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Bistability in Biochemical Signaling Models

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Abstract

This Teaching Resource provides lecture notes, slides, and a student assignment for a two-part lecture on the principles underlying bistability in biochemical signaling networks, which are illustrated with examples from the literature. The lectures cover analog, or graded, versus digital, all-or-none, responses in cells, with examples from different types of biological processes requiring each. Rate-balance plots are introduced as a method for determining whether generic one-variable systems exhibit one or several stable steady states. Bifurcation diagrams are presented as a more general method for detecting the presence of bistability in biochemical signaling networks. The examples include an artificial toggle switch, the *lac* operon in bacteria, and the mitogen-activated protein kinase cascade in both *Xenopus* oocytes and mammalian cells. The second part of the lecture links the concepts of bistability more closely to the mathematical tools provided by dynamical systems analysis. The examples from the first part of the lecture are analyzed with phase-plane techniques and bifurcation analysis, using the scientific programming language MATLAB. Using these programs as a template, the assignment requires the students to implement a model from the literature and analyze the stability of this model's steady states.

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Educational Details

Learning Resource Type: Lecture notes, assignment, presentation

Context: Graduate

Intended Users: Teacher, learner

Intended Educational Use: Learn, plan, teach

Discipline: Bacteriology, biochemistry, bioengineering, biophysics, cell biology, developmental biology, education, enzymology, reproductive biology, theoretical biology

Technical Details

Format: PowerPoint (.ppt)

Size: 3.86 MB

Requirements: Microsoft PowerPoint

Format: MATLAB (.m)

Requirements: Mathworks MATLAB

Format: PDF (.pdf)

Requirements: Adobe PDF Reader

Supplementary Materials

<http://stke.sciencemag.org/cgi/content/full/sigtrans;4/192/tr10/DC1>

Slides. Bistability in biochemical signaling models.

Problem set MATLAB code. Two MATLAB programs for computational analysis of bistability.

Answer key. MATLAB code and PDF of the answers are available upon request

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Keywords

Dynamical systems; mathematical modeling; differential equations; protein kinase signaling; fertilization; lac operon; rate-balance plot; MATLAB; nullcline; simulation

Lecture Notes

Analog Versus Digital Responses

Biological processes can be graded or digital (Slides 2 to 5). Most biological processes taught to undergraduates and beginning graduate students are analog and graded in nature. For instance, the following represent physiological examples of analog responses: (i) the increase in cardiac output during exercise, (ii) the secretion of insulin by pancreatic beta cells in response to elevated blood glucose, and (iii) the increased concentration of a reaction's product when the concentration of the catalyzing enzyme increases. In these cases, the output is a smoothly varying function of the input, and the output returns back to the original level when the input is removed (albeit sometimes with a delay) (Slide 4).

For some other biological processes, however, such an analog response is inadequate, and the cell (or organism) must make a digital, yes-or-no decision. Examples include fertilization, differentiation, cell division, apoptosis, and immune cell activation (Slide 5). In these situations, the cell's decision is difficult to reverse, such that once a commitment is made, small fluctuations in the initiating stimulus are insufficient to cause a change in the output. In some cases, cell decisions are irreversible.

Rate-Balance Plots for Quantitative Analysis of Bistability

Digital, yes-or-no cell decisions generally result from biochemical signaling networks that exhibit bistability. This means that either a low activity (“No”) state or a high activity (“Yes”) state are possible, but intermediate levels of activity are observed only rarely (Slide 3). At the level of biochemical reactions, bistability typically results from “mutual activation” or “mutual repression” feedback loops (Slide 6) (1, 2). However, these arrangements do not by themselves guarantee that a particular network will be bistable. A particular signaling network will frequently be monostable (one steady state) under some conditions and bistable under others (Slides 7 and 8). Quantitative analyses are required to determine whether a particular signaling network exhibits bistability. One method for determining bistability is the rate-balance plot (Slides 9 and 10). The work of Ferrell and Xiong (3) is used to illustrate how, when considering a single variable, rate-balance plots provide an intuitive, yet rigorous, means to establish the stability characteristics of a network (Slides 10 to 19). If both the forward rate and the backward rate of a reaction depend linearly on the quantity of substrate, then forward and backward rates will be equal at a single point, and the system will be monostable (Slide 10). Two system properties that can lead to bistability are (i) “ultrasensitive” positive feedback and (ii) a back reaction that saturates with increasing amounts of product (Slides 16 to 19) (3).

Bifurcation Diagrams to Illustrate Bistable Regimes

Systems that have the capacity for bistability frequently only exhibit this behavior under particular conditions. For instance, if an external stimulus activates a participating enzyme, it is possible that bistability will only be present for intermediate values of stimulus and that high or low levels of stimulus will produce monostability. In such a case, the value of stimulus where the system switches from monostable to bistable represents a bifurcation, and important system properties can frequently be inferred by plotting stimulus on the abscissa and a relevant dependent variable on the ordinate. Examples of such bifurcation diagrams from several generic reaction schemes are presented in Slides 20 to 23.

Phase-Plane Analysis of Steady States of Biological Systems

The second method of analysis that can determine the presence of bistability is the plotting of trajectories in the phase plane (Slides 24 and 25). Given a set of differential equations defining the evolution of the state variables of the system, a “nullcline” is defined as the set of points at which the derivative of a variable is zero. For a system with two variables, nullclines can be plotted as a function of the two variables to illustrate important characteristics of the system (Slides 24 and 25). For instance, each time a nullcline is crossed, the direction the system travels in the phase plane changes (Slide 24). Examples illustrate that when the two nullclines intersect at only a single point, the system must have only one steady state. This steady state may be stable, indicating a system that always returns back to this point, or it can be unstable, in which case the system oscillates continuously. However, if the nullclines intersect three times, the system can be bistable. These principles are illustrated with proven bistable biological signaling systems, including the artificial toggle switch built by Collins and co-workers (Slide 26) (4); the mitogen-activated protein kinase (MAPK) cascade in *Xenopus* oocytes (Slide 27) (5, 6); and the MAPK cascade in mammalian cells (Slide 28) (7, 8).

Analysis and Simulation of Biological Systems with MATLAB

The second part of this lecture reinforces the quantitative concepts introduced previously by presenting analysis and simulation scripts written in MATLAB, then illustrating the results obtained with these scripts. This lecture begins with a brief review of certain key points from the previous lecture (Slides 29 to 35). Students are provided with MATLAB scripts that (i) generate rate-balance plots and (ii) simulate the temporal evolution of a generic two-variable system that can potentially exhibit bistability. These scripts are used by the students as templates for completing the Problem Set (see below). Explaining these scripts and allowing the students to run simulations with them helps to reinforce the pertinent concepts. For instance, the dynamical simulations show that when a system is bistable, the initial conditions determine whether the “high” steady state or the “low” steady state is eventually attained. Initial conditions close to the unstable steady state can lead to either outcome, and the time required to reach steady state is typically quite long with such initial conditions (Slides 36 to 38). Finally, a simple graphical method for determining the stability characteristics of steady states in phase-plane plots is described and applied to a two-variable example system (Slides 39 to 42). The result obtained with this method is shown to

be consistent with the answer obtained through the classical method from dynamical systems, computing the roots of the characteristic equation (Slides 43 and 44).

Conclusion

The lecture ends with a review of some of the biological systems that can exhibit bistability (5, 7) (Slides 45 to 48), including the now-classic example of the *lac* operon of *Escherichia coli* (Slides 47 to 48) (9, 10, 11). The latter system is the subject of the homework assignment, which is discussed in Slide 49.

Problem Set

Introductory Details

The homework assignment requires the students to apply the methods of analysis taught in the lectures. In Part 1, students generate rate-balance plots for a system with linear positive feedback but a back reaction that can become saturated. The assignment includes a MATLAB script that generates rate-balance plots for a simpler system, and the students convert this into a program that addresses the assignment. In Part 2, the students implement a two-variable model of the *E. coli lac* operon. This system exhibits either one or two stable steady states, depending on the value of extracellular lactose. In addition to performing dynamic simulations with this model, the students perform a phase-plane analysis to determine the stability characteristics. The second assignment is the more difficult of the two.

The Supplementary Materials include two MATLAB programs: “ratebalance.m,” which generates rate-balance plots for cases with (i) no feedback or (ii) ultrasensitive feedback, and “repression.m,” which consists of an ordinary differential equation (ODE) model of a generic two-variable system with mutual repression.

Student Assignment: Part 1

In a system with a single variable, rate-balance plots can be used to determine whether bistability is present (see Slides 10 to 20 for examples). The MATLAB script “ratebalance.m” plots these curves for two simple cases: (i) a constant forward rate and (ii) ultrasensitive autocatalytic feedback that causes the forward rate to be nonlinear. The variable of interest A^* (Astar in the script) is assumed to be normalized to the quantity $[A]_{\text{TOTAL}}$, so this ranges from 0 to 1. For several values of the stimulus S , the program plots forward and backward rates, as well as the steady states where the forward and backward rates cross.

Bistability can be produced with ultra-sensitive feedback, as in the example shown in Slide 17. It is not possible with linear feedback and a first-order reverse rate. However, bistability can be produced if linear autocatalytic feedback is combined with a “back reaction” that becomes partially saturated when the amount of product (A^* in this case) increases (see Slide 19).

1. Modify “ratebalance.m” to simulate the condition of linear autocatalytic feedback with a partially saturated back reaction. You can assume that the stimulus [S] is zero. Start with the following constants: $k_{\text{minus}} = 5$; $K_{\text{mb}} = 0.1$.
2. Assuming $S = 0$, test several different values of K_{mb} and determine which values of K_{mb} produce bistability and which do not. You will have to determine the “interesting” range of K_{mb} values to examine. Plot your results as a bifurcation diagram: K_{mb} on the abscissa, steady-state values of $[A^*]$ on the ordinate. For the bistable values of K_{mb} , there will be three possible steady-state values of $[A^*]$, so this plot will be most clear if you plot each steady-state value with a distinct symbol and do not connect the points. Lines 57 to 65 in the program accommodate the situation in which one or multiple steady states are possible.
3. From your analysis in problem 2, how large does K_{mb} have to become before bistability is no longer observed?
4. From your analysis in problem 2, why do small values of K_{mb} allow for bistability, whereas large values do not?

Student Assignment: Part 2

To complete this assignment, you must implement a simple two-variable model of the *E. coli lac* operon (Slides 47 and 48) and use nullclines to evaluate bistability (Slides 25 and 38 to 42). The differential equations governing this system are as follows:

$$\begin{aligned}\frac{dl}{dt} &= \beta l_{\text{ext}} \text{LacY} - \gamma l \\ \frac{d\text{LacY}}{dt} &= \delta + p \frac{l^4}{l^4 + l_0^4} - \sigma \text{LacY}\end{aligned}$$

For this question, assume that the following parameter values are fixed:

$$\begin{aligned}\beta &= 1, \gamma = 1, \delta = 0.2, \\ l_0 &= 4, p = 4, \sigma = 1.\end{aligned}$$

The variable l_{ext} , representing extracellular lactose, is the variable that can vary and potentially cause the bacteria to change states.

1. Plot the nullclines of this model for $l_{\text{ext}} = 2.5$. Because of the l^4 term appearing in the second equation, it is easiest to treat LacY as the dependent variable (ordinate) and l as the independent variable (abscissa). Your nullclines should intersect in three places.
2. From examining the equations in problem 1, above the LacY nullcline (that is, increased LacY), is LacY increasing or decreasing?
3. From examining the equations in problem 1, above the l nullcline, is l increasing or decreasing? Draw arrows on the LacY and l nullclines to illustrate these results. From these arrows, determine in qualitative terms the stability of the three steady states.

4. Modify the provided program “repression.m” so that the new model now simulates the temporal evolution of l and $LacY$. To reach steady state, a total simulation time of 20 s is reasonable, and the solution converges with a time step of 0.01 if Euler's method is used.
5. With the modified program, for $l_{\text{ext}} = 2.5$, plot the time evolution of $LacY$ and l for the following initial conditions: (i) $l = 8$, $LacY = 3$; (ii) $l = 3.2$, $LacY = 1.3$; (iii) $l = 3$, $LacY = 1.2$; (iv) $l = 2$, $LacY = 1$. Explain the different results obtained.
6. With the modified program, vary l_{ext} over the range 1 to 7 and compute the temporal evolution of l and $LacY$. For each simulation, store the final value of $LacY$. Run two sets of simulations: one in which the initial conditions are $l = 8$, $LacY = 3$, and a second in which the initial conditions are $l = 2$, $LacY = 1$. Generate a bifurcation diagram of how the final value of $LacY$ varies as a function of l_{ext} . Plot the results of the two sets of simulations in different colors.
7. From the analysis in problem 6, how small must l_{ext} become before bistability is no longer observed?
8. From the analysis in problem 6, how large must l_{ext} become before bistability is no longer observed?
9. From the analysis in problem 6, explain in biological terms why bistability is only observed at intermediate values of l_{ext} .

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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