Bifurcation

Fish in a tank

Take a simple model for fishes growing in a tank,

$$\frac{dx}{dt} = kx\left(1 - \frac{x}{C}\right) - r \tag{1}$$

This is a logistic growth model with removal of fish at a constant rate (r). In logistic growth, the rate of growth depends upon the current population size but is limited by the carrying capacity of system (C). In Equation 1, when $x << C$, there will be exponential growth of fishes. But as they increase in number, there will be shortage of space and resources. Eventually, when $x = C$, the growth rate will become zero.

Let's identify the steady states for Equation 1, algebraically by setting $dx/dt = 0$,

$$kx\left(1 - \frac{x}{C}\right) - r = 0$$

$$kx^2 - kCx + rC = 0 \tag{2}$$

The solution of this quadratic equation is,

$$x = \frac{kC \pm \sqrt{k^2C^2 - 4rkC}}{2k} \tag{3}$$

Take different values for r in Equation 3. When $r = 0$, there are two steady states, $x = 0$ and $x = C$. When $r > kC/4$, there is no real solution. But, if $r = kC/4$ there is only one steady state, $x = C/2$.

When $0 < r < kC/4$, there are two steady states,

$$x = \frac{kC + \sqrt{k^2C^2 - 4rkC}}{2k} \quad \text{and} \quad x = \frac{kC - \sqrt{k^2C^2 - 4rkC}}{2k}$$

Therefore, the number of possible steady states for Equation 1 depends upon the value of the parameter *r*. This behavior is shown graphically in Figure 1. In this plot, *r* is in the horizontal axes and steady states of *x* are in the vertical axes.

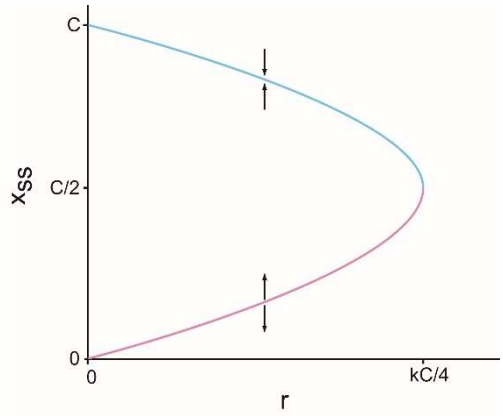

Figure 1: Bifurcation diagram for the fish in a tank model. The blue and pink lines are stable and unstable steady states respectively.

We have shown that number of steady states for this system changes with *r*. But what about the stability of those steady states? Does *r* also affect the stability of those steady states?

Again, we will take *r* in different domains and check the stability of steady states. For stability analysis we will use the derivative-based method discussed earlier.

$$\frac{dx}{dt} = f(x) = kx\left(1 - \frac{x}{C}\right) - r$$

$$\therefore f'(x) = \frac{d}{dx}\left[kx\left(1 - \frac{x}{C}\right) - r\right] = k - \frac{2k}{C}x \tag{4}$$

When $r = 0$, we have two steady states, $x = 0$ and C. Following Equation 4, for $x = 0$, $f'(x) > 0$. Therefore, this steady state is unstable. For $x = C$, $f'(x) < 0$. Therefore $x = C$ is a stable steady state.

When $0 < r < kC/4$, there are two steady states.

For $x = \dfrac{kC + \sqrt{k^2C^2 - 4rkC}}{2k}$, $\quad f'(x) = -\dfrac{\sqrt{k^2C^2 - 4rkC}}{C} < 0$

Therefore, this steady state is stable.

For $x = \dfrac{kC - \sqrt{k^2C^2 - 4rkC}}{2k}$, $\quad f'(x) = \dfrac{\sqrt{k^2C^2 - 4rkC}}{C} > 0$

So, this steady state is an unstable one. In Figure 1, the blue line represents stable steady states and the pink line represents unstable steady states.

The steady state of x for $r = kC/4$ is $x = C/2$. At this steady state $f'(x) = 0$. So we need to use graphical method or the numerical tabular method to ascertain the stability. However, from Figure 1 you can convince yourself the $x = C/2$ is a semi-stable steady state.

This dynamical system for fishes growing in a tank has a unique characteristic– the number of steady states and the stability of steady states changes with the numerical value of r, a parameter. It is said that this system has Bifurcation.

In general, for some dynamical systems the qualitative behavior changes with variation of a parameter. By change in qualitative behavior, we mean, a) change in number of steady states and b) change in stability of the steady states. Change in either of these two or both, will change the phase portrait. Such qualitative changes in the dynamics of the system depending upon a parameter is called Bifurcation. That particular parameter is called a control parameter and the value of the control parameter where the bifurcation happens is called the bifurcation point. In the fish tank problem, r is the control parameter and the bifurcation point is $r = kC/4$.

Figure 1 is called a bifurcation diagram. In a bifurcation diagram, the control parameter and the steady states of a dependent variable are shown on the horizontal and vertical axes, respectively. Usually stable and unstable steady states are represented by different colors or by different line patterns. You can also place arrows near the steady states to represent stability. Check Figure 1. Both the arrows on the line for the stable steady state are pointing towards the steady state. For the unstable one, those arrows are pointing in opposite directions.

Bifurcation in this system can be detected using a graphical approach too. For ease of analysis, let's consider specific numerical values for parameters, k = 1 and C = 100. In Figure 2 we plotted the function on the righthand side of Equation 1, for different values of r, in the dx/dt vs. x plane. When r = 0, the curve intersect the horizontal axes at two positions, x = 0 and x = 100. Therefore, these two points are steady states. Stability analysis using the tabular method, discussed in Chapter 3, shows that these two steady states are stable. Similarly, the system has two stable steady states when r = 20. However, we increase r to 25, the curve intersects the horizontal axes only at one point and this is a semistable steady state. When r > 25, the curve does not intersect the horizontal axes, indicating that there is no steady state. So this graphical analysis shows that the number of steady states and the stability of those steady states changes with r.

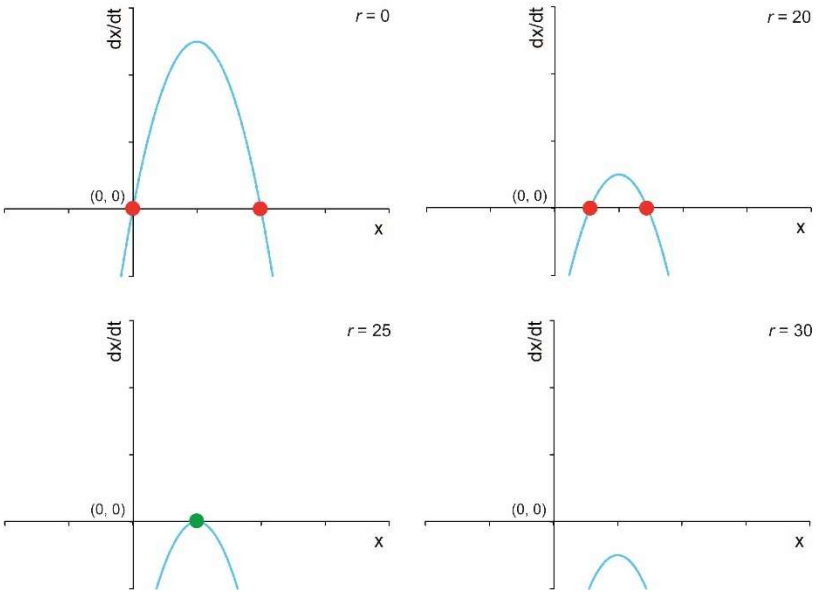

Figure 2: Graphical method for detection of bifurcation. The blue is the right hand side function of the fish in a tank model. Steady states are marked by filled circles. Red and green circles represent stable and semistable steady states, respectively. r is varied from 0 to 30; k = 1 and C = 100.

Different types of Bifurcation

The bifurcation observed for the fish in a tank model, is called a saddle node bifurcation. In saddle node bifurcation, as the control parameter changes, two nodes (or steady

states) collide and annihilate each other. This type of bifurcation is also called fold bifurcation. Though called saddle, we do not see any shape similar to a saddle in Figure 1. But the name will be more meaningful when we will deal with higher dimensional models.

There are several other types of bifurcations. We will introduce some of those here. The fish in a tank problem is an one-dimensional problem. Let's take a model involving two dependent variables. The following system of ODEs is a simplified model of a genetic circuit with mutual repressors. In this model *x* and *y* are transcription factors and they repress each other's expression.

$$\frac{dx}{dt} = \frac{k}{\left(1+y^n\right)} - cx \tag{5}$$

$$\frac{dy}{dt} = \frac{k}{\left(1+x^n\right)} - cy \tag{6}$$

here, $n \geq 1$, $x \geq 0$, and $y \geq 0$

Figure 3 shows the phase portraits of this system of ODEs for different values of *n*. For this analysis we have considered $k = 3$, $c = 1$. When $n = 1$, there is only one steady state at (1.3028, 1.3028) and it is a nodal sink. However, changing *n* to 3, changes the phase portrait. For $n = 3$, there are three steady states. Steady states at (0.1075, 2.9963) and (2.9963, 0.1075) are nodal sinks. However, the third steady state at (1.164, 1.164) is a saddle point. As the phase portrait changes with change in the parameter *n*, this system has bifurcation.

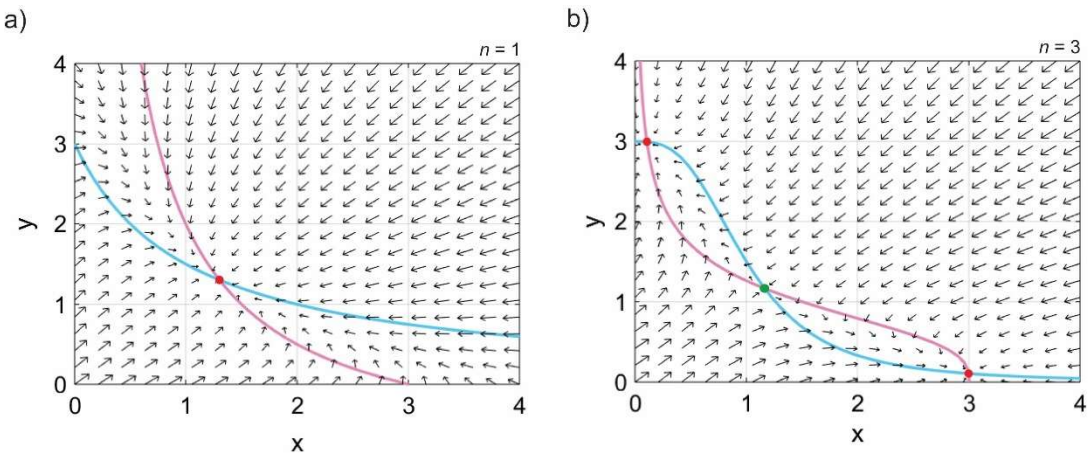

Figure 3: Phase portraits for the model of mutual repressors. a) $n = 1$, and b) $n = 3$. Stable and unstable steady states are shown by red and green circles, respectively. The blue and pink lines represent x- and y-nullclines, respectively.

The bifurcation diagram of the model, for control parameter n, is shown in Figure 4. When n is below the bifurcation point ($n = 1.699$), both x and y have only one stable steady state. However, above the bifurcation point, the system has two stable steady states and one unstable steady state.

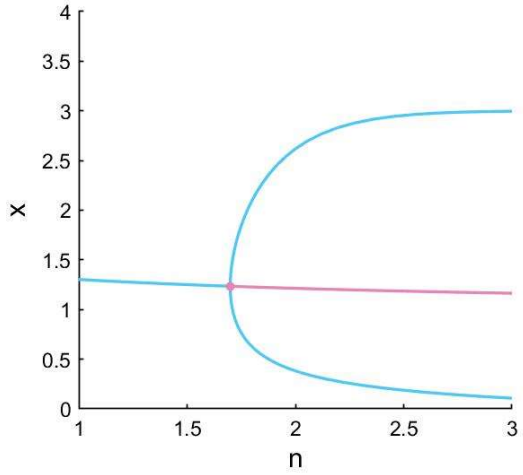

Figure 4: The bifurcation diagram for the mutual repressors. The steady state values of x are on the vertical axes. Blue and pink lines are for stable and unstable steady states respectively. For this analysis we have considered $k = 3$, and $c = 1$. y shows similar bifurcation.

The bifurcation observed in the system of mutual repressor is called Pitchfork bifurcation. Pitchfork bifurcation can be of two types- supercritical and subcritical. In supercritical pitchfork bifurcation, two new stable steady states emerge at the bifurcation point, as in Figure 4. However, the subcritical one is an inversion of supercritical pitchfork. In subcritical pitchfork, beyond the bifurcation point, the system has only one unstable steady state (Figure 5). At the bifurcation point a pair of unstable steady states emerge along with a stable steady state.

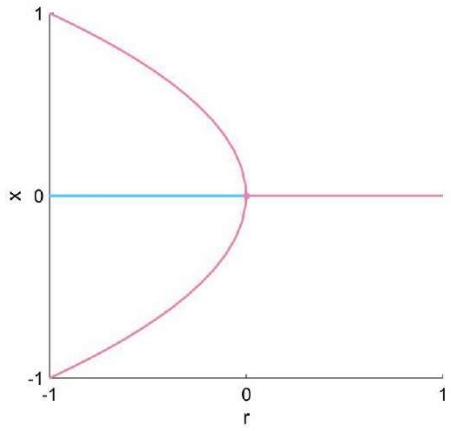

Figure 5: Subcritical pitchfork bifurcation observed in $\frac{dx}{dt} = rx + x^3$. Blue and pink lines are for stable and unstable steady states respectively.

There are few other types of bifurcation. In Figure 6 we show another one called transcritical bifurcation. In a system with transcritical bifurcation, a stable, and unstable steady states coexists and beyond the bifurcation point these steady states exchange their stabilities.

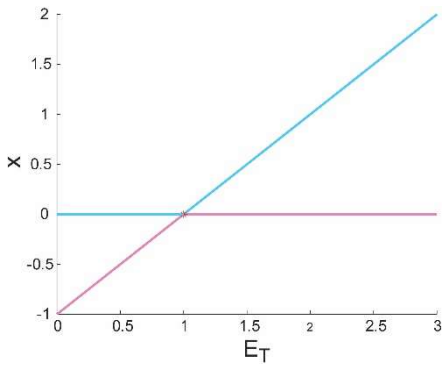

Figure 6: Transcritical bifurcation observed in $\frac{dx}{dt} = k_1(E_T - x)x - k_2 x$. Blue and pink lines are for stable and unstable steady states respectively. The parameter values used for the analysis are: $k_1 = k_2 = 1$.

Bifurcation and hysteresis

Various types of bifurcation are observed in dynamical models in biology. However, does bifurcation affect biology, or is it just a fascinating mathematical property? We will discuss two examples to answer this question.

Consider a simplified mathematical model of the cell cycle. Between two mitotic cell division, a cell goes through different phases. The first phase is G1, where the cell duplicates its cellular machinery, copies organelles, and increases size. DNA replication happens in the subsequent S phase, followed by the G2 phase that makes the cell ready for mitosis or M-phase.

Here, we will discuss a very simplified model to understand how a cell gets committed to different stages of the cell cycle. For simplicity, we will consider S-G2-M as a single phase. Between G1 and S, the cell commits to cell division by entering the S-G2-M phase. We can call that as the start point. Once in the M phase, the cell takes another crucial decision- to segregate daughter chromosomes and divide the cell. This event happens between the metaphase and anaphase of mitosis. We will call this the endpoint.

This cyclic transition through G1–S–G2–M is tightly controlled by intricate molecular signaling. In Eukaryotes, three critical molecules involved in cell cycle regulation are- Cyclins, Cyclin-dependent kinases (Cdks), and Anaphase-promoting Complex (APC).

There are several cyclins and their cognate Cdks. Binding of a cyclin to its Cdk activates the Cdk. In the present model, we consider a single cyclin-Cdk complex (C) that is enzymatically active. The active cyclin-Cdk complex phosphorylates proteins that regulate DNA replication and chromosome condensation. Therefore, the high activity of the cyclin-Cdk complex is required to cross the start point and commit to the S-G2-M phase.

The other key player in this story is APC, which is a ubiquitin ligase. APC tags target proteins for proteasomal degradation. Several cell cycle molecules, including cyclins in cyclin-Cdk complexes, are targets of APC. APC and cyclin-Cdk complex have a mutually antagonizing relation. APC deactivates cyclin-Cdk complex by tagging the cyclin for

degradation, whereas active cyclin-Cdk complex inactivates APC through phosphorylation of a component of this complex.

In our model, A represents active APC. The formation of active APC is regulated by different molecules, including Cdc14 a phosphatase. Cdc14 is, in turn, regulated by cyclins and cell size. To reduce the clutter, in our model, we have considered p that represents all processes and molecules that promote the formation of active APC.

The following system of ODEs is our simplified model for cell cycle regulation.

$$\frac{dC}{dt} = \beta - (J_1 + A)C \tag{7}$$

$$\frac{dA}{dt} = \frac{p(1-A)}{J_2 + (1-A)} - \frac{AC}{J_3 + A} \tag{8}$$

C is formed at a constant rate, and its degradation is regulated by A. The production of A is regulated by p and C regulates the degradation of A.

The bifurcation diagram for this system is shown in Figure 7. p is the bifurcation parameter. As C and A are mutually antagonizing, they have opposite behaviors in the bifurcation diagrams (Compare Figure 7a and b).

This system has two saddle-node bifurcation points S_1 and S_2. For $p < p_1$, the system has only one steady state, with very high C and very low A. This steady state is stable. Similarly, for $p > p_2$, the system has one stable steady state, with very low C and very high A. However, between p_1 and p_2 the system has two stable steady states separated by one unstable steady state. This region is called the bistable region.

In G1 phase, p is high (say 0.4), and the steady state concentration of C is very low. For the same p, A remains at a higher stable, steady state. High A and low C maintains the cell in G1 phase. As the cell grows, p drops. As long as $p > p_2$, the cell stays at a stable steady state with low C and high A.

Further decrease in p will take the system into the bistable zone. However, as the cell is already in a stable steady state with low C and high A, it will continue with the lower steady state.

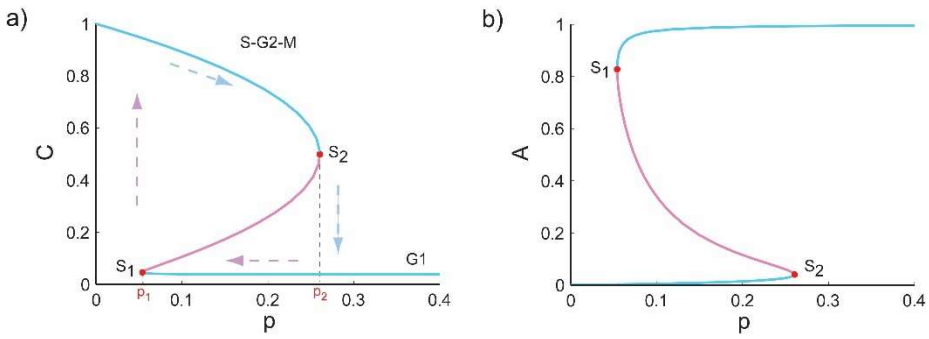

Figure 7: Bistability and hysteresis in the cell cycle model. Blue and pink lines represent stable and unstable steady states, respectively. S1 and S2 are two saddle-node bifurcation points. Dashed arrows show the movement of the cell from one steady state regime to another. Parameter values considered for the bifurcation analysis are: $J_1 = J_2 = J_3 = \beta = 0.04$.

However, there will be a drastic switching once p gets lower than p_1. For $p < p_1$, there is only one stable steady state, and the cell has to jump to that one. At this steady state, C is very high, and A is very low. This will force the cell to enter into S-G2-M phase.

As the cell moves through S-G2-M phase, p starts dropping. Once in the bistable zone, two stable steady states are possible. However, as the cell is currently at the higher stable steady state, it will follow the higher stable steady state as long as $p < p_2$.

Beyond the bifurcation point S2, the cell will be in the monostable zone and jump to the steady state with very low C and very high A. This will trigger the cell to go into the G1 phase.

The dashed arrows in Figure 7a show the journey of a cell through different steady states. When p decreases from a high initial value (say from p = 0.4) it follows the path shown by the pink dashed arrows. However, while p increases from a low value (say from p = 0) the cell follows the path marked by blue dashed arrows. That means the paths of onward and backward journeys are different. This behavior is called Hysteresis.

In general, the path followed by a system depends upon its initial position on the phase space. If there are more than one steady states, the system will move to one of those following the trajectories in the phase space. If there are more than one steady states, a system with hysteresis will move to a new one depending upon the current steady state. In the bistable region in Figure 7a, the cell will move to the lower steady state if it is already in the lower one.

In that sense, the system with hysteresis remembers the past. That is why this type of behavior in a cellular system is called cellular memory. Hysteresis is crucial for successful completion of cell cycle. Once the cell has committed to a particular cell cycle phase, it will be in that phase unless there is considerable change in the control parameter. Imagine that we have treated a cell with a growth factor. That caused a rapid decrease in p and the cell moved to S-G2-M phase. Even if the signal by the growth factor gets decayed, the cell will complete the S-G2-M phase till the p remain lower than p_2. Irrespective of the status of the external cue the cell remains committed to the process it started. Therefore, hysteresis helps the cell to maintain the function even when there are environmental and cellular fluctuations.

Bifurcation and cellular heterogeneity

Gene expression in a cell is tightly regulated. Transcription factors are the key regulators of gene expression. Transcription factors often control each other expression. Using genetic engineering, one can create a new transcriptional circuit and investigate the dynamics of gene regulatory networks.

Figure 8 shows the architecture of a simple synthetic transcriptional circuit made of two transcription factors L and T. Suppose, L is fused to a fluorescence protein like GFP so that we can measure the level of expression of L by flow cytometry or imaging. The promoter regions of these two genes are designed in such a way that these two transcription factors repress each other's expression. Activities of both the transcription factors can be regulated by small molecule inhibitors I and S. You can image L as *lacI* and I as IPTG. We can easily regulate the doses of I and S in an experiment.

The system of ODEs in Equations 9-10, represents this mutual repressor circuit.

$$\frac{dL}{dt} = \frac{a}{1+\left(\frac{T}{S}\right)^2} - \beta L \tag{9}$$

$$\frac{dT}{dt} = \frac{a}{1+\left(\frac{L}{I}\right)^2} - \beta T \tag{10}$$

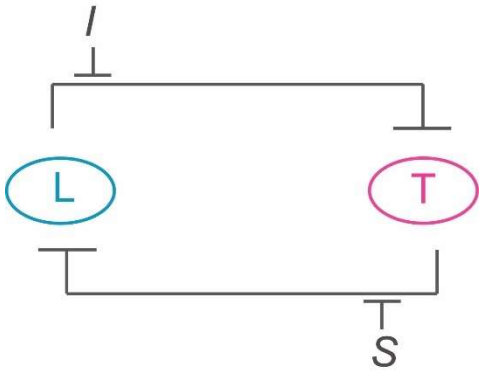

Figure 8: A mutually repressing transcriptional circuit. L and T are transcription factors. L is fused to a fluorescence protein. I and S are small molecule inhibitors of L and T, respectively.

Bifurcation analysis of this system of ODEs shows that this system has saddle-node bifurcation. The bifurcation diagram for L is shown in Figure 9a. S_1 and S_2 are two saddle-node bifurcation points. S is the bifurcation parameter. When the inhibitor S is low, T remains high, and that leads to a monostable steady state with low expression of L. For a very high dose of S, the system is monostable with high expression of L. However, between S_1 and S_2, the system is bistable.

Imagine we have a population of bacterial cells having this transcriptional circuit, and these cells are treated with a very low dose of S. We wait for the system to reach the steady state and then analyze these cells by flow cytometry. At a low dose of S, the expression of L will be very low.

However, like many other processes in a cell, gene expression is stochastic and shows random fluctuation. Even when the gene expression is tightly regulated, all cells in a sample can not have precisely the same level of expression. Further, in a culture flask, all cells will not receive an equal amount of S. Due to such random variations, the level of expression of L will vary among the cells. Therefore, in flow cytometry experiment, we will get a distribution for L, as shown by the histograms in Figure 9b.

For a high dose of S, we will again get a distribution for L, but the mean of that distribution will be high (Bottom histogram in Figure 9b). An interesting behavior will be observed if we use a moderate dose of S near the bifurcation point S_1. At this position, the system is near the junction of bistable-monostable states. Due to noise in gene expression and random variation in S, some of the cells will reach the higher steady state, whereas the rest will stay at the lower steady state.

In this situation, we will observe two subpopulations in the flow cytometry experiment - one with a lower expression of L and the other with higher expression. The frequency distribution of L will have two modes, as shown in the middle panel of Figure 9b. A frequency distribution with two modes is called bimodal. Therefore, gene expression with two subpopulations having high and low expression is called a bimodal expression.

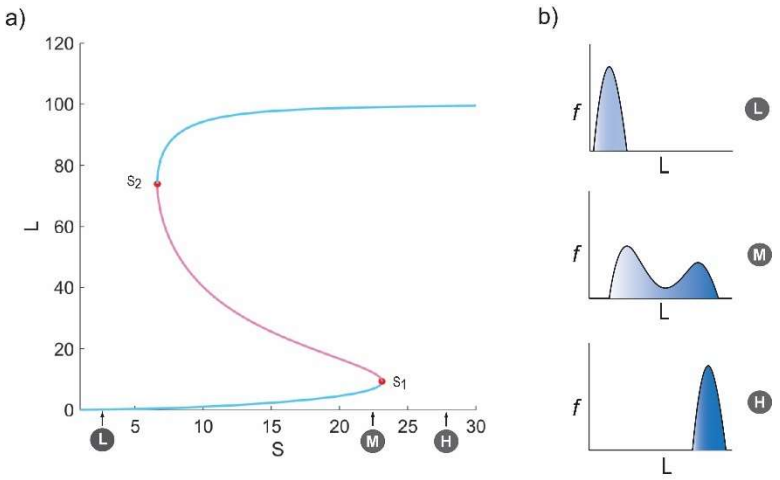

Figure 9: Bistability and bimodal gene expression. a) Bifurcation diagram for L. Blue and pink lines are stable and unstable steady states, respectively. S_1 and S_2 are two bifurcation points. L, M, and H, written below the horizontal axis, are low, moderate, and high doses of S, respectively.

Bimodal gene expression due to bistability has been observed in several synthetic genetic circuits as well in may wildtype or natural cellular systems.

We can even observe hysteresis in such bistable systems. Imagine for the genetic circuit in Figure 8, initially, we first treat the cells with a low dose of S, say S = 1. The expression of L would be around the lower stable steady state. We add some more of S in the media so that total S = 15. Even though the cells are in the bistable region, the expression of L will be around the lower stable steady state at that S. Add some more of S to the media, to increase S to 30. That will push all the cells to jump to the higher stable steady state. Imagine that we measure the expression of L by flow cytometry. Hypothetical data of such an experiment is shown in Figure 10.

Now dilute/change the culture media to reduce the concentration of S to 15 again. All though we are in the bistable zone, all the cells will stay on the higher stable steady state. As we reduce S further to 1, all the cells will jump to the lower stable steady state. The

difference in the paths followed by cells with change in S, can be easily detected in the flow cytometry data (Figure 10).

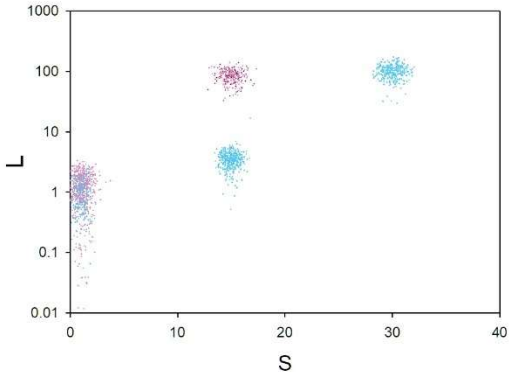

Figure 10: Hypothetical result of a flow cytometry experiment for the genetic circuit shown in Figure 8. Each dot represents one cell. The blue dots are for cells when S was increased stepwise from 1 to 30. The pink dots are for cells when S is reduced stepwise from 30 to 1. This data is generated by using random numbers and not by simulation of the model.

Bifurcation and cell fate determination

You must have noticed that in the example of the bimodal expression, we have combined the idea of random or stochastic variations in gene expression with bifurcation. Our mathematical model (Equations 9-10) is deterministic. Bifurcation is also a deterministic phenomenon. However, when combined with stochastic aspects of cellular processes, the same model can explain many surprising phenomena observed in biology.

In all multicellular organisms, diverse cell types originate from the differentiation of stem cells. Cellular differentiation is controlled by transcription factors and involves drastic changes in the pattern of gene expression of a cell. For example, in hematopoiesis, differentiation of progenitor cell into erythroid and myeloid lineages is controlled by a transcriptional switch involving transcription factors GATA1 and PU.1. High expression of GATA1 makes a cell committed towards the erythroid lineage, whereas PU.1 induces myeloid lineage commitment.

During differentiation, cells originating from the same progenitor cell diverge in different cell types. What could be the switching mechanism that differentiates the journey of two

cells that otherwise are the same? We will try to understand this switching using the concepts of bifurcation in a gene regulatory circuit and stochasticity in gene expression.

Here, we use a toy model involving two transcription factors – *x* and *y*. Suppose, higher expression of *x* triggers a cell to differentiate into E-type, and *y* induces differentiation into M-type. We assume that *x* and *y* repress each other and also autoregulate their expression through positive feedback (Figure 11). Such mutual repression with autoregulation is widespread in molecular circuits that control the differentiation of stem cells.

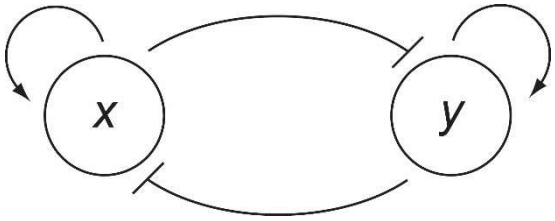

Figure 11: A transcriptional circuit with autoregulation and mutual repression. *x* and *y* are transcription factors.

We model this molecular circuit using the following system of ODEs,

$$\frac{dx}{dt} = \frac{ax^n}{K^n + x^n} + \frac{bK^n}{K^n + y^n} - k_d x \tag{11}$$

$$\frac{dy}{dt} = \frac{ay^n}{K^n + y^n} + \frac{bK^n}{K^n + x^n} - k_d y \tag{12}$$

Imagine, the parameter *b* represents a transcriptional control that could be regulated by an external signal. We investigate bifurcation in this system, considering b as the control parameter.

The bifurcation diagram for *x* is shown in Figure 12a. This system shows supercritical pitchfork bifurcation. The bifurcation point is at $b = 0.498$. When $b < 0.498$, the system has only one steady state with x = y. Beyond the bifurcation point, the system has two stable steady states and one unstable steady state. One of the stable steady states has a higher value for *x* than the other, and with an increase in *b*, these two steady states diverge further.

As the ODEs are symmetric, the bifurcation diagram of *y* is similar to the one for *x*, but when *x* is at the higher stable steady state, *y* remains at the lower one.

Imagine, we have two cells currently at a steady state just before the bifurcation point, with b = 0.45. An external signal increases b to 0.5. This increase in b changes the phase portrait, and the existing steady state is lost. Now the system has two new stable steady states. Our cells must move to one of these two. However, which of the two should they choose?

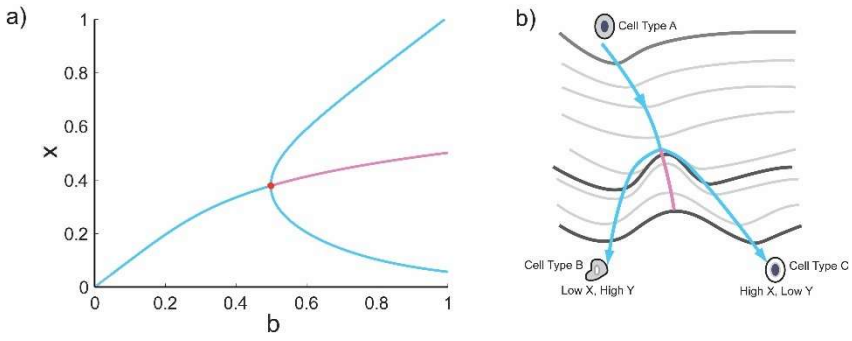

Figure 12: Bifurcation and cellular differentiation. a) The bifurcation diagram of x in the system of Equations 11-12. Blue lines are for stable steady states, and the pink line is for unstable steady state. Parameter values used for the bifurcation analysis are: a = 0.01, n = 4, K = 0.5 and k_d = 1. b) A potential landscape model for differentiation. The surface with grey colored lines represents the potential of the system as it evolves with time. A minimum of this landscape is a stable steady state. As the system moves from one stable steady state to another, there are canals/gorges in the landscape. The bifurcation diagram of x follows those canals. The blue lines of the bifurcation diagram are in the canals, whereas the pink line is on a hill. The arrows represent the direction of time.

Here comes the role of stochastic variation in gene expression. Though both the cells were at the same steady state, they may not have the identical amount of x and y. Even if they have little variations in gene expression, their positions on the phase plane would be different.

Near the bifurcation point with b = 0.5, such difference in position on the phase plain may force them to follow different trajectories and lead them to different stable steady states. Therefore, one cell may reach the steady state with higher x (and lower y), while the other may reach the lower stable steady state with a lower value of x (and higher y).

With further increase in b, the cell that is at the higher steady state for x would follow the same path in the bifurcation diagram, and x will increase further. For the other cell, an increase in b would lead to a further decrease in x. When b reaches 1, there will be two cells, though originally identical, having opposing levels of x and y. One cell will have high

x and low *y*. This cell will differentiate into an E-type cell. The other one will have very low *x*, but very high of *y*. This cell will differentiate into an M-type cell.

A fascinating way to represent cellular differentiation through bifurcation is to use the concept of potential landscape. Imagine the system under investigation has a potential (or pseudopotential) landscape. A stable steady state can be considered as a local or global minimum in that landscape. Therefore, when a cell moves from one stable steady state to another, we can imagine as if it is moving through a canal/gorge on the landscape with hills, valleys, and gorges (*Figure 12*b). At a bifurcation point, the canal or gorge bifurcates. The cell follows one of the paths. The stochastic fluctuation in molecular processes determines the choice between the two paths. Eventually, the cell moving through a particular canal differentiate into a particular cell type.

Though fascinating, generating such a potential landscape for a nonlinear system is not trivial. Even then, it is an excellent metaphor and equivalent to a landscape metaphor proposed by C. H. Waddington in 1957. Waddington is considered as one of the founding fathers of Systems Biology. To understand the directionality and divergences in embryonic development, he proposed a landscape metaphor. Waddington imagined that like a rolling ball, a cell rolls over a landscape with hills and gorges and eventually differentiates into a particular cell type based on the path it followed. He called the landscape as the Epigenetic Landscape (not to be confused with what we now called epigenetics). The landscape model shown in Figure 12b is as close as we can get to Waddington's metaphor.

Exercises

1. Draw the bifurcation diagram for $\frac{dx}{dt} = m - x^2$.

2. Which of the following statements is WRONG?
   a) Bistability in the expression of a molecule can give rise to bimodal cell population.
   b) Change in the numerical value of the steady state due to change in the value of a parameter in a system of ODEs is called bifurcation.
   c) Change in the stability of the steady states due to change in the value of a parameter in a system of ODEs is called bifurcation.
   d) Change in the number of possible steady states due to change in the value of a parameter in a system of ODEs is called bifurcation.

3. For the following system of ODEs draw the phase plane plot with nullclines. Identify the steady state in this plot and mark it on the plot. Will this system show bifurcation if the values of α or β are varied? Explain your answer using the phase plot. Consider $x \geq 0$ and $y \geq 0$.

$$\frac{dx}{dt} = \frac{\alpha}{1+y} - x$$
$$\frac{dy}{dt} = \frac{\beta}{1+x} - y$$

4. For the following system of ODEs, draw the bifurcation diagram for *y*.

$$\frac{dx}{dt} = x(y-1) \qquad \frac{dy}{dt} = \mu - y(1+x)$$

5. The bifurcation diagrams shown in this chapter are created using MatCont, a numerical bifurcation analysis toolbox for Matlab. You can use other free tools like AUTO-07p, XPPAUT, DDE-BIFTOOL for bifurcation analysis. Use any of these tools to perform bifurcation analysis for the following system:

$$\frac{dx}{dt} = y - a\frac{x^2}{1+x^2}$$

$$\frac{dy}{dt} = bx - y$$

here, $x \geq 0$, $y \geq 0$, $a > 0$, and $b > 0$