functions, producing sharper switching behavior as more cascade stages are added. This sensitivity amplification will occur as long as the kinetic constants of each component satisfy certain broad constraints (Chock and Stadtman, 1977; Goldbeter and Koshland, 1981). For example, let $f(x) = x^2/(x^2 + 1)$ (the Hill function of order 2) describe the shape of a typical sigmoidal transfer function. The maximum slope of this function occurs at $x = 1$, at the half-saturation point of f. Let the actual transfer function at stage i have an amplitude a_i and a half-saturation point R_{i-1}, and let $\gamma = 1$ be the decay rate of all species. The time evolution of the species y_i in a cascade of n steps will then

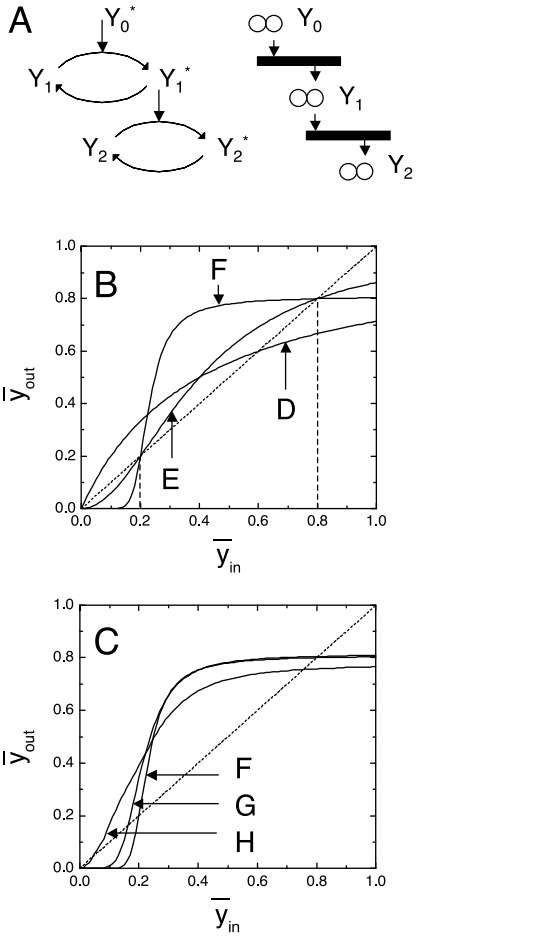

FIGURE 3 Robust thresholded response of ultrasensitive cascades. (*A*) Examples of ultrasensitive cascades. A cascade of proteins (*left*) that exist in two forms, Y_i, and an active form Y_i^*, ultrasensitivity can arise from zero-order covalent modification (Goldbeter and Koshland, 1981). A cascade of genes (*right*) coding for inducer proteins Y_i. Ultrasensitivity results from the protein dimerization reaction (Ptashne, 1992). (*B*) Sensitivity amplification. A hyperbolic Michaelis–Menten-type transfer function $f(\bar{y}/\bar{R}) = \bar{y}/(\bar{y} + \bar{R})$ (*D*) is compared with a typical ultrasensitive transfer function $f(\bar{y}/\bar{R}) = \bar{y}^2/(\bar{y}^2 + \bar{R}^2)$ (*E*) with $\bar{R} = 0.4$. The ultrasensitive function is compounded in a four-step cascade to give a much sharper net transfer function (*F*). The intersections of (*E*) and (*F*) with the dotted line of slope 1 give the fixed points of the cascade. Input signals below the threshold value of 0.2 are driven to zero, and those above it are driven to 0.8. (*C*) Net transfer functions are shown for four-step cascades, averaged over individual cascades that have $\bar{R}_i$ normally distributed about 0.4 with different standard deviations: (*F*) $\bar{R}_i = 0.4$, (*G*) $\bar{R}_i = 0.4 \pm 20\%$, (*H*) $\bar{R}_i = 0.4 \pm 50\%$. We see that sensitivity amplification occurs even for large variations in the values of $\bar{R}_i$.

be given by $\dot{y}_i + y_i = a_i f(y_{i-1}/R_{i-1})$. Rescaling y_i and R_i by a_i gives

$$\dot{\bar{y}}_i + \bar{y}_i = f(\bar{y}_{i-1}/\bar{R}_{i-1}), \tag{23}$$

and the steady-state solutions will then satisfy $0 \le \bar{y}_i \le 1$. For sensitivity amplification to occur, the ultrasensitive portions of successive single-stage transfer functions must "line up" so that their individual slopes can be multiplied to produce a steep net transfer function. Consider the case when all single-stage transfer functions have identical half-saturation points $\bar{R}_i = \bar{R}$. The net transfer function as $n \to \infty$ will then be a step function whose low output, high output, and threshold point are given by the three solutions of the equation $\bar{y} = f(\bar{y}/\bar{R})$. These solutions will exist as long as $\bar{R} \le ½$; choosing $\bar{R}_i = \bar{R} < ½$ is thus sufficient for sensitivity amplification (Fig. 3 *B*). The net response will become more gradual if the spread in $\bar{R}_i$ is increased, because the single-stage transfer functions would then fail to line up exactly. However, sensitivity amplification can still be achieved for fairly large variations in $\bar{R}_i$ (Fig. 3 *C*). Ultrasensitive cascades thus implement a thresholding operation that is robust to significant changes in component parameters. Specifically, the steep central region and the flat or saturated regions of the net transfer function can be achieved without much fine-tuning.

The role of ultrasensitive cascades in the establishment of all-or-none cellular responses has been extensively studied, both theoretically and experimentally (Ferrell and Machleder, 1998; Ferrell, 1996; Chock and Stadtman, 1977). Such studies mainly focus on thresholding as the deterministic modification of some input signal. When noise is introduced, however, the effective cascade transfer function can behave differently than deterministically predicted. For example, fluctuations in biochemical reaction rates can cause the sharp transition region of ultrasensitive systems to become more gradual (Berg et al., 2000), just as less-than-ideal transfer parameters can reduce cascade sensitivity (Fig. 3 *C*). Here we concentrate on the behavior of fluctuations in the saturated regions of the net transfer function, close to the high or low outputs and away from the steep or ultrasensitive region.

We want to obtain a limit on c, the differential amplification factor, for an ultrasensitive cascade. We proceed with the following geometric argument: the net transfer function of a multi-stage cascade is obtained by iteratively applying the single-stage transfer function, as shown in Fig. 4 A. Saturation occurs because points above threshold are mapped close to the high fixed point, while those below threshold are mapped close to the low fixed point; these fixed points effectively become the operating points for the cascade after very few iterations. At the fixed points (where the transfer function intersects the line of slope 1) the single-stage transfer function itself has slope $|c| < 1$; this will be true even for cascades whose individual stages show

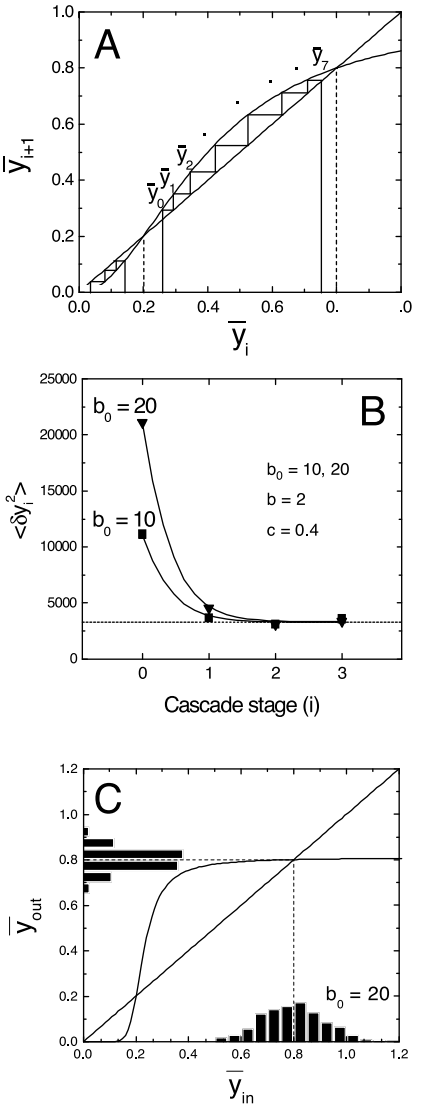

FIGURE 4 Thresholding and noise reduction in an ultrasensitive cascade. (*A*) Iteration of the single-stage transfer function. The single-stage function is $f(\bar{y}/\bar{R}) = \bar{y}^2/(\bar{y}^2 + \bar{R}^2)$ with $\bar{R} = 0.4$, whose stable fixed points are at $\bar{y} = 0.0$ and $\bar{y} = 0.8$, with a threshold point at $\bar{y} = 0.2$. The slope of this function at the low fixed point is $c = 0$, and that at the high fixed point is $c = 0.4$. (*B*) Attenuation of input noise. The size of fluctuations is shown versus the cascade stage. These results are obtained from Monte Carlo simulations of an ultrasensitive genetic cascade (Eq. 23) using Gillespie's algorithm (Thattai and van Oudenaarden, 2001). Steady state is assumed to have been reached after 15 cell generations, and results are averaged over 500 trials. The numerical results (*symbols*) closely match the analytic predictions of Eq. 18 (*lines*). We set the system to operate at the high fixed point $\bar{y} = 0.8$, so that $c = 0.4$. The unscaled mean protein number is chosen to be $\langle y \rangle = 1000$, and $b = 2$ for all cascade stages except for the input stage, which has $b_0 = 10$ (*squares*) and $b_0 = 20$ (*triangles*). Although the input signal is noisy, the output fluctuations all saturate at $\langle \delta y^2 \rangle = 3.27 \times 10^3$ (*dotted line*) as predicted by Eq. 17. The attenuation length for this cascade is $n_0 = 0.55$ (Eq. 20). (*C*) Net transfer function shows thresholding behavior. Noise reduction is illustrated for the cascade with $b_0 = 20$. Histograms obtained from the Monte Carlo simulations show the high input fluctuations ($\langle \delta y^2 \rangle / \langle y \rangle = 0.14$) and reduced output fluctuations ($\langle \delta y^2 \rangle / \langle y \rangle = 0.06$).

variations in $\bar{R}_i$, as long as the variations are not large. The cascade therefore operates in accordance with Eq. 12 with $|c_i| \leq |c| < 1$, implying that fluctuations will *always* be bounded according to Eq. 16, and that noise attenuation can be achieved according to Eq. 20. Therefore, both thresholding and noise reduction are robust properties of ultrasensitive cascades.

To illustrate this noise-reduction effect, we have numerically simulated the behavior of an ultrasensitive cascade of genetic components. The simulation explicitly incorporates the discrete nature of transcription and translation events, and the Poisson noise of biochemical reactions (Thattai and van Oudenaarden, 2001). In Fig. 4 *B*, we compare the results of a Monte Carlo simulation of the complete system of ultrasensitive components (Eq. 23) to the analytic predictions of the linearized system (Eqs. 12 and 18): the match is excellent. The simulations clearly demonstrate that fluctuations in a noisy input signal are reduced by the action of the cascade, resulting in a low-noise output (Fig. 4 *C*).

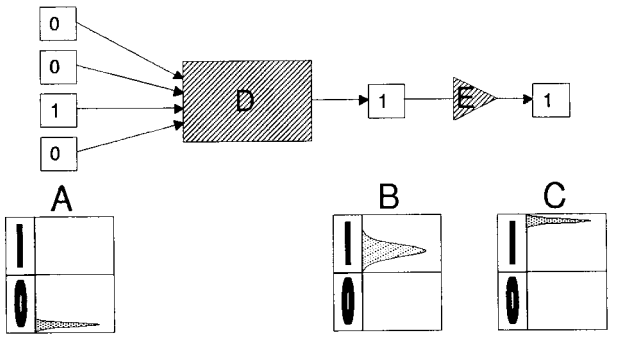

FIGURE 5 Signal and noise in binary systems. (*A*) An example of a well-defined 0 input is shown in a box whose vertical axis indicates the range of values that can represent 0 or 1. (*B*) During some hypothetical computation, the device *D* produces a noisy intermediate signal. (*C*) The component *E* thresholds and sharpens this signal to produce a clean output.

DISCUSSION

Cascades play a crucial part in numerous cellular processes, from cell signaling to gene regulation. In many cases, the output of the cascade is several layers from the input, with each stage introducing potentially harmful noise into the propagated signal. We have shown that cascades constructed within certain wide tolerances can transmit signals through any number of stages, with essentially no addition of noise. Ultrasensitive cascades, commonly used to implement thresholding and frequently found in living systems, are among the cascades that display such a fluctuation bound. We have also discussed systems that can implement noise reduction. To perform this function, cascades must not only be fluctuation bounded, but must also be intrinsically less noisy than the input signal. We argue that this is frequently the case. For example, MAP kinase cascades can convert the input signal from very few active membrane receptors into an output involving a large number of phosphorylated proteins. Let $N_{in} \ll N_{casc}$ relate the mean number of input receptors and output cascade proteins. We can estimate the size of signal fluctuations by assuming that they arise from simple Poisson statistics, giving $q \propto N$. When we normalize mean numbers to unity, Eq. 10 implies that $q \propto 1/N$, giving the familiar $1/\sqrt{N}$ Poisson behavior. Therefore, such cascades satisfy the noise-reduction criterion $q_{casc} \ll q_{in}$. The noisy input from a few membrane receptors is converted to a low-noise signal carried by large numbers of proteins. A more complete analysis of experimental data will be required to see if such cascades are, in fact, optimal in length.

The connection between thresholding and noise reduction can be useful in several contexts. The thresholding operation produces a binary output that is common in biological systems, indicating the presence or absence of ligands or triggering all-or-none responses, for example; but binary circuits have also long been the medium of choice for constructing complex artificial computational devices. Because the two states, 0 and 1, of any binary signal can be easily distinguished, binary systems are robust to noise sources, and also to variations in the transfer parameters of their components. Further, as long as the input and output signals of these components satisfy certain tolerances, each component can be designed independently, allowing small subunits to be assembled in a modular fashion to produce the final circuit. In practice, the physical computational device D (Fig. 5), whether a semiconductor circuit or a biochemical network, will produce an output of less than perfect quality. First, the average output could deviate from the ideal 0 or 1 values. Second, the spread in the output signal could be large due to noise sources in the device D. Such an output must be cleaned up before it can be used in a new computation: 1) The signal must be compared to a threshold value, and rectified to 1 if above threshold, and 0 if below. 2) The spread in the signal must be decreased, producing a well-defined, sharp output. We have shown that ultrasensitive cascades simultaneously perform both these functions without requiring fine-tuning of parameters (compare the input Fig. 5 *B* and output Fig. 5 *C* to the cascade behavior in Fig. 4 *C*). Such cascades might be used in the design of artificial biological networks.

The widespread natural occurrence of cascades, and their importance in the execution of virtually every cellular process, is probably due to their many virtues: sharp switching characteristics (Ferrell, 1996); multiple control points for tuning input–output functions (Stadtman and Chock, 1977; Chock and Stadtman, 1977); and the capacity for high amplification or ultrasensitivity (Detwiler et al., 2000). We have shown that, in addition to all these features, they are also able to reduce signal fluctuations. This further justifies their ubiquity in biological networks, and perhaps accounts in part for the robustness of living systems.

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