Review

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Ultrasensitivity part III: cascades, bistable switches, and oscillators

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Switch-like, ultrasensitive responses – responses that resemble those of cooperative enzymes but are not necessarily generated by cooperativity – are widespread in signal transduction. In the previous installments in this series, we reviewed several mechanisms for generating ultrasensitivity: zero-order ultrasensitivity; multistep ultrasensitivity; inhibitor ultrasensitivity; and positive feedback (or double negative feedback) loops. In this review, we focus on how ultrasensitive components can be important for the functioning of more complex signaling circuits. Ultrasensitivity can allow the effective transmission of signals down a signaling cascade, can contribute to the generation of bistability by positive feedback, and can promote the production of biochemical oscillations in negative feedback loops. This makes ultrasensitivity a key building block in systems biology and synthetic biology.

Why is ultrasensitivity important?

In the first two parts of this series [1,2], we examined a diverse collection of mechanisms that can generate ultrasensitive responses: switch-like responses that resemble those of cooperative enzymes (see Glossary). These include zero-order ultrasensitivity, multistep ultrasensitivity (including multisite phosphorylation, reciprocal regulation, and other forms of feed forward regulation), stoichiometric inhibitors and substrate competition, and positive feedback or double negative feedback. Experimental studies have implicated all of these mechanisms in signal transduction.

In and of itself, an ultrasensitive response seems like a potentially important biochemical property; it allows a signal transducer to ignore small stimuli and then respond decisively to modest changes in input stimulus. There is a cost to this, of course. The steady-state output of an ultrasensitive system is, by definition, more sensitive to fluctuations in the input stimulus than that of a Michaelian system, and the range of inputs over which the system responds is more limited. Nevertheless, there are many biological processes where the combination of decisiveness and filtering provided by an ultrasensitive system may be extremely valuable.

However, perhaps the greater importance of ultrasensitivity lies in how it enables more complicated systems, such as cascade amplifiers, bistable switches, and oscillators, to operate effectively. In this article, we finish our series by reviewing how ultrasensitivity can contribute to each of these processes.

Ultrasensitivity in signaling cascades

Before examining how ultrasensitive responses can contribute to the function of a signaling cascade, it is helpful to first examine how a cascade would be expected to behave in the absence of ultrasensitivity. Suppose we have a three-step signaling cascade, like the mitogen-activated protein kinase (MAPK) cascade, and suppose that there is no

Glossary

Bistable: having two stable steady states for a single value of the input, as contrasted with a monostable response.
Conservation equation: in the present context, it is an algebraic equation of the form $X_{tot} = X_1 + X_2 + \ldots$, where species X is interconverted among various forms (X_1, X_2....) but the total concentration of X (X_{tot}) does not change with respect to time.
Cooperativity: a characteristic of some multistep processes where completing some of the early steps makes a later step more favorable. Examples include the multistep binding of oxygen to hemoglobin and priming in multisite phosphorylation.
***EC*50:** effective concentration 50; the concentration of a stimulus required for a half-maximal (50%) response.
Hill function: an input-output relationship of the form $Output = \frac{Input^n}{K^n + Input^n}$, where n is the Hill exponent or Hill coefficient. The larger the Hill exponent, the more ultrasensitive the response.
Mass action kinetics: a simple kinetic scheme where the rate of a reaction is directly proportional to the concentration of the substrate or substrates involved in the reaction. This contrasts with Michaelis–Menten kinetics or kinetic schemes involving Hill functions.
Michaelis–Menten kinetics: a model for the rate of an enzymatic reaction, premised on the assumption that the enzyme is small in concentration compared to its substrate, and that the concentration of the enzyme-substrate complex is unchanging with respect to time. In the Michaelis–Menten model the rate of an enzymatic reaction is given by: $\frac{dProduct}{dt} = V_{\max}\frac{Substrate}{K_m + Substrate}$.
Michaelian response: a hyperbolic relationship between the input to a system and its steady-state response, described by an equation of the form $Output = \frac{Input}{EC50 + Input}$. The equation for the response resembles the Michaelis–Menten equation (above), hence the name. Note though that a Michaelian response is obtained when the activities of the enzymes that produce the output are described by the law of mass action, not the Michealis–Menten equation. See Part 1 of this series [1] for further discussion.
Monostable: having one stable steady-state output for each value of the input, as contrasted with bistable or multistable responses.
Multistable: having two or more stable steady-state output for each value of the input, as contrasted with a monostable response.
Relaxation oscillator: a type of limit cycle oscillator with a bistable trigger, where one slowly changing species causes a second rapidly-changing species to switch between discrete states.
Ultrasensitivity: a property of steady-state input-output relationships that makes them switch-like in character. Goldbeter and Koshland defined input-output relationships to be ultrasensitive if it took less than an 81-fold change in input stimulus to drive the output from 10% to 90% of maximum.
Zero-order: a zero-order chemical or biochemical reaction is one where the rate of the reaction is independent of the substrate concentration. Enzyme reactions approach zero-order when the enzyme is saturated with substrate.

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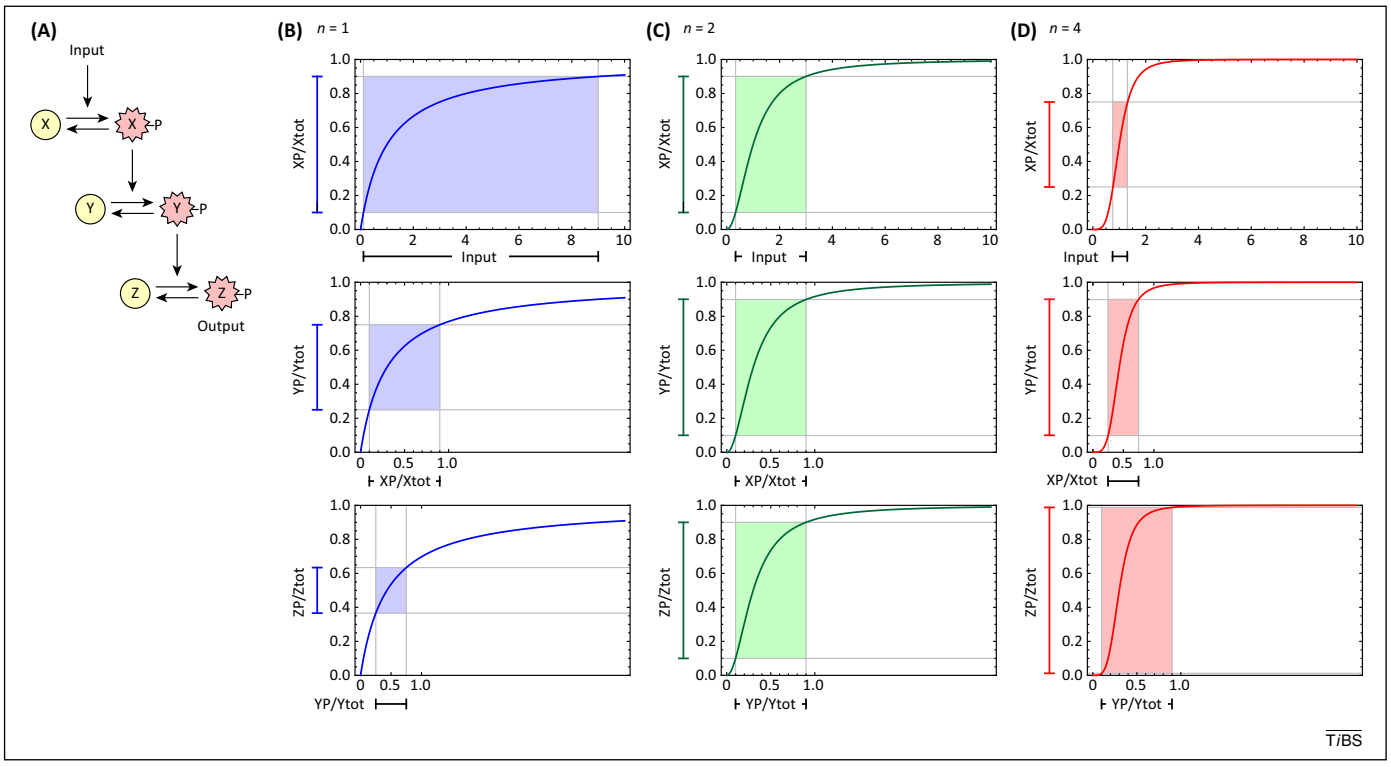

Figure 1. Signaling down Michaelian versus ultrasensitive cascades. **(A)** Schematic view of a three-tier kinase cascade, like the mitogen-activated protein kinase (MAPK) cascade. **(B)** The response of each kinase to an 81-fold change in input, assuming each level of the cascade exhibits a Michaelian response. Note that XP/X_{tot} is the output of the first tier and the input to the second. Likewise, YP/Y_{tot} is the output of the second tier and the input to the third. **(C,D)** The response of each kinase to an 81-fold change in input, assuming ultrasensitive responses with a Hill exponent of 2 (C) or 4 (D).

ultrasensitivity in the cascade; each kinase exhibits a Michaelian (hyperbolic) response to its upstream regulator (Figure 1A,B), so that in each level of the cascade:

$$Output = \frac{Input}{EC50 + Input} \quad [1]$$

How would a signal change as it descended the cascade?

To make the example concrete, let us suppose that initially we have an 81-fold change in input stimulus (a reasonably large number). If we want to maximize the difference between the initial and final levels of *XP*, which is the output of the first tier, we should center the *EC*50 at the geometric mean of the range of inputs; that is, have the initial level of input be one-ninth the *EC*50 and the final level be nine times the *EC*50. Plugging these inputs into Equation 1, we find that the steady-state fraction of phosphorylated *XP* prior to the stimulus was 0.1, and after the stimulus it was 0.9. Thus, an 81-fold change in the input has yielded a nine-fold change in output (Figure 1B, top plot).

If we feed this nine-fold change in *XP* into the second level of the cascade, we get a three-fold change in output (Figure 1B, middle plot), and if we feed this into the third level of the cascade, we get a 70% change in output ($\sqrt{3}$-fold) (Figure 1B, bottom plot). Thus, in three steps of signal transduction, a nearly black-and-white difference in input has deteriorated into a muddled, middle-grey change in output. One can avoid some of the deterioration by using only the left-most parts of the stimulus–response curves, where the response is closer to linear, but this would mean expressing the signaling proteins in great excess of the amounts that would actually get activated in response to input signals.

Ultrasensitive responses can keep signals from degrading. Assume that each level's steady-state response to its upstream activator is ultrasensitive, described by a Hill function:

$$Output = \frac{Input^n}{EC50^n + Input^n} \quad [2]$$

Initially let us assume that the Hill exponent is two, and again center each input geometrically on its *EC*50 to maximize the change in output. In this case, a nine-fold change in input produces a nine-fold change in *XP*, which produces a nine-fold change in *YP*, which produces a nine-fold change in *ZP* (Figure 1C). The same holds true for any other fold-change in input, as long as the fold-change is centered on the *EC*50. If the input is shifted to the left of the *EC*50, then the fold-change will increase as the cascade is descended.

This increase becomes more pronounced if the individual levels of the cascade are described by Hill functions with $n > 2$. As shown in Figure 1D, if we assume $n = 4$, then a $\sqrt{3}$-fold change in input is translated into a three-fold change in *XP*, a nine-fold change in *YP*, and an 81-fold change in *ZP* – the opposite of what we saw with the Michaelian cascade (Figure 1B).

In general, if the input to the Hill function ranges between $Input_1 = EC50/a$ and $Input_2 = EC50 \times a$, with $a > 1$, so that the ratio of the inputs is:

$$\frac{Input_2}{Input_1} = a^2, \quad [3]$$

then it follows that:

$$\frac{Output_2}{Output_1} = a^n \quad [4]$$

Box 1. Sensitivity amplification from signaling cascades

One way to see why a Michaelian cascade degrades responses while an ultrasensitive cascade makes them more switch-like is to examine the local sensitivity functions. Consider a three-tier cascade, where an input promotes the phosphorylation of *X* to *XP*, *XP* promotes the phosphorylation of *Y* to *YP*, and *YP* promotes the phosphorylation of *Z* to *ZP* (see Figure 1A in main text). As defined in Part I of this series [1], the local sensitivity function for the whole cascade is:

$$S_{local} = \frac{d \ln ZP}{d \ln Input} \quad [I]$$

By the chain rule, it follows that this overall sensitivity function can be expressed as the product of the individual levels' sensitivity functions [25]:

$$\frac{d \ln ZP}{d \ln Input} = \frac{d \ln ZP}{d \ln YP} \cdot \frac{d \ln ZP}{d \ln XP} \cdot \frac{d \ln XP}{d \ln Input} \quad [II]$$

Since a sensitivity function is the polynomial order of a response, and for a Michaelian response the polynomial order is always greater than zero but less than one for any non-zero input, the product of these less-than-one numbers will be smaller than any of the individual numbers. Thus, for every input level, the polynomial order of the response gets smaller as you descend a Michaelian cascade, which makes the response more and more graded. Conversely, the polynomial order can get bigger if there are ultrasensitive monocycles in the cascade, at least for those inputs where the polynomial order of the monocycle is >1.

The fold-change in the output is bigger than the fold-change in the input if $n > 2$, and smaller if $n < 2$. Therefore, an ultrasensitive signaling cascade can convert a modest change in input signal into a highly switch-like output. The sigmoidal shape of an ultrasensitive response function allows it to filter out background levels of input, and then respond decisively to suprathreshold inputs.

An increase in fold-change output relative to the fold-change input has been termed 'sensitivity amplification' [3], and a signaling cascade composed of modestly-ultrasensitive monocycles can be highly effective as a sensitivity amplifier. Sensitivity amplification has now been demonstrated experimentally, both through the quantitative analysis of natural signaling cascades [4] and through the construction of synthetic transcriptional cascades [5], as shown in Box 1 and Figure 2.

Ultrasensitivity in bistability

A highly ultrasensitive response can approach a step function, which is one kind of switch – a monostable switch with no built-in memory. A doorbell buzzer is an everyday example of this type of switch. However, systems with positive feedback loops (either implicit or explicit) can function as a different kind of switch, a bistable switch with hysteresis or irreversibility built into the system. A good example of this type of switch is a toggle switch, like a light switch. Flip the switch and the light turns on and stays on indefinitely. Bistability is not an inevitable consequence of positive feedback. However, if we assume that somewhere in the circuit there is an ultrasensitive response, we can fairly easily obtain a bistable response for the whole system. Ultrasensitivity facilitates the generation of a bistable response, and under some circumstances is essential for bistability.

Here we begin with a positive feedback system: a single phosphorylation–dephosphorylation monocycle where the phosphorylated product (*X**) promotes its own production

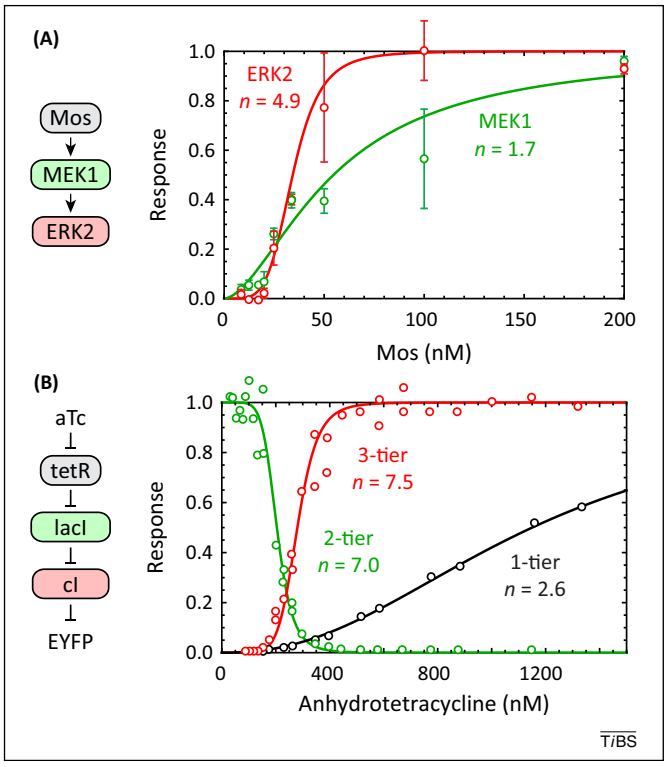

Figure 2. Responses become more ultrasensitive as a cascade is descended. **(A)** A physiological protein kinase cascade. Steady-state response of the mitogen-activated protein kinase kinase MEK1 and extracellular signal-regulated kinase 2 (ERK2) to recombinant bacterially-expressed *Mos* in *Xenopus* oocyte extracts. Adapted from [4]. **(B)** A synthetic transcriptional cascade. In all cases the input is anhydrotetracycline (aTc) and the output is enhanced yellow fluorescent protein (EYFP) production. In the three circuits for which data are shown, aTc regulates EYFP transcription and translation through one intermediary (tetR), two (tetR regulating lacI), or three (tetR regulating lacI which regulates cI). Adapted from [5].

from *X* (Figure 3A). We will then assume either that the feedback depends linearly upon the amount of active *X**, or that there is ultrasensitivity in the feedback loop and the feedback is proportional to a Hill function of *X**. The details of this model are presented in Box 2.

First, let us assume that the positive feedback is described by simple one-step, mass action kinetics, so that the feedback increases linearly with *XP*. Then the phosphorylation rate curve becomes an inverted parabola whose intercept with the *y*-axis depends upon the value of *Input* (Figure 3B, each green curve corresponding to a different value of *Input*), and the dephosphorylation rate curve is a straight line (Figure 3B, red). For the parameters chosen, one can obtain two intersection points and hence two steady states, but only when *Input* = 0 (Figure 3B, lowest green curve). The higher steady state (at XP/X_{tot} = 0.5, filled circle) is stable. However, the lower steady state (at XP/X_{tot} = 0.0, open circle) is unstable: adding any finite amount of *XP* to a system in this steady state would move the system to the right, where the phosphorylation (green) curve is higher than the dephosphorylation (red) curve. This moves the level of *XP* further to the right. The process continues until the system converges upon the stable XP/X_{tot} = 0.5 steady state. We have produced multiple steady states, but only one is stable; the system is still monostable. If we plot the steady-state value of *XP* as a function of the *Input*, the result is a single-valued, monotonically-increasing input/output relationship (Figure 3B, right panel).

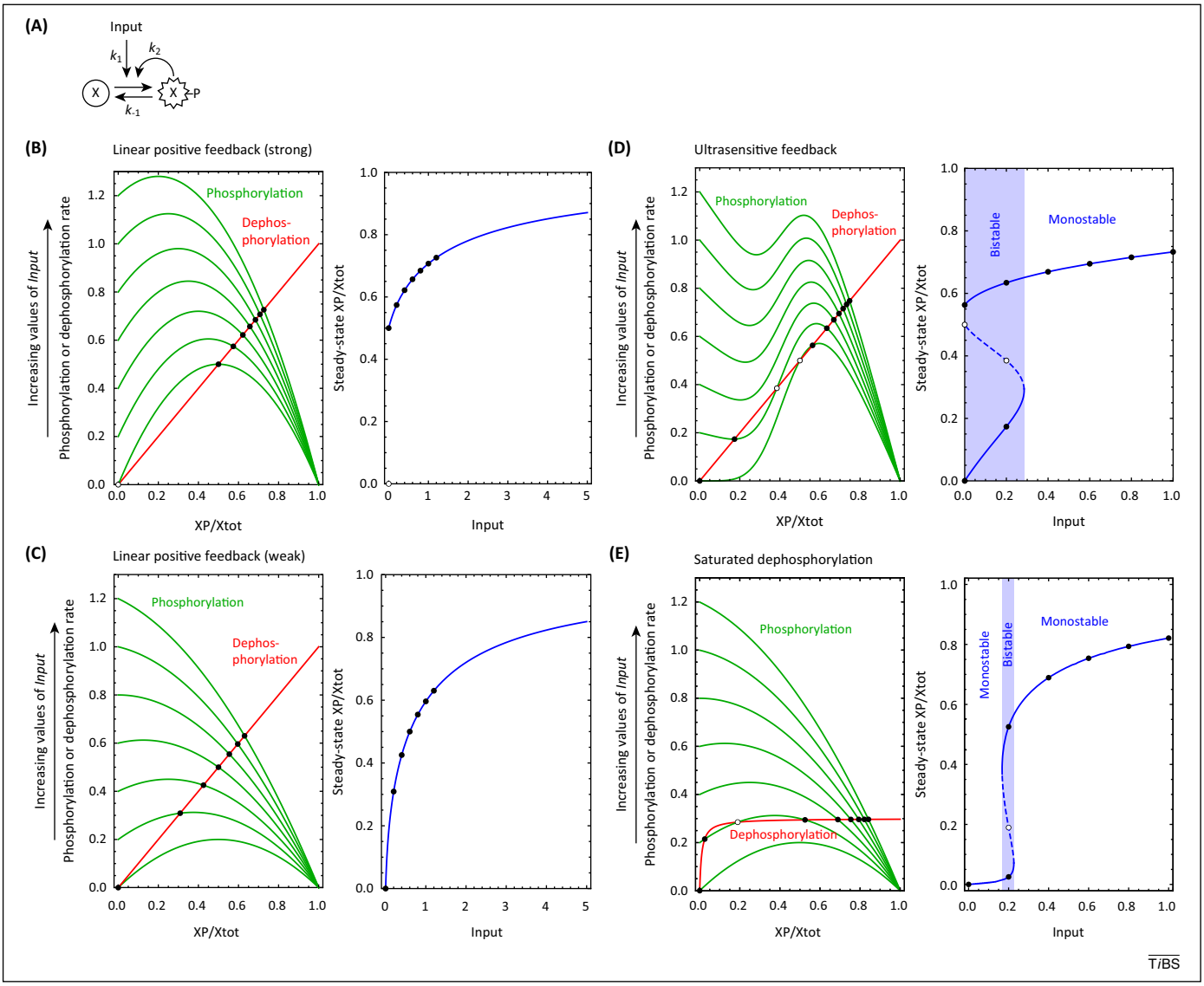

Figure 3. Bistability depends upon an ultrasensitive feedback in a simple positive feedback model. **(A)** Schematic view of a simple positive feedback loop. **(B–E)** Rate–balance plots (left) and steady state input–output curves (right). In all panels, the filled circles represent stable steady states and the open circles represent unstable steady states. In the right side of panel (D), the unbroken blue curve denotes the stable steady state(s) and the broken blue line the unstable steady state. The assumed kinetic values and response functions were:
(B) $k_1 = 1$, $k_{-1} = 1$, $k_2 = 2$, $f[XP] = XP$; (C) $k_1 = 1$, $k_{-1} = 1$, $k_2 = 0.8$, $f[XP] = XP$;
(D) $k_1 = 1, k_{-1} = 1, k_2 = 2$, $f[XP] = \frac{XP^5}{0.5^5 + XP^5}$; and
(E) $k_1 = 1, k_{-1} = 0.3, k_2 = 0.8, K = 0.01$, $f[XP] = XP$, *dephosphorylation rate* $= \frac{XP}{K+XP}$.
In all cases the green curves correspond to *Input* = 0, 0.2, 0.4, 0.6, 0.8, 1, and 1.2 (from bottom to top). Note that if the dephosphorylation rate curve is saturable rather than linear, it becomes possible to generate bistability without having ultrasensitive feedback (panel E; also see [26]).

If we decrease the strength of the positive feedback – decrease the value of k_2 – we can make the $XP/X_{tot} = 0$ steady state stable, but it only becomes stable when the two rate curves no longer intersect twice, and so the system no longer has two steady states (Figure 3C). No matter what we choose for the system's parameters, we cannot produce multiple stable steady-states. The same is true if we assume that the feedback is Michaelian, as long as we have a mass action dephosphorylation curve.

However, the situation changes if we assume that the feedback is ultrasensitive. As shown in Figure 3D, we can now arrange for the phosphorylation rate curve to snake around the dephosphorylation curve and intersect it three times [6,7]. When there are three intersections, as there are for the two lowest values of *Input* shown here, the outer steady states are stable, corresponding to an off-state and an on-state, and the middle one is unstable. The system has become bistable and, in this case, irreversible – the strength of the positive feedback is high enough that once the system switches to the upper limb of the response curve, it will stay there indefinitely, at least in the absence of perturbations large enough to push the system beyond the unstable steady state and back down to the off-state. In addition, this bistability and irreversibility has been made possible by the ultrasensitivity in the positive feedback loop. In intuitive terms, the initial concave-up shape of the ultrasensitive feedback allows the system to filter out small stimuli, making the off-state stable, but then

Box 2. Simple positive feedback loops that can or cannot yield bistable responses

The single rate equation for this system shown in Figure 3A in the main text is:

$$\frac{dXP}{dt} = (k_1 Input + k_2\, f[XP])(X_{tot} - XP) - k_{-1}XP \quad [I]$$

The quantity $f[XP]$ is some response function that defines how XP promotes its own production. In the simplest case, mass action kinetics, $f[XP]=XP$. Alternatively, $f[XP]$ could be a Michaelian response function or an ultrasensitive one.

If we assume simple mass action kinetics for the feedback from XP to XP production, the rate equation becomes

$$\frac{dXP}{dt} = (k_1 Input + k_2 XP)(X_{tot} - XP) - k_{-1}XP \quad [II]$$

The first term is the phosphorylation rate, with its independent contributions from *Input* and from positive feedback. Irrespective of parameter choice, this curve will be an upside down parabola (see Figure 3B, C, green curves, in main text). The dephosphorylation rate curve is a straight line with a slope of $-k_{-1}$ (see Figure 3B, C, red curves, in main text). The curves can intersect once (yielding a stable steady state) or twice (yielding a stable steady state and an unstable one), but not three times.

If instead we assume that the feedback from XP to XP production is described by a Hill function, the rate equation will be:

$$\frac{dXP}{dt} = \left(k_1 Input + k_2 \frac{XP^n}{K^n + XP^n}\right)(X_{tot} - XP) - k_{-1}XP \quad [III]$$

As n gets larger, it becomes easier to arrange for the phosphorylation rate curve to snake around the dephosphorylation rate curve and intersect it three times (see Figure 3D in main text), yielding two stable steady-states and an unstable one in between.

overtake the dephosphorylation rate curve once the system has reached a critical input level.

Note that if we assume that the back reaction is saturable rather than linear with respect to substrate concentration, then it becomes possible to generate a bistable response in the absence of ultrasensitivity in the feedback loop (Figure 3E). However, even in this situation, adding ultrasensitivity to the feedback loop increases the robustness of the bistability.

The question then is whether known bistable switches are actually built out of components with highly ultrasensitive responses, as these theoretical arguments would suggest. One well-studied bistable switch is the cyclin-dependent kinase 1–cell division cycle 25 M-phase inducer phosphatase 3–Ser/Thr nuclear kinase (Cdk1–Cdc25C–Wee1A) system, a circuit that regulates mitotic entry in eukaryotic cells. The system consists of a positive feedback loop – cyclin B–Cdk1 activates Cdc25C, which in turn activates Cdk1 – and a double-negative feedback loop, in which Cdk1 inactivates Wee1A, which in turn inactivates cyclin B–Cdk1 (Figure 4). Experimental studies showing that the circuit does, in fact, function as a bistable switch [8,9] were followed by studies demonstrating that the response functions for both Cdc25C and Wee1A are highly ultrasensitive, with Hill exponents of approximately 11 and 3.5, respectively [10,11] (Figure 4). We suspect that ultrasensitive responses will be found in the components of many other bistable biological systems.

Chang *et al.* [12] have provided a nice synthetic biology demonstration of how the robustness of a bistable response depends upon the ultrasensitivity built into the feedback loops. They expressed artificial gene regulatory systems

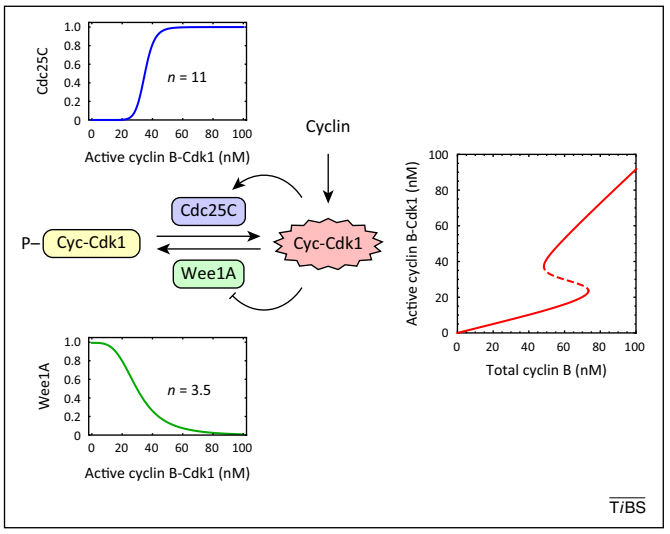

Figure 4. Ultrasensitive feedback in the mitotic trigger. Experimental studies have shown that the steady-state response of the mitotic phosphatase Cdc25C to cyclin B–cyclin-dependent kinase 1 (Cdk1) is highly ultrasensitive, with a Hill exponent of 11 (blue curve; [10]) and the response of the Cdk1-inhibitory kinase Wee1A exhibits a Hill coefficient of 3.5 (green curve; [11]). These ultrasensitive responses account for the bistable steady-state response of Cdk1 to cyclin B (red curve; [9]).

based on isopropyl β-D-1-thiogalactopyranoside (IPTG) regulation of the *lac* operon in *Escherichia coli*. One circuit possessed a single positive feedback loop, one a single double-negative feedback loop, and one included both interlinked loops. The expectation was that the double loop system, which can be regarded as a reciprocal regulation system, would effectively have a higher degree of ultrasensitivity within the feedback loops [2], and thus would exhibit bistability over a larger range of IPTG concentrations than would either of the single loop systems [13]. This was in fact the case – the single loop systems were bistable over approximately four-fold and 12-fold ranges of IPTG, respectively, whereas the double loop system was bistable over a ~480-fold range. These findings are consistent with the hypothesis that ultrasensitivity is critical for robust bistable responses.

Bistability in oscillations

In this section, we turn our attention to biological oscillator systems that exhibit unending, limit cycle oscillations. Limit cycle oscillations approach the same dynamical pattern of behavior – the same frequency and amplitude, plus or minus a phase shift – regardless of the initial conditions, as contrasted with harmonic oscillators, where the amplitude of the oscillations varies with the initial conditions. Biological examples of limit cycle oscillations include the once-per-second pulse of the cardiac pacemaker and the once-per-25 min pulse of the *Xenopus* embryonic cell cycle [14].

Does the steady-state behavior of the components that make up the oscillator circuit have anything to do with this inherently dynamical behavior? The answer is yes – ultrasensitive components not only promote switch-like steady-state behaviors (above), but also oscillatory dynamical behaviors. This connection between ultrasensitivity and oscillations was appreciated even before the term ultrasensitivity was coined, dating back to the pioneering studies of Goodwin in the 1960s [15].

Box 3. A simple protein oscillator circuit

Starting with a triple-negative feedback loop, as shown in Figure 5A in main text, we will assume that each protein is activated by an unregulated, mass action process like constitutive phosphorylation, and inactivated (dephosphorylated) at a rate that is proportional to a Hill function of its upstream reporter and to the amount of active protein available to be inactivated. If, for simplicity, we assume that the loop is perfectly symmetrical, the rate equations become:

$$\frac{dX}{dt} = k_1(X_{tot} - X) - k_{-1}X\frac{Z^n}{EC50^n + Z^n} \quad [I]$$

$$\frac{dY}{dt} = k_1(Y_{tot} - Y) - k_{-1}Y\frac{X^n}{EC50^n + X^n} \quad [II]$$

$$\frac{dZ}{dt} = k_1(Z_{tot} - Z) - k_{-1}Z\frac{Y^n}{EC50^n + Y^n} \quad [III]$$

The parameter k_1 is the rate constant for the constitutive activation of *X*, *Y*, and *Z*, and k_{-1} represents the maximal rate of inactivation of *X*, *Y*, and *Z*. If we choose our units such that $k_{-1} = 1$ and each total protein concentration = 1, there are only three adjustable parameters in the model: the activation rate constant k_1, the *EC*50 for the inactivation process, and the Hill exponent *n*. We can then determine what range of parameters yields oscillations for several assumed values of *n*, as shown in Figure 5B in main text.

To illustrate this, let us examine one of the simplest oscillator circuits: a triple negative feedback loop like the Repressilator, a transcriptional circuit famous for being one of the first triumphs of synthetic biology [16]. In keeping with the other examples in this review, we will make our oscillator a protein circuit rather than a gene circuit, as shown in Figure 5A and detailed in Box 3. A model of a simple triple negative gene circuit can be found in [16], and it yields similar behavior.

In Box 3, we make our circuit symmetrical and thereby simplify the model to include just three adjustable parameters: the rate of phosphorylation relative to dephosphorylation for each of the reactions (k_1), the *EC*50 value, and the Hill exponent *n*. We then examine what range of values of k_1 and *EC*50 will support limit cycle oscillations for various values of the Hill exponent *n*. As shown in Figure 5B, oscillations did not occur for any choices of k_1 and *EC*50 until $n > 2$. Once oscillations emerged, the higher the value of *n*, the larger the range of k_1 and *EC*50 values that supported oscillations (Figure 5B). The amplitude of the oscillations also increased as *n* increased (Figure 5C). Therefore, in this three-protein oscillator circuit, ultrasensitivity makes oscillations possible, and higher degrees of ultrasensitivity improve the amplitude of the oscillations and the robustness of the oscillations with respect to changes in parameter values.

There are other more complicated ways to construct an oscillator circuit. The circuit could contain more than three components and, in general, the longer the loop, the greater the chances for oscillations, and there is a trade-off between how long the loop is and how much ultrasensitivity is required for oscillations [17]. Alternatively, the circuit could include a bistable trigger and behave like a relaxation oscillator [18,19], or it could be a long positive feedback loop interlinked with shorter negative feedback loops [20,21], or the model of the circuit could include explicit time delays [22,23]. Nevertheless, in all of these models, it always seems to be the case that ultrasensitivity is one of the key ingredients in the oscillatory circuit.

Motivated by these various theoretical studies, two recent papers experimentally addressed the question of whether ultrasensitive response functions are present in the negative feedback loop at the heart of the *Xenopus* embryonic cell cycle. The answer was a resounding yes. The response of the anaphase-promoting complex (APC^{Cdc20}) to Cdk1 was found to be extremely ultrasensitive (n = 17–464) in both egg and embryo extracts [22,24].

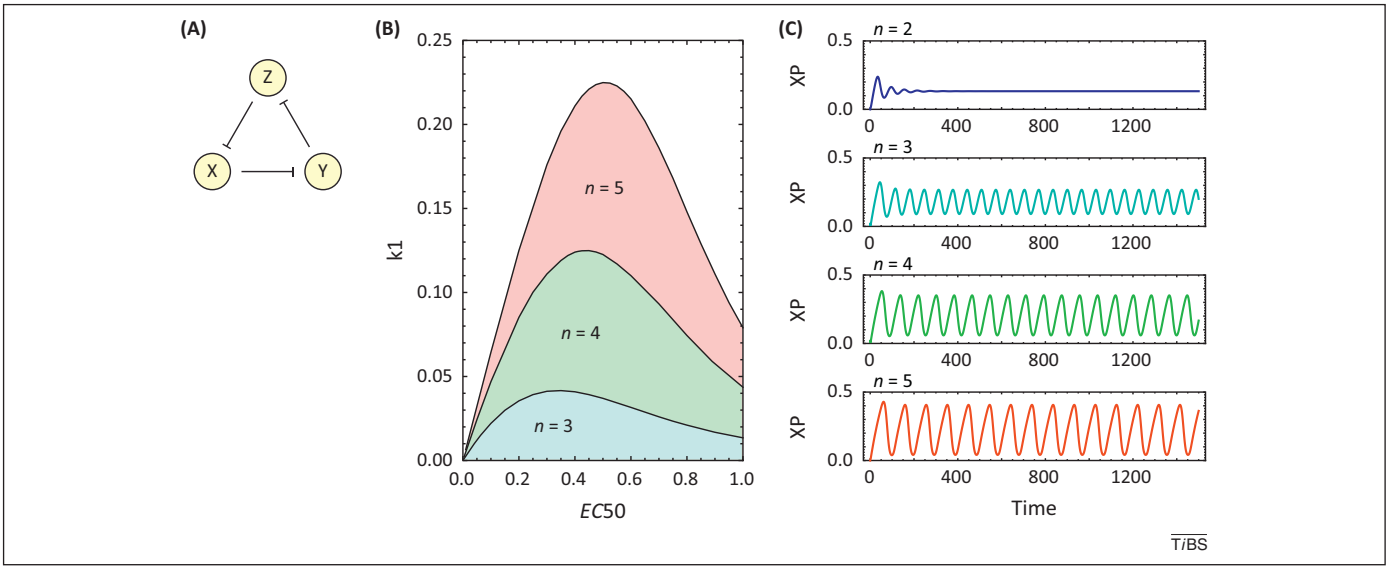

Figure 5. Ultrasensitivity promotes oscillations. **(A)** A three-protein oscillator circuit similar to the transcriptional Repressilator [16]. The details of the circuit are described in Box 3. **(B)** Values from the model that yield limit cycle oscillations. The model has four parameters, k_1, which is the activation rate constant, k_{-1}, which is the inactivation rate constant, *EC*50, the concentration of the upstream protein required for a half-maximal rate of inactivation of its target protein, and *n*, the Hill exponents for the inactivation reactions. We have assumed that the total concentration of each of the proteins is equal to 1, and that the system is symmetrical, with the same parameters applying to each reaction. We have also chosen units such that k_{-1} = 1. The cyan region corresponds to the values of the parameters that yield oscillations for n = 3. The green area corresponds to the additional values that yield oscillations for n = 4, and pink for n = 5. Only damped oscillations were found for n = 2. **(C)** Time courses of *XP* oscillations for different assumed values of *n*. The amplitude increases with *n*. In each case we assumed k_1 = 0.01, *EC*50 = 0.5.

Concluding remarks

As shown in this review series [1,2], at least four classes of distinct mechanisms can generate ultrasensitive responses. These include zero-order ultrasensitivity, the saturation mechanism discovered by Goldbeter and Koshland; multisite ultrasensitivity, which is the closest to classical cooperativity in its conceptual basis; inhibitor ultrasensitivity, which is a particularly simple and powerful mechanism; and positive feedback loops, which can generate ultrasensitive responses even under circumstances where they cannot yield bistable responses. Each of these mechanisms was proposed before there was any clear experimental evidence that they occur *in vivo* – they were invented by theoreticians before they were established by experimentalists.

Moreover, ultrasensitive responses can be critical for the generation of more complex systems-level behaviors. Whether a circuit is built out of Michaelian or ultrasensitive response functions can determine whether it is easy, difficult, or even impossible to obtain interesting emergent behaviors like oscillations and bistability from a given circuit. This makes ultrasensitivity an important brick in the wall of systems biology, and a highly useful ingredient for the engineering of synthetic biology circuits.

Acknowledgments

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References

1 Ferrell, J.E., Jr and Ha, S.H. (2014) Ultrasensitivity part I: Michaelian responses and zero-order ultrasensitivity. *Trends Biochem. Sci.* 39, 496–503
2 Ferrell, J.E., Jr and Ha, S.H. (2014) Ultrasensitivity part II: Multisite phosphorylation, stoichiometric inhibitors, and positive feedback. *Trends Biochem. Sci.* 39, 556–569
3 Goldbeter, A. and Koshland, D.E., Jr (1982) Sensitivity amplification in biochemical systems. *Q. Rev. Biophys.* 15, 555–591
4 Huang, C-Y.F. and Ferrell, J.E., Jr (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. *PNAS* 93, 10078–10083
5 Hooshangi, S. *et al.* (2005) Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3581–3586
6 Angeli, D. and Sontag, E.D. (2003) *Multistability in Monotone I/O Systems, Preliminary Report,* arXiv math.OC/0212357, Cornell University Library submitted December 2002
7 Angeli, D. *et al.* (2004) Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. *Proc. Natl. Acad. Sci. U.S.A.* 101, 1822–1827
8 Sha, W. *et al.* (2003) Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc. Natl. Acad. Sci. U.S.A.* 100, 975–980
9 Pomerening, J.R. *et al.* (2003) Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nat. Cell Biol.* 5, 346–351
10 Trunnell, N.B. *et al.* (2011) Ultrasensitivity in the regulation of Cdc25C by Cdk1. *Mol. Cell* 41, 263–274
11 Kim, S.Y. and Ferrell, J.E., Jr (2007) Substrate competition as a source of ultrasensitivity in the inactivation of Wee1. *Cell* 128, 1133–1145
12 Chang, D.E. *et al.* (2010) Building biological memory by linking positive feedback loops. *Proc. Natl. Acad. Sci. U.S.A.* 107, 175–180
13 Ferrell, J.E., Jr (2008) Feedback regulation of opposing enzymes generates robust, all-or-none bistable responses. *Curr. Biol.* 18, R244–R245
14 Ferrell, J.E., Jr *et al.* (2011) Modeling the cell cycle: why do certain circuits oscillate? *Cell* 144, 874–885
15 Goodwin, B.C. (1965) Oscillatory behavior in enzymatic control processes. In *Advances in Enzyme Regulation, Vol 3* (Weber, G., ed.), pp. 425–438, Oxford UK, Permagon Press
16 Elowitz, M.B. and Leibler, S. (2000) A synthetic oscillatory network of transcriptional regulators. *Nature* 403, 335–338
17 Tyson, J.J. and Othmer, H.G. (1978) The dynamics of feedback control circuits in biochemical pathways. *Prog. Theor. Biol.* 5, 1–62
18 Novak, B. and Tyson, J.J. (1993) Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. *J. Cell Sci.* 106, 1153–1168
19 Novak, B. and Tyson, J.J. (1993) Modeling the cell division cycle: M-phase trigger, oscillations, and size control. *J. Theor. Biol.* 165, 101–134
20 Orlando, D.A. *et al.* (2008) Global control of cell-cycle transcription by coupled CDK and network oscillators. *Nature* 453, 944–947
21 Sevim, V. *et al.* (2010) Reliability of transcriptional cycles and the yeast cell-cycle oscillator. *PLoS Comput. Biol.* 6, e1000842
22 Yang, Q. and Ferrell, J.E., Jr (2013) The Cdk1–APC/C cell cycle oscillator circuit functions as a time-delayed, ultrasensitive switch. *Nat. Cell Biol.* 15, 519–525
23 Stricker, J. *et al.* (2008) A fast, robust, and tunable synthetic gene oscillator. *Nature* 456, 516–519
24 Tsai, T.Y. *et al.* (2014) Changes in oscillatory dynamics in the cell cycle of early *Xenopus laevis* embryos. *PLoS Biol.* 12, e1001788
25 Brown, G.C. *et al.* (1997) Why do protein kinase cascades have more than one level? *Trends Biochem. Sci.* 22, 288
26 Ferrell, J.E., Jr and Xiong, W. (2001) Bistability in cell signaling: how to make continuous processes discontinuous, and reversible processes irreversible. *Chaos* 11, 227–236