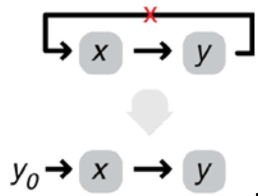
Supplemental Box

One method to understand feedback is to "break the loop," turning the closed-loop feedback system into one with a defined input and output. In the resulting open-loop system, the output would feed back into the input. In the following system, we break the loop in a system with an output *y* that feeds back into *x*:



We can then measure the sign and strength of the now-broken feedback loop with the concept of sensitivity: how much the output *y* changes for a small change in the input (y_0). In this particular case, the sensitivity is called an open-loop gain because it measures the gain (or how much *y* changes with respect to changes in y_0) of the open-loop form of the closed-loop system. Mathematically, sensitivity of *y* to changes in the point of feedback regulation (y_0) at a given steady state ($\hat{x}, \hat{y}$) is calculated with the formula $\gamma = \left.\frac{\partial y}{\partial y_0}\right|_{y_0=\hat{y}}$. The sign of γ tells us the sign of feedback. Note that the sign of feedback may not be obvious, and may emerge from complex interactions in the network that connects *x* to *y*.

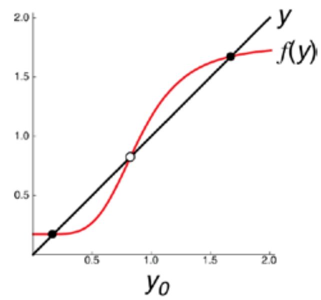
For instance, we can find the open-loop gain in a simple mathematical model of a two-component system (Figure 1), by defining mathematical forms with one or more rate constant for each process embedded in the system. Sparing the details of computation, we found that γ is proportional to $k_{pt}k_d - k_{ph}k_e$ where k_{pt} and k_{ph} are respective rates of regulator phosphorylation and dephosphorylation by the sensor, k_d is the rate of dilution from growth, and k_e is the rate parameter for exogenous phosphorylation. This simple relationship between the gain and rate constants tells us several things about transcriptional feedback in two-component systems:

(*i*) regulator dephosphorylation and exogenous phosphorylation are both necessary for negative feedback and thus overshoot kinetics;

(*ii*) because the dilution rate k_d is small even for fast exponential growth, the rate of exogenous phosphorylation can be small and still permit negative feedback;

(*iii*) different magnitudes of external signaling that modulate phosphorylation and dephosphorylation of the regulator via the sensor (k_{pt} or k_{ph}) can result in different effective feedback signs.

If the circuit diagram of a network (Box 2) has only unambiguously *positive* feedback loops, and its open-loop form has a single steady state, breaking the loop can determine if the intact system is bistable[47]. The basic idea is to use the open-loop form of the system to quantify *y* at steady state as a function of y_0. We denote this function, the open-loop characteristic curve, by $f(y_0)$. Then graph $y = f(y_0)$ along with the feedback strength of the system (*i.e.* open-loop gain, here for simplicity $y_0 = y$) and look for intersections:



If the system is bistable as in the graph above, the system will have two stable steady states (black points) and one unstable (open circle).