Published in final edited form as:

Methods Mol Biol. 2012 ; 880: 53–67. doi:10.1007/978-1-61779-833-7_4.

Bistability in One Equation or Fewer

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Abstract

When several genes or proteins modulate one another's activity as part of a network, they sometimes produce behaviors that no protein could accomplish on its own. Intuition for these emergent behaviors often cannot be obtained simply by tracing causality through the network in discreet steps. Specifically, when a network contains a feedback loop, biologists need specialized tools to understand the network's behaviors and their necessary conditions. This analysis is grounded in the mathematics of ordinary differential equations. We, however, will demonstrate the use of purely graphical methods to determine, for experimental data, the plausibility of two network behaviors, bistability and irreversibility. We use the *Xenopus laevis* oocyte maturation network as our example, and we make special use of iterative stability analysis, a graphical tool for determining stability in two dimensions.

Keywords

Graphical method; Bistability; Irreversibility; Iterative stability analysis; Steady state; Ultrasensitivity; *Xenopus laevis*; Oocyte maturation

1. Introduction

Let us begin by considering the point in describing a biological circuit at which natural language fails. We will trace a chain of causality through a schematic of the *Xenopus* oocyte maturation machinery (Fig. 1a).

Xenopus oocytes are arrested in Meiosis I early in a female frog's development. In adulthood, oocytes are triggered to mature, an all-or-nothing and irreversible (1) process of cell growth and progression to metaphase of meiosis II, at which point the oocyte again arrests awaiting fertilization. Maturation is initiated by progesterone. Imagine an oocyte is exposed to a small amount of progesterone, which leads to a small amount of phosphorylated and activated Mos. This kinase in turn phosphorylates and activates the kinase MEK, which does the same to the kinase p42 MAPK. But then p42 MAPK phosphorylates and activates a little more Mos, which activates a little more MEK, and so on. In the absence of any phosphatases, this positive feedback loop (2) would clearly continue on in this fashion, leading to the total activation of all the available components of the cascade. In the presence of phosphatases, however, things get trickier.

We could imagine that there is enough phosphatase activity acting throughout the loop to balance the collective activity of the kinases. In this case, the positive feedback loop will not explode to total activation, but will come to rest at some intermediate level. Now what happens if we add just a little more progesterone to a loop at rest at this intermediate

position? Will the loop then go on to explode up to total activation? Or will the loop instead remain at rest, but with a little more kinase activity?

Furthermore, what if not all the kinases in the loop pass on activation in the same way? Specifically, what if some kinases respond with a nonlinear response that most of us know as *cooperativity*? If this were true, then, for example, MEK may not phosphorylate p42 MAPK very much when just a small fraction of MEK is phosphorylated, but when phosphorylated MEK reaches higher levels, it may begin to become active and phosphorylate p42 MAPK with great alacrity. If you think imaginatively and with a bit of foresight, you might even imagine that in this case of a cooperative MEK, the feedback loop could be balanced by phosphatase activity twice—once when MEK activity is still not responding well to phosphorylation and again when MEK kicks into gear.

As you can see, analyzing a feedback loop presents an inherent challenge. We understand the world in terms of cause and effect, but in a feedback loop, cause and effect are all mixed up. The preceding exploration of the oocyte maturation network's potential behaviors was reaching the limit of what natural language can describe precisely. Mathematics is a good choice for the language of our conversation, but we will keep our dialogue focused on the cognates that natural language and math share in common: curves and graphs. In doing so, we will explore some of the more biologically relevant concepts in *dynamical systems theory*, the branch of mathematics used to describe the behavior of sets of nonlinear differential equations.

2. Steady State and Stability

Let's begin by obtaining a clear definition of two useful concepts: steady state and stability.

Imagine a valley with very high and steep sides. At the bottom of the valley floor is a small ridge, which effectively divides the valley in two. Additionally, the valley is smooth, uniform, and symmetric—like a half-pipe—so it is enough to consider the cross section of the valley, as we do in Fig. 2. Now, drop a perfectly spherical rock into the valley. Where will it come to rest? Intuition tells us that as long as there is some friction to slow things down, the rock will come to rest at the bottom on one side or the other of the small ridge. Now what if the rock is centered exactly on the top of the ridge? In an idealized situation, the rock will stay perched atop the ridge forever.

Every location where the rock is able to come to rest is a *steady state*. There are three steady states in total: the two bottoms of the mini-valleys on either side of the ridge and the top of the ridge itself. Only the two valley bottoms, however, can be occupied by the rock in anything less than an idealized world. If the rock should fall perfectly atop the ridge, the slightest breeze or tremor would knock it to one side or the other. The valley top is, it follows, an *unstable* steady state. When the rock is at the valley bottom, on the other hand, breezes, tremors, even hard shoves may push the rock up the valley wall, but it will always roll back down and come to rest at its original location. These are *stable* steady states.

Hopefully, the parable of the spherical rock and the oddly shaped valley has allowed you to begin mapping the mathematical definition of stability onto the common-sense idea of

stability. Explicitly, dynamical stability occurs when small perturbations from a steady state shrink over time. This is clearly true of the rock at a valley bottom—a shove up the wall is reversed due to gravity. You’ve probably already guessed that instability occurs when small perturbations grow over time. This happens when a tiny breeze pushes the ridge-top rock to one side, and the displacement quickly grows as the rock rolls down the ridge.

These definitions of steady states and stability will serve us well in analyzing biochemical circuits, but we still need a few more tools in our kit.

3. Open Loops

We seek a method of understanding a feedback loop that is both graphical in nature and closely related to experiments. In the case of the MAPK system governing *Xenopus* oocyte maturation, we can divide the system into two interacting pieces: (1) The cascade from Mos to p42 MAPK, and (2) the positive feedback from p42 MAPK to Mos (Fig. 1b). We can then imagine measuring a dose–response curve for each piece in isolation. We will call this curve an *open-loop dose–response curve*. The open-loop dose–response curve from Mos to p42 MAPK has been experimentally measured (1), a fact we will return to. For now, let’s start our analysis by assuming both open-loop dose–response curves for the MAPK system are saturating Michaelis–Menten shaped curves.

Each curve represents a series of steady states that would exist for p42 MAPK and Mos when half of the feedback loop is operating in isolation. So, if we can find a pair of values for p42 MAPK and Mos that are steady states in both open-loop dose–response curves, that pair of values are also a steady state for the entire MAPK system. In other words, any states that are steady for all the isolated pieces of a system must also be steady for the integrated system. Graphically, this statement has compelling simplicity: steady states for a positive feedback loop occur at intersections of the component open-loop dose–response curves. To see this, we will graph open-loop curves for both separated pieces of the MAPK system on the same set of axes (Fig. 3a). The Mos $\rightarrow$ p42 MAPK curve is plotted as a saturating Michaelis–Menten shaped curve, and the MAPK $\rightarrow$ Mos curve, with the same shape, is reflected and rotated in order to be displayed on the same set of axes.

Now, we just have to find the intersection of these two curves to find the steady states of the entire positive feedback loop. But in order to analyze the potential for interesting behaviors and phenotypes, we need to be able to determine the stability of any steady state we come across. To see if our rock was in a stable state, we simply pushed it and observed what happened. We’ll now give the MAPK feedback loop a shove and watch what happens.

Because we have separated the MAPK signaling system into two parts, each with its own steady-state dose–response curve, our version of “watching what happens” will exist within this piecewise framework. In real life, the consequences of a small perturbation to a steady state of MAPK signaling simultaneously involve both the cascade from Mos to MAPK and the feedback from MAPK to Mos. But for small enough perturbations, we can get away with examining each piece in turn. If we begin at a steady state, then “push” the amount of active MEK away from the steady state, we can follow the causality through each piece of the

MAPK system in series, then determine if our perturbation has grown, indicating an unstable steady state, or has shrunk back toward the fixed point, indicating a stable steady state.

For the first iteration of our piecewise method, start at the systemwide steady-state value of MAPK on the x -axis of Fig. 3b, then move a bit to the right or left to create a small perturbation. In this case, a small enough perturbation is one over which the slopes of the steady-state curves do not change sign. Now, trace a line up to the cascade steady-state curve (black) and read off the y -value of MAPK activity. For the second step, begin with that same y -value of MAPK activity, and trace a line over to the feedback steady-state curve (gray). You should now arrive at a value on the x -axis that you can compare to the starting point. Is the new value of MEK activity closer to the fixed point or farther away? If it is closer, then we know that the MAPK circuit has decreased the size of the perturbation after one round of our iterative analysis, and it will do so again on the next iteration. This fits our definition of a stable steady state. On the other hand, if the perturbation has grown, and one round of iterative analysis has brought MEK signaling away from the fixed point, we know that the steady state is unstable.

4. Bistability

In Fig. 3b, 3c, we apply iterative stability analysis to two different steady states. We find that the steady state at the origin is unstable. Any small perturbation climbs toward the second steady state, which is stable. The biochemical interpretation is that any small amount of activation in the MAPK system will lead to activation at the level of the stable steady state. In the presence of any perturbation, whether biological noise or progesterone, the loop will turn on and stay on. If we were to add progesterone, we could push this steady state to a higher degree of activation (not pictured), and if we took progesterone away, we could lower the steady state. But without a second stable steady state, the system is irreversibly on. This constitutes a trivial case of irreversibility, one in which there is no “off” state. In fact, we wouldn’t really consider this to be an irreversible cell-fate decision. We would just consider it to be a constitutively activated signaling pathway.

What could create a second stable steady state in the MAPK positive feedback loop? We made some guesses about nonlinear activation in the introduction, but let’s keep our thinking constrained for now by the system’s open-loop dose–response curves. One way to make more steady states might be to bend one of these curves, such that they intersect at more points. This makes sense—more bends in a curve provide more chances for intersecting a straight curve.

It turns out that the experimentally measured $Mos \rightarrow p42$ MAPK curve does contain such a bend. In Fig. 4a, we reprint data from (1). On a log horizontal axis, even a Michaelis–Menten-shaped curve appears in an “S”-shape, but you can see that the experimental $Mos \rightarrow p42$ MAPK curve is significantly sharper than a Michaelis–Menten curve. We plot the same data on a linear axis for further comparison in Fig. 4b.

After adding a similar bend to our idealized representation of the $Mos \rightarrow p42$ MAPK curve, the two open loop dose–response curves now intersect at three points (Fig 5a). Furthermore,

using our iterative stability analysis, we find that a small perturbation in Mos from the modified first steady state now translates into a smaller perturbation, not a larger one, after a trip through the feedback loop (Fig. 5b). Before bending the Mos $\rightarrow$ MAPK curve, we obtained the opposite result, that perturbations in Mos are magnified. Really, the only thing that has changed here at the origin is the relative slopes of the steady-state curves. The Mos $\rightarrow$ p42 MAPK curve (black) previously exited the origin above the p42 MAPK $\rightarrow$ Mos curve (gray). But now it exits below, and the stability of the steady state has switched from unstable to stable.

The newly created middle steady state has the property that the origin used to have: The Mos $\rightarrow$ p42 MAPK curve (black) exits above and to the right of the p42 MAPK $\rightarrow$ Mos curve. If all that matters for stability is the relative slopes of the curves at a steady state, we would predict this state to be unstable, just like the origin was in Fig. 3. Iterative stability analysis shows that this is indeed the case (Fig. 5c), as perturbations from the middle steady state inevitably arrive at the first or the third state.

What does this mean for the MAPK feedback loop? There are now clearly two stable steady states that the loop may come to rest in, one low and one high. More importantly, there are no stable steady states connecting the “off” and “on” states. Just by bending one of the open-loop dose–response curves in our positive feedback loops, we have created *bistability*.

5. Ultrasensitivity

What actually happened when we bent the Mos $\rightarrow$ p42 MAPK curve? What is the biological meaning of such a change? It turns out that the exact shape we bent the curve into is not as important as how steep the curve was in the bent region. A steeper curve is more likely to intersect another curve at more than one point. Indeed, steep *ultrasensitive* dose–response curves are often present in networks that display the hallmarks of bistability (3; 4; 5). But what sort of biological mechanisms could affect the steepness of a dose–response curve?

There are in fact too many steepening mechanisms to list here (6; 7; 8; 9; 10; 11), but one that many biochemists and cell biologists are familiar with is cooperativity. Cooperativity occurs whenever some process is required to be completed more than once in order for an activation to occur. It shows up when more than one transcription factor is required to bind to a promoter for full gene expression, and when one of the subunits of hemoglobin must be occupied with oxygen in order for the other subunits to display an increased affinity. The *Xenopus* p42 MAPK cascade may exhibit cooperativity due to the distributive phosphorylations required to activate each kinase (12).

But there are other noncooperative ways to bend a dose–response curve (13; 14). For example, ultrasensitivity may arise when enzymes in the MAPK cascade are nearly saturated with substrate. When this happens, an opposing kinase and phosphatase engage in a tug-of-war for modifying their shared substrate. Any excess of the kinase’s activity over the phosphatase’s, for example, results in nearly all of the substrate becoming phosphorylated. The transition in this tug-of-war is sharp, leading to a sigmoidal dose–response curve,

though there was never a cooperative mechanism at work. We will call the general property of a nonlinear, bent, sigmoidal curve ultrasensitivity. In practice, we quantify the degree of ultrasensitivity present in experimental data by fitting a Hill equation

$$Y = \min + (\max - \min) \frac{X^{n_H}}{K^{n_H} + X^{n_H}} \text{ where } Y \text{ is any biological output, } X \text{ is any biological input,}$$

$\max$ and $\min$ are the bounds of the output, K is the level of X at which Y is halfway activated between $\min$ and $\max$, and n_H is the Hill coefficient, which controls ultrasensitivity. Equations fit to more bent, sigmoidal graphs will have higher Hill coefficients. You may also notice that this is a Michaelis–Menten equation with renamed constants and the addition of the Hill coefficient. In fact, a Michaelis–Menten equation is a Hill equation with $n_H = 1$.

Using a standard nonlinear curve-fitting algorithm in MATLAB we can fit the Hill equation to experimentally measured data to find that the $\text{Mos} \rightarrow \text{p42 MAPK}$ dose–response–curve has an n_H value of 4.9 (1). In fact, we have based our example $\text{Mos} \rightarrow \text{MAPK}$ open-loop dose–response curve on precisely these data. The curve from $\text{MAPK} \rightarrow \text{Mos}$ has not been measured, so we will for now stick with our assumption that this dose–response curve is a saturating Michaelis–Menten-shaped curve.

6. Reversibility

What do we do with these steady states now that we’ve found them and characterized their stability? We already know that the existence of more than one stable steady state is bistability. But we probably want to know if bistability exists over a broad range of the values of some parameter, or if bistability was more of an ephemeral property of the system. And what happens when bistability appears and disappears as we vary a parameter?

Let’s choose that varied parameter to be progesterone concentration. In the *Xenopus* oocyte, progesterone initiates maturation and leads to self-sustaining MAPK signaling. The specific way in which progesterone interacts with the MAPK feedback loop is poorly understood, so we will simply assume for now that it leads to an amount of activated p42 MAPK proportional to progesterone concentration. This progesterone-derived p42 MAPK signaling is added to the activation already brought about by Mos, and it shifts the $\text{Mos} \rightarrow \text{MAPK}$ open-loop dose–response curve vertically by an amount proportional to progesterone concentration. The open-loop response curve accounts for MAPK activation coming from all sources. MAPK activation coming from progesterone is constant at all levels of Mos, so it shifts the curve vertically without altering its shape.

Now, let’s simply add a series of concentrations of progesterone, vertically shifting the $\text{Mos} \rightarrow \text{MAPK}$ curve (Fig. 6a), keeping track of where the steady states are found (Fig. 6b). (In these figures, we’ve adjusted the saturating values of the two open-loop dose–response curves to make the following points more clear.) We notice right away that adding progesterone moves the first stable steady state (lower black line in Fig. 6b) and the unstable steady state (gray line in Fig. 6b) closer together. Eventually, the two points meet, and with another addition of progesterone, disappear. At this high level of progesterone, the two open-loop curves intersect at only one point (top black line in Fig. 6b).

What happens to the MAPK system if it occupied the stable steady state that has now disappeared? This problem at first seems difficult, since we haven't encountered a previous case in which the system was not already at a steady state. But we can perform iterative stability analysis and get very close to the answer we would arrive at with more sophisticated treatments. We find that after the lower stable steady state disappears, the feedback loop shoots up to the upper stable-steady state, undergoing a rapid activation of MAPK signaling.

This type of behavior exactly matches descriptions of the all-or-nothing nature of oocyte maturation (1). Furthermore, it is an example of how a bistable system moves between its two stable steady states. The system never comes to rest in any state that is intermediate between the two stable steady states. From the point of view of an experiment conducted at steady state, the transition is absolute and discontinuous. This change in the number and type of steady states is termed *bifurcation*. In the bifurcation we just examined, the lower stable steady state and the unstable steady state merge and annihilate. If a system occupies a steady state that is annihilated in a bifurcation, the system will be forced to move to a remaining stable steady state, creating an all-or-nothing transition.

Of course, lowering the concentration of progesterone will now allow the lower stable steady state and the middle unstable steady state to reappear. The bifurcation will occur again, but in reverse. Will the MAPK loop then decrease its activity to reoccupy the newly formed lower stable steady state? No, it will not. Because the upper stable steady state is still present, the MAPK system will remain in its high-activity state, even though the lower-expression option exists (15).

Importantly, these persistent states and the all-or-nothing behaviors they produce are properties of the network of kinases itself, rather than of any individual enzyme. If the connections between kinases were altered, the number and type of steady states might change. Conversely, individual kinases can be degraded and resynthesized while the network remains in a persistent steady state (16; 17).

Through this simple graphical method, we have uncovered something quite profound. The behavior of our model of the MAPK feedback loop depends on its history. In the bistable region, the loop will occupy the lower stable steady state if it was previously in this state, and it will occupy the upper stable steady state if it was instead previously in that state. This history dependence of a system's behavior is *hysteresis*. You are already familiar with hysteresis in blowing up a rubber balloon. The first time you inflate a balloon, it requires quite a bit of pressure. But this extra pressure goes into stretching the rubber inelastically, and it never returns to its original state after being stretched. The next time you blow up the balloon, it requires much less pressure to inflate the balloon to the same degree.

Will the MAPK feedback loop ever return to its original state once it is activated? In Fig. 6a, we continue reducing the concentration of progesterone bit-by-bit, but we find that the upper stable steady state never disappears. We record this fact in Fig. 6b as an upper steady state (top black line) that intersects the *y*-axis at 0 nM progesterone. Like a stretching balloon, the MAPK system undergoes an irreversible transition the first time it is activated. Unlike our

first encounter with this property in Fig. 3c, there is now a stable “off” state that can be initially occupied.

Our model now displays an “off” state, an “on” state, and an irreversible all-or-nothing transition from “off” to “on”. With just graphs and one equation, we’ve recapitulated some of the most biologically significant behaviors of cell-fate determination. We can even make a simple prediction. Because the all-or-nothing nature of this decision is a consequence of bistability, which emerges from the interaction of the cascade from $Mos \rightarrow p42 \text{ MAPK}$ and the feedback from $p42 \text{ MAPK} \rightarrow Mos$, we should be able to abolish irreversibility and the all-or-nothing character by inhibiting one of the legs of the feedback loop. Graphically, if we remove the $p42 \text{ MAPK} \rightarrow Mos$ curve (gray in Figs. 3 and 5), then every point along the remaining $Mos \rightarrow p42 \text{ MAPK}$ curve will be a steady state. The output of the cascade will depend continuously on the input, with no irreversibility. Indeed, these predictions that abolishing positive feedback in the *Xenopus* MAPK cascade also converts the normally all-or-nothing and irreversible response of the cascade to progesterone into a continuous, reversible response have been born out experimentally (18; 19).

7. Conclusion

The concepts of bistability, bifurcations, and hysteresis have made their way into mainstream cell biological and biochemical literature, though many working scientists did not have access to these concepts as a part of their training. Fortunately, only basic calculus and graphical methods are required for a working knowledge of these dynamical phenomena. By separating a nonlinear system such as a feedback loop into at least two chunks, one can graph the open-loop dose–response curves, or estimates thereof, and then, by applying iterative stability analysis, arrive at the number and location of stable and unstable steady states. Whether achieved using graphical techniques, as we have here, or using analytical techniques, as you may go on to master, identifying and characterizing steady states is the foundation of dynamical systems analysis.

Once identified and characterized, one can observe how the steady states change as parameters of a biological system are varied. Here, we chose to vary progesterone concentration, though we could have chosen to vary the sensitivity of the cascade instead, and we would have obtained the graph of steady-state position as a function of sensitivity. Bistability is simply the property that two stable steady states are present at a single parameter value. Bifurcations occur whenever the number of steady states (stable or unstable) changes as the parameter varies, and hysteresis, always present in the case of bistability, refers to the history dependence a system displays as it moves along the graph of stable steady states as the parameter is varied.

Bistability, when coupled with some degree of cell-to-cell variability, often presents itself in a population as two peaks in the histogram of some single-cell measurement. To be sure, this bimodality is a hallmark of bistability, but not a sufficient condition (20). Hysteresis can be thought of as a more reliable hallmark of bistability (21).

If this was your first intellectual jaunt into the methods of dynamical systems, we recommend adding one additional graphical method to your toolbox, rate–balance analysis (22). It is the graphical method of choice for systems that can be written in a single variable, and characterizing steady states in rate–balance analysis has a more obvious link to the mathematical definition of stability.

Finally, we can recommend Stephen Strogatz’s textbook *Nonlinear Dynamics and Chaos* (23) as a lucid and eminently readable next step in your dynamical systems education.

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Fig. 1.
A schematic of the *Xenopus laevis* MAP kinase pathway (**a**) as connected *in vivo* and (**b**) separated into a cascade and a positive feedback, for purposes of theoretical analysis.

Fig. 2.

Cross section of a landscape containing a perfectly spherical rock. The rock can come to rest at three positions: the two valley floors, and at the very top of the small hill. If the rock is perched at the hilltop, and it is pushed, it will clearly accelerate in the direction it was pushed and roll down the hillside. Thus, the hilltop position is unstable. A rock perched at a valley bottom, however, if pushed, will stop rolling, then reverse direction and eventually settle again at the valley bottom. The two valley bottom positions are stable. You can map the mathematical concept of stability onto an intuitive understanding of stability you already possess.

Fig. 3.

Open-loop dose-response curves for the separated legs of the MAPK system, assuming Michaelis-Menten-shaped curves. Note the two intersection points (a), indicating two steady states. Suppose the system comes to rest at the nonzero steady state (b). Following a small perturbation with iterative stability analysis, we end up back at the steady-state; the nonzero steady-state is stable. If the system is at rest at the graph's origin (c), then a small perturbation will grow with each iteration until the system comes to rest at the stable steady state. Thus, the steady state at the origin is unstable.

Fig. 4.

The in vivo p42 MAPK $\rightarrow$ Mos curve displays a sigmoidal, not a Michaelis–Menten shape. A hill coefficient of 4.9 causes p42 MAPK activity to markedly accelerate with respect to Mos concentration before saturating. For reference, a Michaelis–Menten-shaped curve contains no such acceleration. The difference in shape can be seen on both semi-log (a) and linear (b) axes. Data reprinted from (1).

Fig. 5.

We induce some sigmoidal curvature in the $\text{Mos} \rightarrow \text{p42 MAPK}$ open-loop curve (a). Note there are now three intersections of the two open-loop curves and three steady-states. A perturbation to a system at rest at the origin now results in a return to the origin—the origin has become a stable steady state (b). Perturbations from the middle steady state will grow, coming to rest at either the origin or the upper steady state (c). In total, there are now two stable steady states and one unstable.

Fig. 6.

We incrementally increase the basal activity of p42 MAPK, which represents the effect of progesterone in vivo. This shifts the Mos $\rightarrow$ p42 MAPK open-loop curve in the vertical axis (a). Each shift has the effect of changing the positions of the three steady states in the Mos axis. Now, we can plot the location of these steady states while varying basal p42 MAPK activity over a wide range (b). As basal p42 MAPK activity increases, the two stable steady states move up the Mos axis (now displayed as the vertical axis), while the unstable steady state moves down the Mos axis. At a high-enough basal p42 MAPK activity, the lower stable

steady state and the unstable steady state meet and annihilate, leaving only the upper stable steady state. A system at rest at the lower stable steady state, when experiencing this annihilation event, will explosively jump from a low Mos concentration to a high Mos concentration without coming to rest at any intermediate levels. Even if the basal p42 MAPK level is lowered after this explosive event, the system will forever remain at the higher of the two stable steady states. The transition from low to high Mos was irreversible.