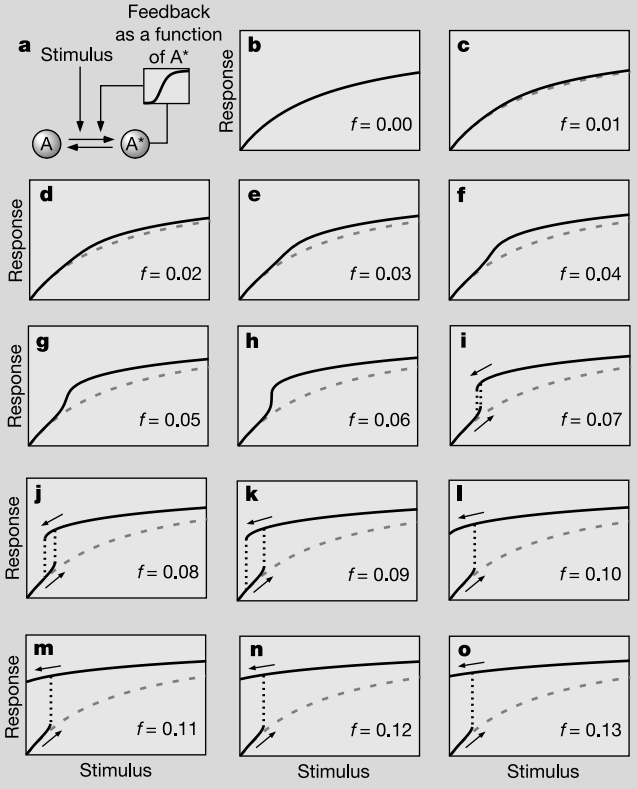
Box 1

Behaviour of a simple positive feedback loop: sensitivity amplification, bistability, hysteresis and irreversibility

Positive feedback loops have the potential to convert a transient stimulus into a self-sustaining, irreversible response. But irreversibility is not an inevitable consequence of positive feedback, nor is irreversibility the only useful systems-level property that can emerge from systems with positive feedback loops. This is true in the case of complicated positive feedback systems, such as the p42 MAPK/Cdc2 system in oocytes, and is also true (and can be more easily seen and understood) in simpler systems, such as the one shown here (**a**).



This system consists of a signalling protein that can be reversibly converted between an inactive form (A) and an active one (A*). The activation reaction is assumed to be regulated in two ways: by an external stimulus (equation (1), first term); and by positive feedback, with a nonlinear Hill equation relationship between the amount of A* produced and the rate of production of more A* (equation (1), second term). The inactivation reaction is assumed to be unregulated; its rate is proportional to the concentration of A* (equation (1), third term). Thus,

$$\frac{d[A^*]}{dt} = \{\text{stimulus} \times ([A_{tot}] - [A^*])\} + f\frac{[A^*]^n}{K^n + [A^*]^n} - k_{inact}[A^*] \qquad (1)$$

where n denotes the Hill coefficient, K is the effector concentration for half-maximum response (EC_{50}) for the feedback as a function of [A*], and f represents the strength of the feedback.

This differential equation was solved numerically (by using Mathematica 2.2.2) to determine the relationship between stimulus and steady-state response ([A*]), assuming that $n = 5$, $K = 1$, $k_{inact} = 0.01$ and stimulus = 0–1, and assuming a range of values of f. The results are shown in **b**–**o**, with the calculated steady-state responses shown as unbroken lines and the discontinuities shown as dotted lines; the no-feedback response (dashed lines) is included for comparison.

When $f = 0$, the response is monostable and the stimulus–response curve is a smooth michaelian hyperbola (**b**). As the strength of the feedback increases, the stimulus–response curve acquires a sigmoidal shape (**c**–**h**). This occurs because the feedback has been assumed to be cooperative or ultrasensitive. The sigmoidicity makes the response of A* more switch-like (but still monostable).

At $f = 0.07$ (**i**), the stimulus–response curve splits into two curves: one representing the amount of stimulus needed to induce the system to turn on, the other representing the amount of stimulus needed to maintain the system in its on state. At this point, the system becomes bistable for some values of stimulus (that is, there are two discrete, stable steady states for a single value of stimulus) and the system shows hysteresis, meaning that the dose–response relationship is a loop rather than a curve. The range of stimulus over which the system is bistable and the extent of the hysteresis both increase as f increases (**i**–**k**).

Eventually, the feedback becomes strong enough to maintain the system in the on state even when the stimulus is decreased to zero (**l**–**o**). At this point, the system may be able to convert a transient stimulus into an essentially irreversible response. But even in a system such as this, stochastic effects still have the potential to make the response reversible[29,30].

Box 1. Feedback and bistability in homogenous reaction networks

The response properties of a homogenous reaction network are determined by the topology and kinetic parameters of the reactions in the network. For autonomous systems, the reaction rate constants k are time invariant and the reaction rates v are functions of the time-dependent concentrations of reactants $S_i(t)$. Figure Ia details a typical cyclic reaction scheme in which a substrate (S) is phosphorylated (SP) by a kinase and dephosphorylated by a phosphatase ($ppase$) (Figure Ia). The input of the system is the kinase activity (kin) and the output the phosphorylated substrate (SP). Can this system generate discrete states? We assume that the kinase and phosphatase reactions obey saturatable Michaelian kinetics. The response of the system (SP) to the input (kin) at steady state is calculated by setting the change in output reactant SP over time to zero in the differential equation that describes the change in SP over time owing to the forward (v_+) and backward (v_-) reactions (Equation I):

$$\frac{d[SP]}{dt} = v_+ - v_- - \frac{k_{cat}^{kin}[kin][S]}{K_m^{kin} + [S]} - \frac{k_{cat}^{ppase}[ppase][SP]}{K_m^{ppase} + [SP]} = 0 \qquad \text{[Eqn I]}$$

where K_m is the Michaelis constant and k_{cat} is the catalytic constant for the kinase (^{kin}) and phosphatase (^{ppase}). By using the conservation of substrate and product, we eliminate the variable S and simplify the equation by normalizing SP by the total of substrate and product concentration $S0$ (sp: molar fraction of SP) (Equation II)

$$\frac{k_{cat}^{kin}[kin][1-sp]}{J_m^{kin} + [1-sp]} - \frac{k_{cat}^{ppase}[ppase][sp]}{J_m^{ppase} + [sp]} = 0 \qquad \text{[Eqn II]}$$

where J is the Michaelis constant normalized to $S0$. Solving for [sp] yields an ultrasensitive response to the kinase activity kin (Figure Ia). This type of cyclic reaction can thus generate a steep response to input that resembles the behavior of a switch. However, for a true switch that is characterized by a discontinuity in the dose response relationship, a positive feedback must be incorporated in the cyclic reaction scheme. This can be achieved for example when S is a kinase that is activated by phosphorylation and, in turn, activates a kinase that catalyses the phosphorylation of S (Figure Ib). The time-dependent concentrations of the phosphorylated reactants of this reaction network are now given by two coupled differential equations (Equations III and IV):

$$\frac{d[SP]}{dt} = v_+ - v_- = \frac{k_{cat}^{kin}[kin][S0-SP]}{K_m^{kin} + [S0-SP]} + \frac{k_{cat}^{S'P}[S'P][S0-SP]}{K_m^{S'P} + [S0-SP]} - \frac{k_{cat}^{ppase}[ppase][SP]}{K_m^{ppase} + [SP]} \qquad \text{[Eqn III]}$$

$$\frac{d[S'P]}{dt} = v'_+ - v'_- = \frac{k_{cat}^{SP}[SP][S'0-S'P]}{K_m^{SP} + [S'0-S'P]} - \frac{k_{cat}^{ppase}[ppase][S'P]}{K_m^{ppase} + [S'P]} \qquad \text{[Eqn IV]}$$

in which the concentrations S and S' have been eliminated by using the conservation of substrate and product for both reactions. Solving for SP at steady state can yield a discontinuity in the input-response curve for certain settings of the kinetic parameters. This system can thus generate a true switch in the response SP to the input signal (kin). As shown in Figure Ib this system also exhibits hysteresis or complete irreversibility, which depends on the strength of the feedback. The curves shown in Figure Ib show that the system contains two stable steady states (low and high SP) and an intermediate unstable steady state that defines the threshold of the system. The necessary ingredients for bistability are thus ultrasensitivity and positive or double negative feedback. For reviews, see [66,67].

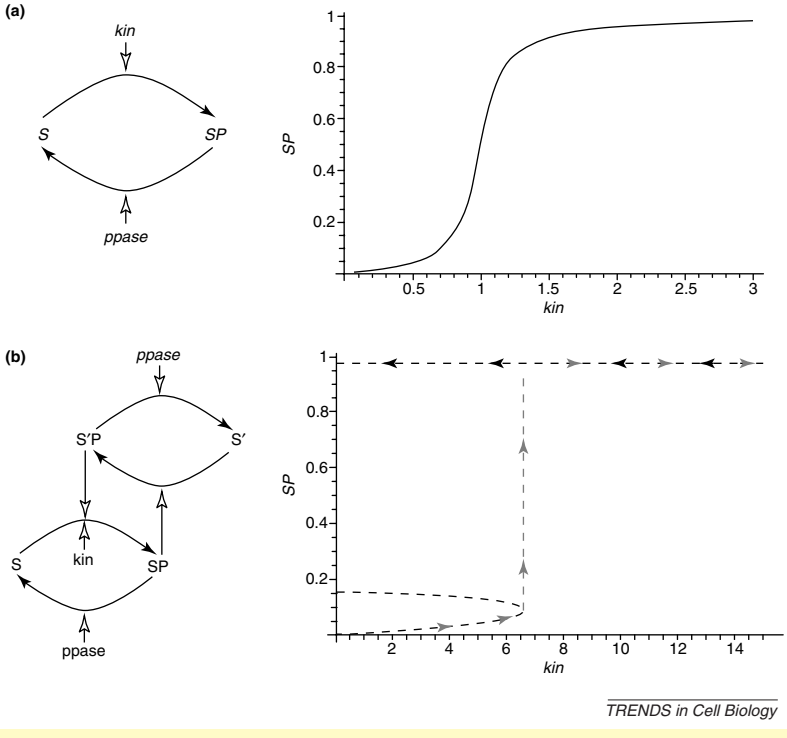


Figure I. Dose-response properties of reaction cycles at steady state. **(a)** A substrate (S) is phosphorylated (SP) by a kinase (kin) and dephosphorylated by a phosphatase ($ppase$), creating a reversible system in which the amount of SP at steady state depends on the relative activity of kin and $ppase$ in a way that can be ultrasensitive. If two such phosphorylation cycles are coupled in such a way that SP is the active kinase for a second substrate S' and $S'P$ is the active kinase for S **(b)**, a positive feedback loop is created that can lead to an irreversible switch in the steady-state concentration of SP as a function of [kin]. Grey arrows show [SP] trajectory with increasing [kin]. Black arrows show the reverse trajectory in which [kin] is decreased, starting from a situation in which all S is phosphorylated. The difference in the two trajectories shows that this system exhibits hysteresis, which in this case results in the complete irreversibility in the phosphorylation of S.