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Supplemental Data

Substrate Competition

as a Source of Ultrasensitivity

in the Inactivation of Wee1

Sun Young Kim and James E. Ferrell, Jr.

Supplemental Experimental Procedures

Ultrasensitivity and bistability in a two-component feedback loop

Consider a simple double-negative feedback loop, where protein A inhibits protein B and protein B inhibits protein A (Figure S1). If the steady-state response of B to A is represented by a simple hyperbolic Michaelian function, then

$$B = \frac{b_0}{K_b + A} \quad [\text{Eq 1}]$$

where B denotes the concentration of active protein B , A denotes the concentration of active protein A , b_0 is a constant such that b_0/K_b equals the total concentration of protein B , and K_b represents the concentration of protein A at which protein B is half-maximally inhibited. Similarly,

$$A = \frac{a_0}{K_a + B} \quad [\text{Eq 2}]$$

where a_0 is a constant such that a_0/K_a equals the total concentration of protein A and K_a represents the concentration of protein B at which protein A is half-maximally inhibited. For the whole A/B feedback loop to be in steady-state, both Eq 1 and Eq 2 must be satisfied. This occurs when:

$$B = \frac{b_0}{K_b + \frac{a_0}{K_a + B}} \quad [\text{Eq 3}]$$

Solving for B , it follows that:

$$B = \frac{(b_0 - a_0 - K_a K_b) \pm \sqrt{(b_0 - a_0 - K_a K_b)^2 + 4b_0 K_a K_b}}{2K_b} \quad [\text{Eq 4}]$$

Since $4b_0K_aK_b$ must be greater than zero, the square root in the numerator must be greater than $|b_0 - a_0 - K_aK_b|$. Thus only the choice of the + sign before the square root will yield a physically meaningful positive number for the concentration of active protein B . This means the system will have a single steady-state, with one value of B and one corresponding value of A . For any physically meaningful choice of parameters, the curves defined by Eqs 1 and 2 will have one and only one intersection (Figure S1B) and the system therefore cannot be bistable.

However, if either of the individual response functions is sigmoidal, rather than hyperbolic, then the system may exhibit bistability. Suppose that the inhibition of B by A is given by a Hill function with a Hill coefficient n_H greater than 1:

$$B = \frac{b_0}{K_b^{n_H} + A^{n_H}} \quad [\text{Eq 5}]$$

As shown in Figure S1C, for some choices of a_0 , b_0 , K_a , and K_b the curves can be made to intersect at three points. Two of these points represent alternative stable steady-states, one where A dominates over B and one where B dominates over A . The third point is also a steady-state, but is unstable; it represents a saddle point threshold. With other things being equal, the higher the Hill coefficient, the easier it is to find parameters that will produce three intersection points, two of which represent alternative stable steady-states.

Similar algebraic and graphical arguments can be made for a positive feedback loop rather than a double-negative feedback loop (not shown).

These simple arguments apply to systems with two variables. Similar graphical arguments can be extended to systems of arbitrarily many variables if the network within the feedback loop constitutes a strongly monotone system (Angeli et al., 2004). The basic approach is to cut the loop at one point, in effect making one leg of the intact feedback loop be the this open loop system and the other leg be the identity function. These two curves can intersect each other three times only if the open loop steady-state response curve is S-shaped. The properties of strongly monotone systems can then be used to demonstrate the stability properties of the three intersection points (Angeli et al., 2004).

Zero-order ultrasensitivity and the Goldbeter-Koshland function

Goldbeter and Koshland (Goldbeter and Koshland, 1981) first showed that under certain assumptions (e.g. the concentrations of the kinase-substrate and phosphatase-substrate complexes are negligible compared with the total substrate concentration), the fraction of phosphorylated substrate is given by:

$$\frac{PhosphoS}{S_{tot}} = \frac{\left(\frac{V_1}{V_2} - 1\right) - K_2 \left(\frac{K_1}{K_2} + \frac{V_1}{V_2}\right) + \sqrt{\left[\frac{V_1}{V_2} - 1 - K_2 \left(\frac{K_1}{K_2} + \frac{V_1}{V_2}\right)\right]^2 + 4K_2 \left(\frac{V_1}{V_2} - 1\right) \frac{V_1}{V_2}}}{2 \left(\frac{V_1}{V_2} - 1\right)} \quad [\text{Eq 6}]$$

where $K_1 = K_{m-kinase}/S_{tot}$; $K_2 = K_{m-phosphatase}/S_{tot}$; $V_1 = V_{max-kinase}$; and $V_2 = V_{max-phosphatase}$. When the kinase and phosphatase are both far from saturation, Eq 6 can be reduced to a Michaelian hyperbola. When the kinase is close to saturation, the fraction of phosphorylated substrate increases linearly with V_1 until all the substrate has been phosphorylated (Fig. S2, blue curve). When the phosphatase is close to saturation, the result is a sigmoidal dose-response curve with a prominent threshold (Fig. S2, green curve). Simple graphical approaches (rate balance plots) can be useful in understanding why saturating the kinase or phosphatase has these effects on the shape of the stimulus/response curve (Ferrell, 1996; LaPorte and Koshland, 1983).

Note that saturating the phosphatase, not the kinase, is what would produce a threshold in the stimulus/response curve (Fig. S2). Experimentally we found that the reconstituted Wee1/Cdk1 exhibited a threshold in the absence of added phosphatase (Fig. 2C). This argues against zero-order ultrasensitivity as being an important contributor to the ultrasensitivity observed in the in vitro system.

Quantifying ultrasensitivity

Although Eq 6 is different in form from a Hill equation, for many reasonable parameter values it yields a sigmoidal curve that is well-approximated by the Hill equation. Thus it is reasonable to calculate an effective Hill coefficient for a Goldbeter-Koshland curve. One way of defining the effective Hill coefficient is by comparing the overall steepness of the curve to that of a Hill function (Goldbeter and Koshland, 1981). For a Hill function, the ratio of the EC_{90} to the EC_{10} is a reasonable measure of the overall steepness, and there is a simple relationship between this ratio and the Hill coefficient:

$$n_H = \frac{\log[81]}{\log[EC_{90}/EC_{10}]} \quad [\text{Eq 7}]$$

Likewise, for a Goldbeter-Koshland curve one can define an effective Hill coefficient in terms of the EC_{90} and EC_{10} values, which can be calculated by setting Eq 6 equal to 0.9 or 0.1 and solving for V_1 :

$$n_{H\text{ eff}} = \frac{\text{Log}[81]}{\text{Log} \left[\frac{(9 + 90K_1)(9 + 90K_2)}{(9 + 10K_1)(9 + 10K_2)} \right]} \quad [\text{Eq 8}]$$

For the K_m values (566 nM and 97 nM) and S_{tot} value (60 nM) appropriate to Fig. 4, the Eq 8 yields an effective Hill coefficient of 1.1.

For experimental data that are well approximated by a Hill function, the apparent Hill coefficient can be determined by non-linear least squares curve fitting.

Multisite ultrasensitivity, assuming the concentration of kinase-substrate complexes is negligible

We begin by considering a two-site phosphorylation/dephosphorylation, as shown in Figure 5A, and assume (1) the two phosphorylations occur non-processively; (2) the kinase and phosphatase are operating far from saturation; and (3) the concentrations of any Cdk1-Wee1 complexes are negligible. This system contains three phospho-states; unphosphorylated Wee1, monophosphorylated Wee1, and bisphosphorylated Wee1. We can write an ordinary differential equation for the production and destruction of each species:

$$\frac{dWee1}{dt} = -k_1 Cdk1Wee1 + k_{-1} Wee1P \quad [\text{Eq 9}]$$

$$\frac{dWee1P}{dt} = k_1 Cdk1Wee1 - k_{-1} Wee1P - k_2 Cdk1Wee1P + k_{-2} Wee1PP \quad [\text{Eq 10}]$$

$$\frac{dWee1PP}{dt} = k_2 Cdk1Wee1P - k_{-2} Wee1PP \quad [\text{Eq 11}]$$

plus a conservation equation:

$$Wee1 + Wee1P + Wee1PP = Wee1_{tot} \quad [\text{Eq 12}]$$

It follows that:

$$\frac{Wee1PP}{Wee1_{tot}} = \frac{Cdk1^2}{\frac{k_{-1}k_{-2}}{k_1k_2} + \frac{k_{-2}}{k_2} Cdk1 + Cdk1^2} \quad [\text{Eq 13}]$$

If $k_{-2}/k_2 \gg k_{-1}/k_1$, then Eq 13 approaches a Michaelian function ($n_H = 1$). However, if $k_{-2}/k_2 \ll k_{-1}/k_1$, Eq 13 approaches a Hill function with $n_H = 2$, which would be more than sufficient to account for the observed intrinsic ultrasensitivity (Figure 2B, 5B). Note that this relationship between the rate constants and resulting the degree of ultrasensitivity provides a connection between classical notions of cooperativity and multisite ultrasensitivity; if the first phosphorylation event greatly promotes the second, then k_2 should be much greater than k_1 and, assuming the dephosphorylation rate constants for the two phosphorylations are equal, k_{-2}/k_2 will be much less than k_{-1}/k_1 and the Hill coefficient will approach 2.

This same strategy can be extended to much more complex networks. Suppose a network consists of n nodes, with each node representing a phosphostate. One can write a system of n ordinary differential equations (ODEs) for the production and destruction of species represented by the n nodes. Each ODE consists of i production terms and i destruction terms, where i is the number of edges that connect to the node. At steady-state each time derivative must equal zero and the system reduces to a set of n simultaneous algebraic equations. For simple networks this can be solved by hand; for more complicated networks, machine-assisted algebra is useful. For example, for the five-step red path shown in Figure 3, the steady state level of the end product Wee1(1P, 2P, 3P, 8P, 10P) is given by:

$$\frac{Wee1(1P, 2P, 3P, 8P, 10P)}{Wee1_{tot}} = \frac{Cdk1^5}{\frac{k_{-1}k_{-2}k_{-3}k_{-4}k_{-5}}{k_1k_2k_3k_4k_5} + \frac{k_{-2}k_{-3}k_{-4}k_{-5}}{k_2k_3k_4k_5} Cdk1 + \frac{k_{-3}k_{-4}k_{-5}}{k_3k_4k_5} Cdk1^2 + \frac{k_{-4}k_{-5}}{k_4k_5} Cdk1^3 + \frac{k_{-5}}{k_5} Cdk1^4 + Cdk1^5} \quad [\text{Eq 14}]$$

where $k_1, k_2, k_3,$ and k_4 are the rate constants for the four phosphorylation reactions and $k_{-1}, k_{-2}, k_{-3},$ and k_{-4} are the rate constants for the four dephosphorylation reactions. For the proper choice of rate constants, Eq 14 can approach a Hill function with a Hill coefficient of 5. In general, the maximum Hill coefficient that can be obtained for the ordered phosphorylation of x sites is $n_H = x$. However, if all of the rate constant ratios are equal (i.e., there is no ‘‘cooperativity’’ in the phosphorylation/dephosphorylation reactions), the effective Hill coefficient will never rise above 2 (Gunawardena, 2005).

More complicated and complete networks can be handled similarly.

Multisite ultrasensitivity, assuming the concentration of kinase-substrate complexes is not negligible

If the steady-state concentrations of the kinase-substrate complexes, phosphatase-substrate complexes, or both, are substantial, the system can exhibit Hill coefficients higher than the $n_H = x$ limit mentioned above, and can even exhibit bistability (Markevich et al., 2004). The model shown in Figure 5D allows for the possibility that kinase-substrate concentrations are substantial, although the parameters chosen do not produce a bistable response.

Ultrasensitivity from competition between two substrates

A stoichiometric inhibitor can soak up the first increments of a kinase, introducing a threshold into phosphorylation of substrates (Ferrell, 1996). Here we show that a preferred substrate can produce a similar effect. We suppose that a kinase like Cdk1 can phosphorylate a substrate Wee1 in an unsaturated, first-order process opposed by an unsaturated phosphatase. We suppose also that this kinase can phosphorylate a

second substrate, designated Substrate 2, and that substantial amounts of a complex between Cdk1 and Substrate 2 form. Phosphorylated Substrate 2 can then be dephosphorylated by an unsaturated phosphatase. The simple reaction scheme (Fig. S3A) gives rise to a quadratic equation for the steady-state level of Wee1P, which yields the following expression for Wee1P as a function of the kinase activity, the concentration of Substrate 2, and the rate constants:

$$\frac{Wee1P}{Wee1_{tot}} = \frac{\left(k_{-2}(K_1 + 2kin_{tot})(d_2 + k_2) + a_2K_1k_{-2}Sub2_{tot} - a_2K_1kin_{tot}(k_2 + k_{-2}) + \sqrt{K_1^2((d_2 + k_2)^2k_{-2}^2 + a_2^2(k_2kin_{tot} + k_{-2}(kin_{tot} - Sub2_{tot}))^2 + 2a_2k_{-2}(d_2 + k_2)(k_2kin_{tot} + k_{-2}(kin_{tot} + Sub2_{tot})))} \right)}{2(k_{-2}(d_2 + k_2)(K_1 + kin_{tot}) - a_2K_1((K_1 + kin_{tot})(k_2 + k_{-2}) - k_{-2}Sub2_{tot}))}$$

[Eq 15]

where $K_1 = k_{-1}/k_1$. As shown in Fig. S3B, Eq 15 represents a sigmoidal stimulus/response relationship, with the phosphorylation/dephosphorylation cycle for Substrate 2 acting to buffer the kinase and produce a threshold.

Primers for Wee1 mutagenesis

QuickChange mutagenesis was performed with the following primers:

- 1E, 5' ATTAATGAAGGTCCCCAGAAGGGGGAGCCCGTGAGTTCCTGGAGGACCA3' ;
 2E, 5' CCAATAACTGCCCCTTCCCCATCGAGCCCCAGAGGAACGAGAGGGAACTT3' ;
 3E, 5' CCCAGAGGAACGAGAGGGAACTTGAGCCTACTCAAGAGCTGAGCCCAA3' ;
 7E, 5' GGAAGAAGCTCAAGCTCTGTGACGAGCCTTATACCCCAAAGAGCC3' ;
 8E, 5' CTCAAGCTCTGTGACACCCCTTATGAGCCAAAGAGCCTTTTGTACAAAACGC3' .

Supplemental Figures

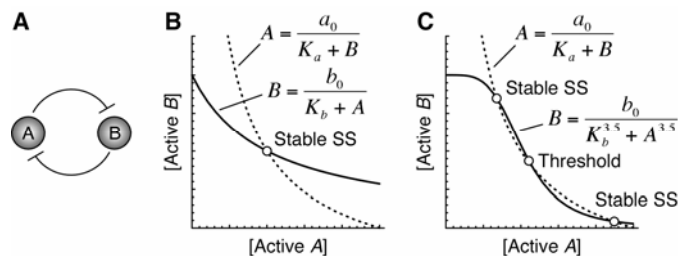


Figure S1. Ultrasensitivity Is Required for Bistability

(A) Schematic depiction of a double-negative feedback loop. Protein A inhibits B, and protein B inhibits A.

(B) Michaelian response functions yield a monostable system. If the steady state response of B to A (solid curve) and A to B (dashed curve) are hyperbolic, Michaelian functions, there will be one and only one intersection.

(C) Ultrasensitivity can produce a bistable response. If inhibition of B by A (solid curve) is given by a sigmoidal function (Hill coefficient $n_H = 3.5$) there can be three intersection points, representing two stable steady-states and one unstable threshold point.

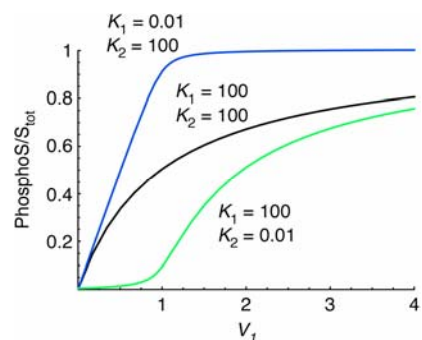


Figure S2. Zero-Order Ultrasensitivity

Goldbeter-Koshland functions are shown for three cases: neither enzyme is close to saturation (black curve); the kinase is close to saturation, but the phosphatase is not (blue curve); the phosphatase is close to saturation but the kinase is not (green curve). The V_{max} values of the phosphatase (V_2) were chosen so that the three curves could be plotted on the same axes ($V_2 = 100$ (blue), 1 (black), or 0.01 (green)).

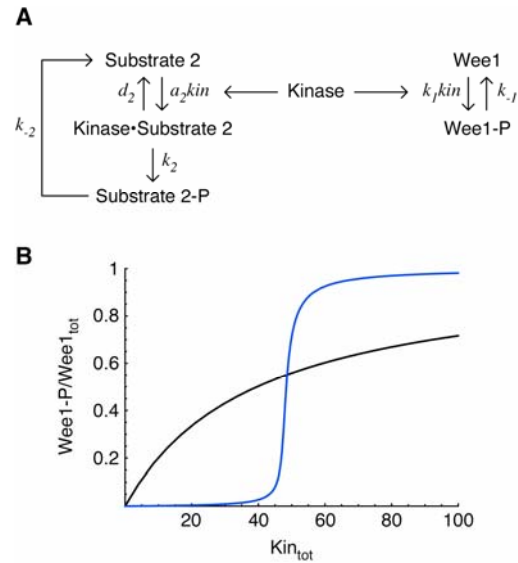


Figure S3. Ultrasensitivity from Competition

(A) Schematic depiction of competition between a high affinity substrate (Substrate 2) and a lower affinity substrate (Wee1).

(B) Steady state stimulus/response curve. Eq 15 is plotted for one choice of parameters (blue): $k_1 = 0.1$, $k_{-1} = 0.1$, $a_2 = 10$, $d_2 = 0.1$, $k_2 = 1$, $k_{-2} = 0.05$, $Wee1_{tot} = 20$, $Sub2_{tot} = 1000$. The result is a steeply sigmoidal curve with a threshold. A Michaelian curve (black) with the same EC50 value is shown for comparison.

Supplemental References

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