Chapter Two

Bistability of Genetic Circuits

2.1 OVERVIEW

P.W. Anderson famously remarked "more is different" (Anderson 1972). This quote serves as an important pre-amble to the material in this chapter. The inner workings of cells represents the central occupation of the biological sciences. This is not meant to valorize nor indict that state of affairs. It is what it is. Moreover, cellular life is arguably the building block of all life, or as some might call it "the basic unit" of life. But in exploring cells, there is a temptation: to continue to reduce the cell into constituent parts until one reaches molecules. There are many molecules inside a cell: nucleotides, proteins, metabolites, lipids, sugars, and water. And why stop with molecules? Why not go all the way to atoms or even quarks? Although the laws of chemistry and biology must be consistent with those of the fundamental laws of physics, this does not mean that we will glean insights from an endless reduction *ad absurdio*. Instead, understanding how cells work should begin at the appropriate scale and with a focus on the appropriate processes – that of gene regulation.

Indeed, molecular and cellular biology does have its own organizing 'law', what is called the central dogma, i.e., that information encoded in DNA is transcribed into a message, i.e., RNA, which is then translated into proteins that carry out the basic functions of cellular life. There are many such functions. (see Figure 2.1). Cells can divide, grow, search for food, deal with stresses and shocks, enter periods of stasis, emerge from quiescence, incorporate and release small molecules, survive (and even 'learn') from interactions with viruses and other mobile genetic elements. Indeed, taking just one of this long list, or an even longer list, of functions in the context of a particular microbe or cell type in a multi-cellular organisms often constitutes the basis for a long, and hopefully interesting, career. But the longer the list becomes the more evident it should also become the these functions represent "potential" functions of a cell. A cell does not do each of these functions all at once. The strange cases of 'teratomas', provide the exception that prove this rule. Teratomas are typically benign; they are tumor cells with multiple kinds of differentiated tissues organized together, e.g., a tooth mixed with brain matter located in an unrelated organ. This sounds somewhat terrifying, and also provides context for the incredible coordination that must take place to ensure that differentiation unfolds during development, both in time and space. Yet the problems of development are, for now, a few steps ahead.

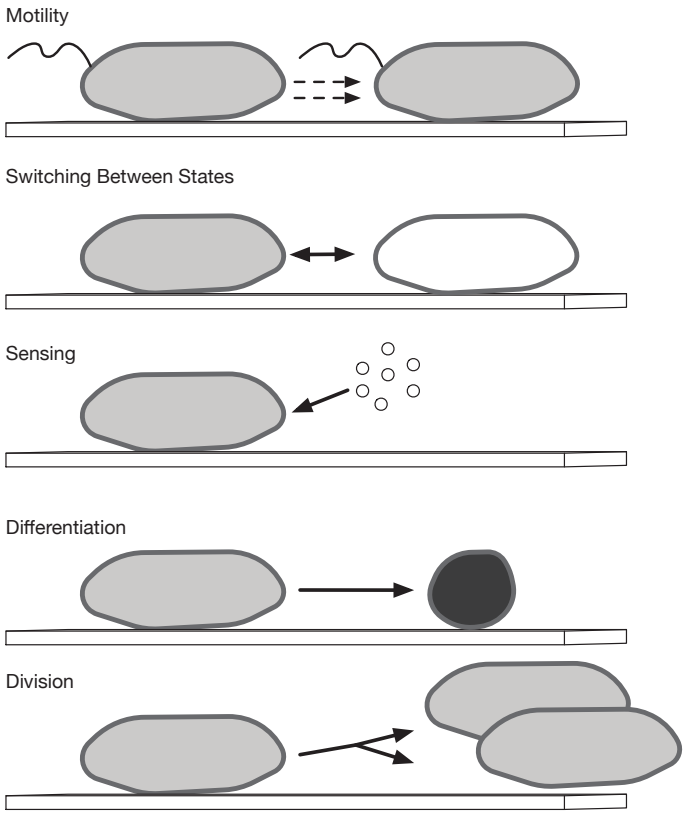


Figure 2.1: A partial view of the many things that cells can do, but not all at the same time - including motility, switching between states, sensing extracellular signals, differentiation, and division from one to two cells.

Here we will approach the problem of how cells do some, but not all, things at once through the lens of bacterial gene regulation. In doing so, it is sensible to follow Anderson's advice and introduce a limited cast of players. In doing so, the text will follow the path laid down by many before to explain ways in which certain genes can turn given the right signals or circumstances. Yet, the other, perhaps more important, point to be made is that the overall behavior of the cell cannot be decomposed into these 'units' of individual elements. Instead, when different molecular cast members start talking to one another, the regulation of individual components can lead to entirely new emergent phenomena. A key emergent phenomena is that of bistability, the notion that cells, given the same environmental cues, can maintain distinct types of cellular states. Having such a property could be quite handy, not only to avoid teratomas, but also to develop from one cell into many, and even for populations of cells to subdivide tasks or

perform a form of evolutionary 'bet hedging'.

Hence, this chapter will provide context to understand core concepts of gene regulation, while moving quickly through many important concepts in molecular and cellular biology. Hopefully the intuition you build will inspire you to go back and fill in the gaps. In doing so, we will be motivated by a relatively simple question: how can a cell exhibit bistability, with distinct phenotypes that have a memory of prior stimuli? These phenotypes include dormancy or division, susceptibility or resistance to antibiotics, able to take up DNA (i.e., competency) or not, motile or non-motile, etc. These are but a few examples of bistability in cellular and indeed bistability extends even to viruses. For example, bacteriophage lambda can infect bacteria of the species *E. coli* and, in some instances, the viral genome is integrated into that of the host. This integration is relatively stable, in part, because of a gene regulatory circuit that represses the genes in the phage genome that would lead to excision from the host and initiation of lysis. Yet such systems – however interesting they might be (Ptashne 2004) – do not necessarily contain a minimal set of ingredients to understand the dynamical origins of bistability. For that, we will turn to the breakthrough work on a genetic toggle switch by Gardner and colleagues, working in the lab of Jim Collins (Gardner et al. 2000a). In doing so, we will also adopt many of the approaches to connecting mechanisms, models, and observations pioneered by Uri Alon and colleagues (and codified in an excellent book on systems biology (Alon 2006)).

The idea that one could rationally design a modular component to be inserted into population of cells seems obvious, in retrospect. Many students have either participated in or at least heard about iGEM, the international genetically engineered machines competition. iGEM has its roots in a 2003 course at MIT (Shetty et al. 2008; Canton et al. 2008; Smolke 2009). The basic premise is that it should be possible for undergraduates – initially those at MIT, but soon worldwide – to design, build, and implement "devices" made of modular building blocks to enable new or altered cellular functions. So easy anyone could do it... even a high school student. This vision is now a reality, at least in part. In practice, there are certain labs and companies whose expertise relies on combining a rational approach to developing parts that work and then, connecting those parts together to achieve a target outcome. As we will see, rationally designing a bistable genetic circuit requires more than just understanding the parts list; it requires understanding feedback of a nonlinear dynamical system. How then does one design a genetic toggle switch based on the principle that two genes can mutually inhibit each other? And even if such ideas are possible, in theory, will they lead to the phenomena that a stimulus that turns one of the genes On (and the other Off) can also lead to cellular memory, i.e., the On gene stays On even when the stimulus is removed (see Figure 2.2)? This is our challenge. And, in focusing on a synthetic switch, this chapter also enables us to move from the lessons of Anderson to that of Feynman who famously cautioned: "what I cannot create, I do not understand".

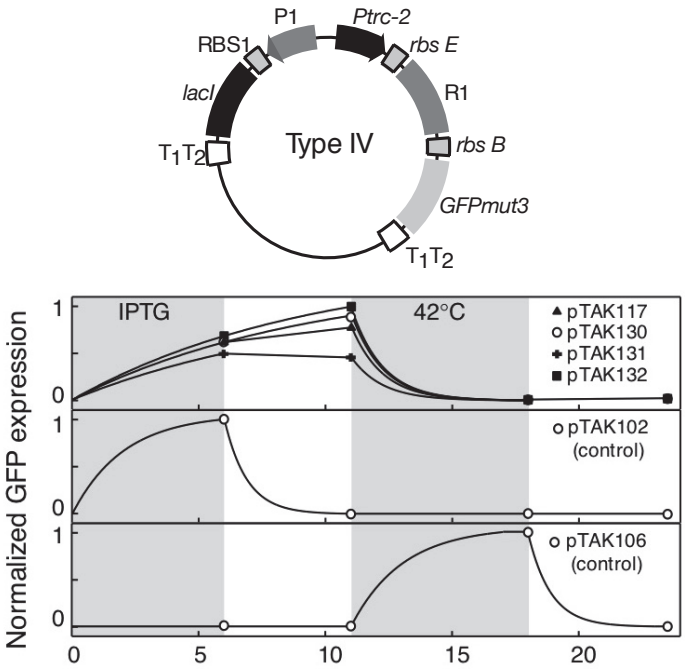


Figure 2.2: Memory and bistability in a toggle switch. (Left) Engineered toggle switch enable bistability in *E. coli*. (Top) The genetic structure of the toggle switch on a plasmid, including mutually repressing genes LacI and R1, which bind and inhbit expression at the promoters *Ptrc-2* and *P1*, respectively. (Bottom) Desmontration of memory and bistability. The key experimental observation is that an inducer 'IPTG' was added and then removed, yet the behavior of the genetic circuit continued unabated (as seen in the increased in the normalized GFP expression). The expression decreased when a new, temperature perturbation was made, denoted by the shaded region 42°. This chapter will explain how and why these simple experiments reveal both memory and bistability as characteristics of genetic circuits and not just the genes themselves. *Figures reproduced from (Gardner et al. 2000b).*

2.2 MOLECULAR CAST AND SCENE

Cells are complex. They involve many components, and we'll begin with a parts list. But a cell cannot be understood simply by counting these parts. Interactions matter, and feedback between components can give rise to new properties of the system that are not embedded in the "local" interactions. This concept of emergence, or "more is different" permeates much of this book. Moreover, given the realized complexity of cells, we will focus on a few components of the cellular landscape that form the essential components of a genetic toggle switch. Now, to the play... beginning with the cast.

The Cast (see Figure 2.3)

- Gene: sequence of nucleotides which encodes a protein.
- Promoter: upstream sequence which allows gene transcription and defines, in part, regulatory relationships.
- Protein: macromolecule that enables the structure, function and regulation of cells.
- RNA polymerase: enzyme that transcribes mRNA.
- Transcription factor (TF): proteins that bind to promoters and modify transcription of target genes.
- Activators: TFs that increase transcription of a target gene.
- Repressors: TFs that decrease transcription of a target gene.
- Inducers (signals): small molecules that modify binding ability of regulatory proteins.

The basic summary of cellular roles helps to think about variations, exceptions, and ways in which the intrinsic dynamics govern evolved behavior or can be influenced or controlled for some engineered outcome. To do so, we will focus on bacterial gene expression. For those familiar with the topic, I hope that the following sections function like a Shakespearean play, sure you know what happens in the end, but it's good enough to sit, watch, and listen. Hence, take a moment to admire a few billion years of evolution's handiwork.

For bacteria, genes are (largely) continuous sequences of DNA, about 1000 base pairs long. These sequences have a start and stop. This information, stored in DNA, is transcribed into a messenger RNA by an enzyme, RNA polymerase. The process is termed transcription. For physicists, mathematicians, and others not versed in the life sciences, it may sometimes seem that terms in biology are invoked out a sense of whim, whimsy, or perhaps even negligence. (And, before casting stones, it may be worthwhile to consider the logic underlying the fanciful description of quarks – though doing so seemed to have prudent

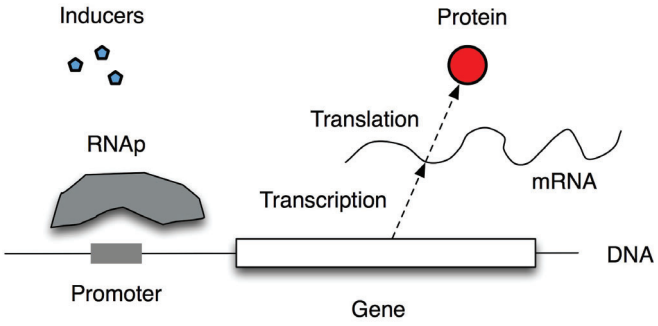


Figure 2.3: The molecular cast, a preamble to understanding the basics of gene expression dynamics and transcriptional networks.

for selling the public on the concepts.) Yet, the term transcription is apt. To transcribe is to copy, faithfully. Here, DNA, and its molecular alphabet of A-s, T-s, C-s, and G-s, is copied into RNA, and its molecular alphabet of A-s, U-s, C-s, and G-s. The swap of T for U reflects the chemical change of the molecule Thymine (T) vs. Uracil (U). In essence, DNA and RNA are written in the same language. The message encoded in RNA is then "translated" into protein at the ribosome. Again, the term is apt. Proteins are comprised of a linear chain of amino acids. There are 20 amino acids. Information is translated from the 4-letter alphabet of DNA/RNA into the 20-letter alphabet of amino acids. Three DNA or RNA letters encode one amino acid. Given the $64 = 4^3$ possibilities of combining three DNA/RNA letters, it is evident that multiple "codons" encode for the same amino acid. In addition, the start and stop of such amino acids are signified by distinct "start" and "stop" codons – a different combination of 3 DNA/RNA letters. This constitutes the central dogma, that information stored in DNA is transmitted via a RNA message into a protein which enable cellular function.

But it turns out that sequence is not enough. Instead, for 70 or more years, the science of gene regulation has laid out principles and mechanisms by which what happens at one gene affects the behavior of another genes, and possible many others. These principles are dynamic, and they are what we turn to next.

2.3 THE FIRST INGREDIENT: REGULATION OF A TARGET GENE

Consider a gene X that regulates a gene Y. This phrase – X regulates Y – means that a protein produced via the translation of the mRNA transcribed from gene X is a transcription factor, and that this transcription factor – typically made active given binding to a small molecular "inducer" – binds to a site upstream

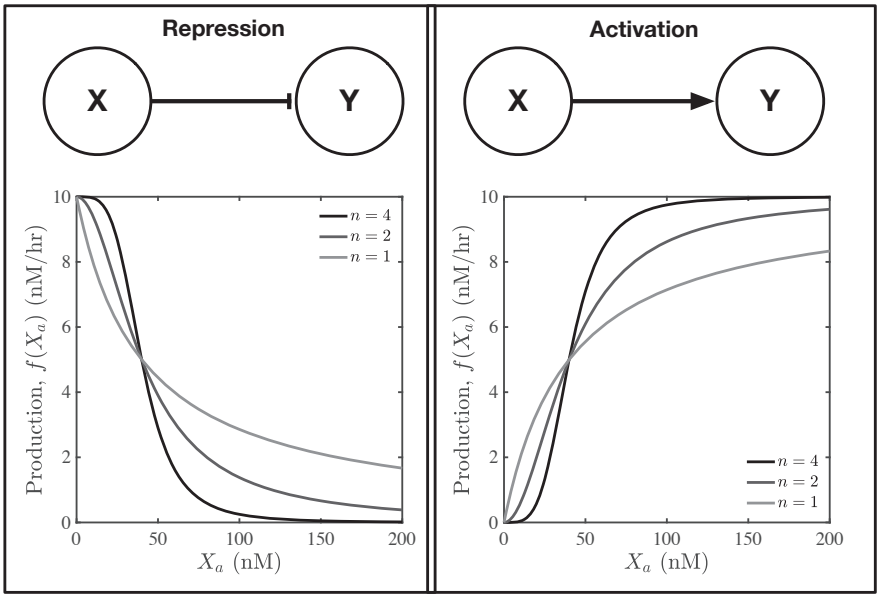


Figure 2.4: Schematics of targeted regulation of Y by X, whether via repression or activation. In both examples, X is a transcription factor that modulates the expression of another gene. The functional form can vary, as seen in the bottom panel in which $\beta = 10$ nM/hr, $K = 40$ nM and $n = 1, 2$, and 4. The functions for the repressor and activator are found in Eq. (2.3) and Eq. (2.2), respectively.

of the promoter of gene Y modulating the rate of RNA polymerase activity of gene Y. That's a mouthful. Instead, let's say stick with the phrase: gene X regulates gene Y. The gene X is called an activator if its binding increases the gene expression of Y and is called a repressor if its binding decreases the gene expression of Y (see left panels of Figure 2.4). For now, we will focus on the concentration of proteins and ignore many of the other processes. These processes range from very fast (like binding of signals to transcription factors and the binding of active transcription factors to DNA) to very slow (like the global evolution of transcriptional modification). For reference, Box **??** provides a sense of scale typical of bacterial gene regulation when division rates are on the order of 30 minutes-1 hr:

Time Scales in the Regulation and Evolution of Bacteria

Bacteria are diverse. Hence, any discussion of time scales must be treated with some caution, what is true for *E. coli* growing in rich medium in the laboratory need not apply precisely to the life of a marine cyanobacterium growing in the surface ocean with scarce nutrients or even to a soil microbe that faces severe variation in environmental conditions conducive to growth vs. dormancy. Nonetheless, the following provide some estimates of time scales of fast, slow, and very slow processes for bacteria about the size of *E. coli* growing in rich medium.

Fast

- Binding of a signal to a transcription factor (1 msec)
- Binding of active transcription factor to DNA (1 sec)

Slow

- Transcription and translation of a gene (5 mins)
- Turnover (50% change) of a protein (1 hr)

Slower still

- Evolution modification of promoter (w/fixation) (days - ???)
- Evolutionary modification of TFs and genes (w/fixation) (days - ???)
- Global evolution of transcriptional regulation (??? - Myrs)

The basic processes by which a gene X regulates a target gene Y can be viewed as a dynamical system. A dynamical system is one in which the state of the system, e.g., the intracellular concentration of proteins, changes with time as a result of feedback and interactions with other components. Here, the dynamical system is:

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \overbrace{f(x)}^{\text{production}} - \overbrace{\alpha y}^{\text{degradation}} \tag{2.1}$$

The production term depends on binding of X upstream of the promoter of Y. The production term depends on the degree of cooperativity of binding, e.g., if X is an activator, then:

$$f(x) = \frac{\beta x^n}{K^n + x^n} \tag{2.2}$$

whereas if X is a repressor then

$$f(x) = \frac{\beta}{1 + x^n/K^n}, \tag{2.3}$$

with examples shown in the bottom panels of Figure 2.4. These Hill functions are often invoked in models of living systems, but where do they come from?

The choice of saturating and declining response functions are biophysically motivated. Sigmoidal activation and repression functions emerge naturally from "cooperative" binding of transcription factors to promoters, where the exponent n denotes the effective degree of cooperativity in the system. As n increases, the system responds every more sharply to changes in x below and above the transition threshold, K (a molecular concentration). In biological terms, one can think of n as indicating the polymer-state of the transcription factor, e.g., $n = 1$ corresponds to monomers, $n = 2$ to dimers, $n = 4$ to tetramers and so on. Hence, as n increases, the change from a gene that is 'On' to one that is 'Off' (and vice versa) becomes ever more stark, and starts to resemble a Boolean logic gate, either On or Off. The detailed derivation of how to go from the molecular cast of players to these sigmoidal functions is described in the technical appendices.

There is one other note required before approaching the question of how to analyze a dynamical system comprised of one gene regulating another gene. Typically, the activity of proteins is controlled by signals, e.g., IPTG and other 'inducers'. These small molecules can bind to the protein changing their activity, enabling them or disabling them from binding to a promoter region and regulating a target gene. The subsequent analysis presumes that the signal is present, but changes in signal presence can also be denoted by modulating the effective level of x. How do we analyze such a mathematical model of a dynamical system of the kind shown in Eq. (2.1)? For those with experience in the study of dynamical systems then this one may appear simple, nonetheless, the models will build in complexity in this chapter. As such, it's good to have a good plan. Here is a good plan:

First, find the steady states.

Then, determine the stability of steady states.

Finally, analyze the dynamics of the system

The following seciont unpack each of these concepts, and the interested reader is encouraged to refer to the Technical Appendices and even full-length treatments of dynamical systems more more details (e.g. (Strogatz 1994; Hastings 1997).

2.3.1 Finding steady states

Consider a cell with a certain amount of protein, but with no inducer. Hence the active concentration begins at $x = 0$. Adding an inducer rapidly changes the feedback properties of the protein, so that they can turn on gene Y. This

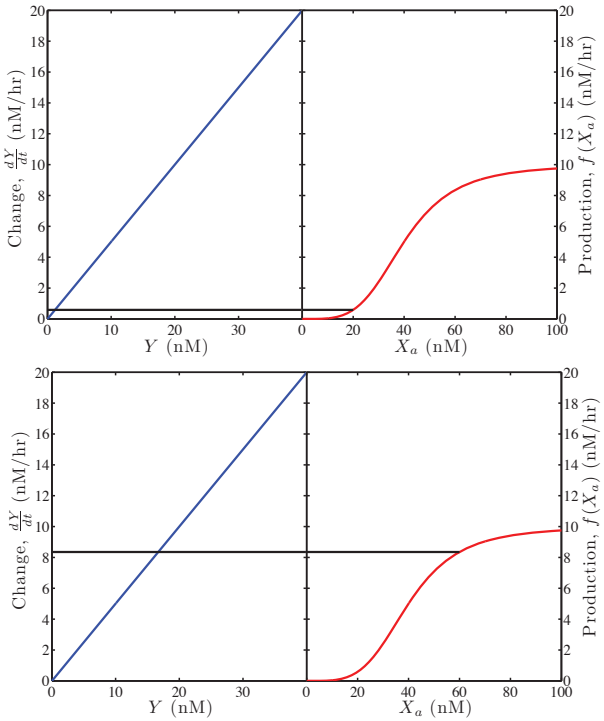


Figure 2.5: Equilibrium of a simple gene regulatory circuit occurs when degradation balances production. In both panels, degradation (left) is compared to production (right). Note that degradation depends only on y whereas production depends on x. The two situations contrast different values of input transcription factor, x, from 20 nM (top) to 60 nM (bottom). The resulting output in y^* increases 14 fold, a strongly *nonlinear* response given the 3-fold change in input.

will then change the concentration of the proteins of Y. Eventually, in theory at least, the concentration of y will reach a "steady state". In the present context the term steady state denotes the fact that there is a particular value $y = y^*$ such that the concentration does not change. In other words, $\frac{\mathrm{d}y}{\mathrm{d}t} = 0$. Note that in general, there can be other more complex kinds of steady states. But the present kind, what one may call an equilibrium, will suffice for now. The status of activation will become important later on in the discussion of the toggle switch.

For the activator, $\frac{\mathrm{d}y}{\mathrm{d}t} = 0$ of Eq. (2.1) occurs when

$$y^* = \frac{\beta x^n}{\alpha\left(K^n + x^n\right)}. \tag{2.4}$$

For example, if β = 10 nM/hr, α = 1 hr^{-1}, K = 40 nM, and n = 4, then y^* = 0.6 and 8.4 when x = 20 nM and 60 nM, respectively. Hence, there is an approximately 14-fold increase in output given a 3-fold change in input (see Figure 2.5). Similarly, for the repressor, $\frac{dy}{dt} = 0$ when

$$y^* = \frac{\beta}{\alpha\left(1 + x^n/K^n\right)}. \tag{2.5}$$

In this case, if β = 10 nM/hr, α = 1 hr^{-1}, K = 40 nM, and n = 4, then y^* = 9.4 and 1.6 when x = 20 nMand 60 nM, respectively. Hence, there is an approximately 6-fold decrease in output given a 3-fold change in input. These nonlinear input-output relationships are an example of one, modest consequence of cooperative binding, e.g., by dimers or tetramers rather than by monomers.

2.3.2 Stability of steady states

The identification of steady states is only part of the puzzle. Imagine that in the case of the activation of Y by X, that the system is close to, but not quite at y^*. If so, will it return, that is will the deviation, $|\epsilon_y| \equiv |y(t) - y^*|$ increase or decrease with time. This "local" change in behavior of the dynamical system defines its local stability. For most applications, equilibria can be classified as either unstable or stable. Occasionally they will be marginally (or neutrally) stable. An unstable fixed point is one in which the deviation grows in time. A stable fixed point is one in which the deviation diminishes in time. A marginal/neutrally stable fixed point is one in which the deviation neither grows nor diminishes.

There are multiple ways to characterize local stability, particularly for a one-dimensional system. In this case, given that s is constant, then $f(s)$ is also a constant. Hence, irrespective of whether or not X is an activator or a repressor, there will be more production than degradation when $y < y^*$ and more degradation than production when $y > y^*$. As a consequence, y will converge to y^* over time, i.e., the equilibrium is stable.

Another way to assess the stability of a dynamical system near its fixed point is to "linearize" the system in terms of its dynamic variables, in this case y. By linearing a system, what was previously an intractable problem can be reduced to a linear differential equation that can be solved completely (albeit insofar as the dynamics remain close to the fixed point). This method is powerful, despite its apparent limitations in scope. The Technical Appendices to this chapter reviews the procedure in detail for systems with 1 or 2 dynamic variables. Similar concepts underlie systems with arbitrary number of variables. The present system is already "linear" in y, which means that such an approach is better reserved for a different type of model. At present, given the monotonicity of either the activation or inhibition case, there can only be one equilibrium when X regulates Y and this equilibrium is stable. To see why, consider that x is

fixed, i.e., it is controlled separately. In that case,

$$\frac{\mathrm{d}y}{\mathrm{d}t} = C(x) - \alpha y \tag{2.6}$$

such that $y^* = C(x)/\alpha$. Denote $u = y - y^*$ as the deviation of the concentration of Y away from its equilibrium value, such that $y(t) = y^* + u(t)$. As a result, we can re-write the dynamics of the deviations, i.e.,:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = C(x) - \alpha y^* - \alpha u. \tag{2.7}$$

However, because $C(x) - \alpha y^* = 0$ at steady state this implies that the dynamics of the deviations are simply:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = -\alpha u. \tag{2.8}$$

This equation implies that small deviations exponentially decay back towards equilibrium with a time scale of $1/\alpha$. Analyzing the full dynamics of the system involves a bit more work.

2.3.3 Dynamics of a simple gene regulatory system

Thus far we have focused on the limited dynamics near a fixed point, but for a single gene regulatory system it is possible to explore further. The prior section provided evidence that a system initialized near $y = y^*$ will approach y^*. In fact the claim is more general – there is only a single value y^* where the system is equilibrium and so the dynamics will always approach the same cellular state irrespective of the initial prepared state, $y = y_0$. But even if the system eventually reaches this equilibrium, there is still a new question to ask: what is the characteristic time scale at which it will approach this equilibrium? In this case, the question can be answered "by hand", e.g., by rewriting this equation as

$$\frac{\mathrm{d}y}{C - \alpha y} = \mathrm{d}t \tag{2.9}$$

and then integrating by parts (details of which are found in the technical appendices). If the cell is initially prepared without protein (i.e., the signal is Off prior to being induced), then the solution is:

$$y(t) = \frac{C(x)}{\alpha}\left(1 - e^{-\alpha t}\right). \tag{2.10}$$

where $C(x)$ is a nonlinear function of the transcription factor concentration. The convergence of proteins to the equilibrium value for different values of x can be seen in Figure 2.6. However, such dynamics can get far more interesting when there is feedback between output and input. Indeed, the takeaway from this analysis is that for directional gene regulation (X regulates Y), then convergence to an equilibrium is typical, that such convergence can be nonlinearly related to input, but that such conclusions must be viewed as tentative when the output levels feeds back to modulate the input.

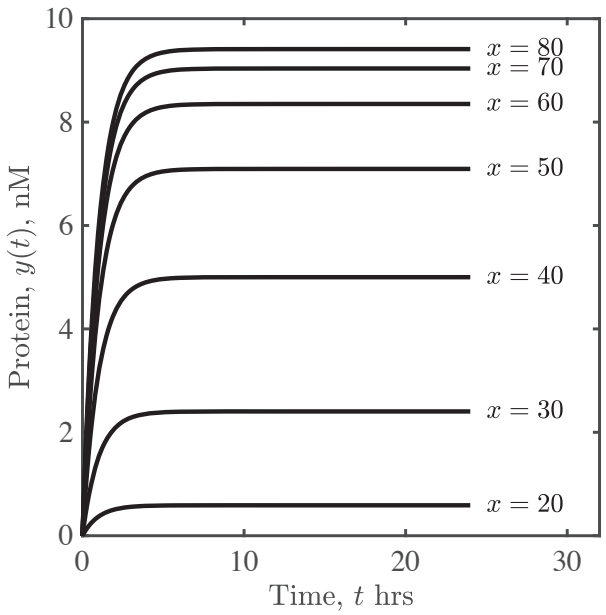


Figure 2.6: Dynamics given positive gene regulation for x = 20 to x = 80 via Eq. (2.1). The dynamics of $y(t)$ converge to y^* that is a nonlinear function of the input, x. In these series of plots the input value of x varies from 20 nM to 80 nM even as the output varies 15-fold.

2.4 FEEDBACK AND BISTABILITY – AUTOREGULATION

How can a cell exhibit bistable gene expression? Gardner et al. (Gardner et al. 2000b) provide one clue near the start of their article:

> Although bistability is theoretically possible with a single, autocatalytic promoter, it would be less robust and more difficult to tune experimentally.

This quote embodies the guidance that serves as one key organizing principle for the entire book, as suggested by Sydney Brenner – that the most important point is not whether a theory is right, but whether it applies to the real world (Brenner 1997). But, to get there, we will focus on the simpler case, in theory, given that a 'single, autocatalytic promoter' provides a more direct route to understand core concepts that will be preserved when shifting towards robust, and experimentally feasible designs – like the toggle switch.

Consider precisely such a promoter, in which a gene encodes for a transcription factor that positive regulates its own expression. If a cell has relatively low levels of gene expression then it will not express whereas it has relatively high levels of gene expression then it will express even more. The consequence

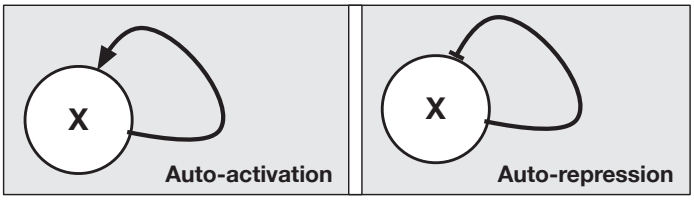


Figure 2.7: Schematic of an autocatalytic and an autoinhibitory loop, in which a gene positively (left) or negatively (right) regulates its own expression. In the autocatalytic loop, the arrow denotes that increasing levels of x increases its own expression. In the autoinhibitory loop, the perpendicular line head denotes that increasing levels of x decreases its own expression.

is that low states get lower and high states get higher, eventually diverging into alternative stable states. This section will explain and explore these mechanisms in more detail by contrasting cases in which a gene can self regulate positively ($X \to X$) negatively ($X \dashv X$) (see Figure 2.7). The former is termed an autocatalytic loop and the latter is an autoinhibitory loop – and is it turns out only the former can potentially exhibit bistability. Motivated by the prior section's plan for analyzing nonlinear dynamical systems, we will define the model, identify steady states, and classify stability. This analysis will form the basis for considering behavior of the toggle switch in the next section.

2.4.1 Negative autoregulation

Consider a self-inhibitory loop, $X \dashv X$:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\beta}{1 + (x/K)^n} - \alpha x \tag{2.11}$$

where, as before, β denotes the maximum production rate, K is a half-saturation constant, α is a decay rate, and n is the degree of cooperativity. In this case, the steady state of the system must satisfy

$$\frac{\beta}{1 + (x^*/K)^n} = \alpha x^*. \tag{2.12}$$

The term on the left hand side (lhs) is a monotonically decreasing function of x. In contrast, the term on the right hand side (rhs) is a monotonically increasing function of x. When $x = 0$ then production is β and degradation is 0. Hence production will steadily decrease from β and degradation will increase from 0. There can only be one crossing of these two curves. This crossing denotes the equilibrium (see left of Figure 2.8). For values of $x < x^*$, then concentration will increase and for values of $x > x^*$ then degradation will exceed production and concentrations will decrease. We expect that the equilibrium is both locally

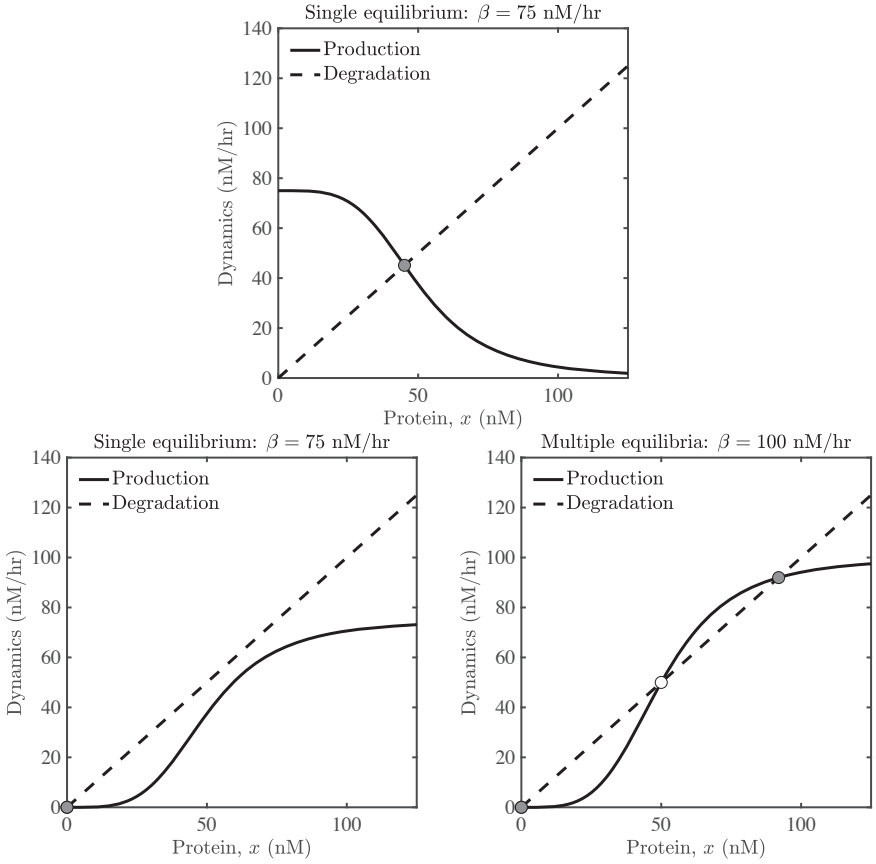


Figure 2.8: Comparison of degradation vs. production for both negative (top) and positive (bottom) feedback loops. For negative autoregulation, there is only a single stable equilibrium. In contrast, for positive autoregulation when $\beta = 75$ there is a single, stable equilibrium and when $\beta = 100$ there are two stable equilibria and one unstable equilibrium. Here $K = 50$ nM, $n = 4$, $\alpha = 1$ hrs^{-1}. In each case, the solid line denotes the corresponding autoregulatory feedback function (whether autocatalytic or autoinhibitory) and the dashed line denotes the net degradation, i.e., αx.

stable and, in fact, globally stable. That means that the system will converge to x^* no matter what its initial conditions The Technical Appendices provides extended details on how to assess the stability of 1- and 2D dynamical systems.

2.4.2 Positive autoregulation

Consider a self-catalytic loop, $X \to X$:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\beta x^n}{K^n + x^n} - \alpha x \tag{2.13}$$

where, as before, β denotes the maximum production rate, K is a half-saturation constant, α is a decay rate, and n is the degree of cooperativity. In this case, the steady state of the system, $x = x^*$ must satisfy

$$\frac{\beta x^n}{K^n + x^n} = \alpha x. \tag{2.14}$$

There are multiple possibilities for the number of times that the function on the lhs can intersect with the function on the rhs. Let's first consider the case when $n = 1$. In that case, then when $x \ll K$, then $\beta x/K = \alpha x$. In other words this is only true in the special case when $\beta/K = \alpha$. However for larger values of n, then the degradation rate will exceed that of the production rate for small values of x. The question then becomes is there a value at which production exceeds degradation? If so, then there is the possibility of two stable states: one low and one high. The dynamics of this system are shown in Figure 2.8 in which a change in the strength of the promoter when turned on leads to a qualitative switch in outcome, i.e., from a system with a single stable equilibrium in the Off state ($x^* = 0$) to one in which there are three equilibria – Off (x_{off}), On (x_{on}), and an intermediate unstable state (x_{mid}). The basis for the bistability is now apparent. When the strength of autocatalytic feedback is sufficiently high, then an On state continues to generate more protein, which ensures that the system stays On. In contrast, when the density of proteins drops below x_{mid}, then protein production drops (nonlinearly), causing x to drop even lower, leading to less production, and so on until the gene is in the Off state again. Hence, the outcome depends on initial conditions in a way that the system can retain a memory of its prepared, i.e., initial, state (see Figure 2.9). These results also suggest that changes in parameters of this autocatalytic loop can lead to qualitative changes in system dynamics – the next topic.

2.4.3 Bifurcation diagram

A bifurcation diagram is a term to denote the visualization of the states of a system which undergoes a qualitative change of state as a function of some parameter or set of parameters. For an autocatalytic loop that may be α, β, K or even n. A particular interesting feature of bifurcation diagrams is that they can reveal so-called 'critical points' at which a very small change in a parameter leads to a very large change in outcome. To explore this concept, let us make the simplifying assumption that $n >> 1$, such that the auto-catalytic production function looks like a "step function", with a sharp transition of production, from

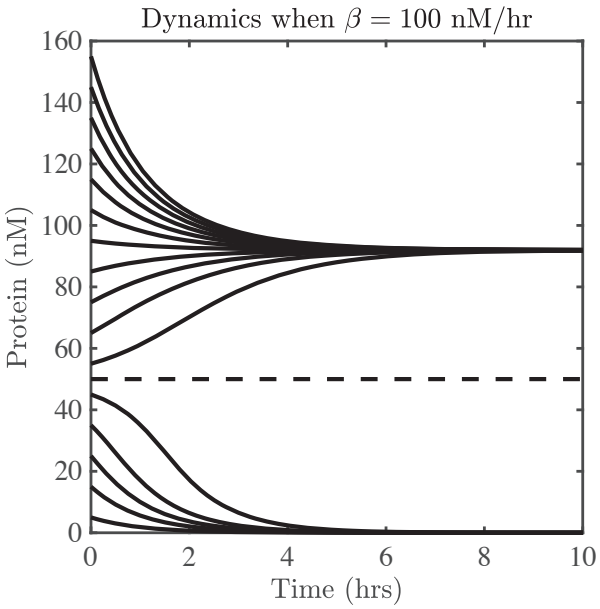


Figure 2.9: Alternative stable states in an autocatalytic gene regulatory loop. In this example, the dynamics correspond to simulations of Eq. (2.13) given positive feedback when $\beta = 100$ nM/hr, $K = 50$ nM, $n = 4$ and $\alpha = 1$. The initial conditions vary from near 0 to nearly 160 nM. Note that the system converges to either $x^* = 0$ (the Off state) or $x^* > 0$ (the On state) depending on whether or not the initial conditions are less than or greater than K, respectively.

0 when $x < K$ to β when $x \geq K$. Formally, we write this as:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \beta\Theta(x - K) - \alpha x \tag{2.15}$$

where Θ is the Heaviside step function which is 1 when its argument is non-negative and 0 when its argument is negative. Given this definition, it is apparent that there is always at least one fixed point, when $x = 0$. In that case, there is no production nor degradation because there is no transcription factor present to degrade. Because of the autocatalytic nature of the gene circuit, then no new production will take place. Note that when $x = K$, then the production is β. Hence, production will exceed degradation when $\beta > \alpha K$. If this condition is met, then instead of just one steady state, there will be three:

$$x^* = 0 \qquad \text{Off state, stable} \tag{2.16}$$

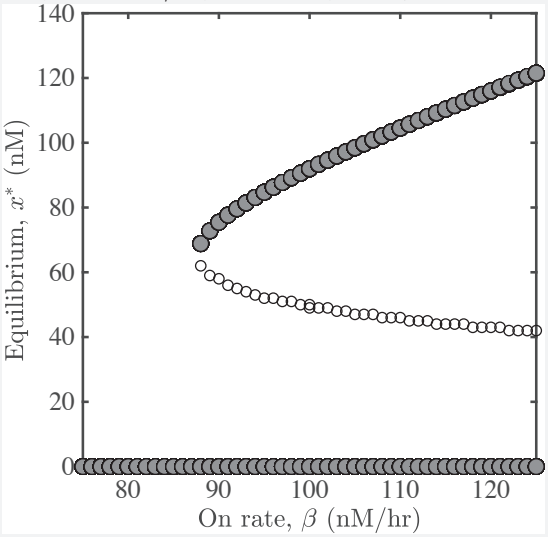
$$x^* = K \qquad \text{Borderline state, unstable} \tag{2.17}$$

$$x^* = \beta/\alpha \qquad \text{On state, stable} \tag{2.18}$$

The stability, denoted here, is shown graphically in Figure 2.9. The important point is that bistability is not inevitable given positive feedback. Instead, bistability depends also on the quantitative rates, i.e., $\beta/\alpha > K$, that is the concentration at the on-state must exceed the carrying capacity. This intuition generalizes to realistic cases with finite levels of cooperativity, i.e., when $n > 1$ (see Boxed text – Bifurcation Diagram in Depth). This intuition will also be useful as we move on to the circuit designed, implemented, and tested by Gardner and colleagues: the toggle switch!

Bifurcation Diagram in Depth

A bifurcation diagram denotes the relationship between output (e.g., steady state) and input (e.g., a driving parameter) in which there is a sudden change in the output as a function of the input. To begin, consider a positive gene regulatory feedback loop as described in the text characterized by a maximum production rate β, half-saturation constant K, degradation rate α and Hill coefficient n. To consider how output gene expression relates to a governing parameter, hold all other parameters fixed while changing β incrementally. For each value of β, simulate the dynamics given a range of initial conditions (or, if possible, identify the fixed points numerically). Then, collect the final states of the simulation, and plot those final states as a function of the parameter. In this particular case, it is also possible to solve for the intersection of the production and degradation functions, e.g., via a zero-finding algorithm, and use these solutions as equivalent to the expected steady states. The final element is to denote which of the fixed points are stable or unstable. In this case, the low and high steady states are both stable and the intermediate value is unstable (denoted by solid and open circles, respectively). This approach is not a comprehensive solution to bifurcation diagrams, but it provides the essential ideas and helps to identify the existence of a critical point. In this particular example when $K = 50$ nM, $\alpha = 1$ nM/hr, and $n = 2$, then $\beta_c \approx 88$.



This is an example of a saddle-node bifurcation in which one one unstable equilibrium and one stable equilibrium 'collide' leaving only a single fixed point for values of $\beta < \beta_c$. Hence, even though the structure of the system is the same throughout, the system can exhibit the feature of alternative stable states only when the strength of the promoter exceeds the critical value. For more details on bifurcation theory consult the excellent introduction to Nonlinear Dynamics and Chaos by Steve Strogatz (Strogatz 1994).

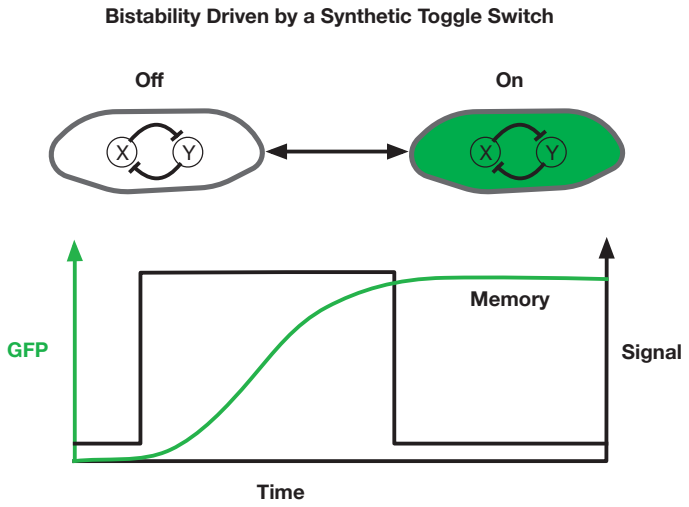


Figure 2.10: A genetic toggle switch in which the genes X and Y mutually inhibit each other can lead to bistability (top) and cellular memory, even when stimuli are removed (bottom). The top schematics indicate the state of the cell as a whole, in which there is not (left) or there is (right) GFP in the system. In a system with alternative stable states, the system can remain in a new state (e.g., 'On') even after the signal is removed; this is a form of cellular memory.

2.5 THE DYNAMICS OF A GENETIC TOGGLE SWITCH

The toggle switch: a network in which two genes mutually repress one another, that is $X \dashv Y$ and $Y \dashv X$. This is the synthetic circuit designed by Gardner et al. (Gardner et al. 2000b) using existing components, i.e., promoter-repressor pairs and green-fluorescent reporters (as reproduced in Figure 2.2). How did the team know that such a switch could possibly work, and what are the relevant governing parameters that could ensure that the toggle switch really does exhibit bistability and memory in practice, not just in theory?

To begin, consider the mathematical representation of an idealized toggle switch, in which x and y denote the concentrations of proteins associated with the two genes:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\beta_1}{1+(y/K)^\beta} - \alpha x \tag{2.19}$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \frac{\beta_2}{1+(x/K)^\gamma} - \alpha y \tag{2.20}$$

This model assumes potentially different levels of cooperativity in the mutual repression. However, this is not the form that Gardner et al. use. To get there,

let's rescale time such that $\tau = \alpha t$, just as we did in our study of Luria and Delbrück. In this case $\mathrm{d}\tau = \alpha \mathrm{d}t$, and by dividing the above equations by α we find:

$$\frac{\mathrm{d}x}{\mathrm{d}\tau} = \frac{\beta_1/\alpha}{1+(y/K)^\beta} - x \tag{2.21}$$

$$\frac{\mathrm{d}y}{\mathrm{d}\tau} = \frac{\beta_2/\alpha}{1+(x/K)^\gamma} - y \tag{2.22}$$

Next, let's also re-scale the concentrations relative to a common half-saturation constant - though in general it is important to keep in mind that such affinities could differ. That is, define $u = x/K$ and $v = y/K$ such that $\mathrm{d}x = K\mathrm{d}u$ and $\mathrm{d}y = K\mathrm{d}v$. The dynamics of the toggle switch can now be written as:

$$K\frac{\mathrm{d}u}{\mathrm{d}\tau} = \frac{\beta_1/\alpha}{1+v^\beta} - Ku \tag{2.23}$$

$$K\frac{\mathrm{d}v}{\mathrm{d}\tau} = \frac{\beta_2/\alpha}{1+u^\gamma} - Kv \tag{2.24}$$

and by dividing both sides by K we find:

$$\frac{\mathrm{d}u}{\mathrm{d}\tau} = \frac{\beta_1/(\alpha K)}{1+(v)^\beta} - u \tag{2.25}$$

$$\frac{\mathrm{d}v}{\mathrm{d}\tau} = \frac{\beta_2/(\alpha K)}{1+(u)^\gamma} - v \tag{2.26}$$

Let's now denote a dimensional production rate $\tilde{\beta}_1 = \beta_1/(\alpha K)$ and $\tilde{\beta}_2 = \beta_2/(\alpha K)$, such that

$$\frac{\mathrm{d}u}{\mathrm{d}\tau} = \frac{\tilde{\beta}_1}{1+(v)^\beta} - u \tag{2.27}$$

$$\frac{\mathrm{d}v}{\mathrm{d}\tau} = \frac{\tilde{\beta}_2}{1+(u)^\gamma} - v \tag{2.28}$$

This is precisely the model proposed for the genetic toggle switch, albeit using different constant names. At this point, to stay consistent, we will use $\alpha_1 \equiv \tilde{\beta}_1$ and $\alpha_2 \equiv \tilde{\beta}_2$. With this definition in place, it is possible to identify steady states and learn about the overall system dynamics.

2.5.1 Theory of robust, toggle switch design

The identification of steady states requires that all variables of the system remain fixed. Another way to say this that the nullclines must intersect. A nullcline defines a set of points (u, v) such the one of the variables does not change. Hence, the nullcline of u must satisfy $\mathrm{d}u/\mathrm{d}t = 0$ and the nullcline of v must satisfy

$dv/dt = 0$. Both variables remain fixed at the intersection of the nullclines, implying that there must be a combination of values (u^*, v^*) such that

$$u^* = \frac{\alpha_1}{1 + (v^*)^\beta} \tag{2.29}$$

$$v^* = \frac{\alpha_2}{1 + (u^*)^\gamma}. \tag{2.30}$$

These two functions define the $\dot{u} = 0$ and $\dot{v} = 0$ nullclines, respectively. The nullclines can, potentially, intersect at 3 points – in which two of these fixed points denote stable states and one is an intermediate unstable state. To see this, substitute the first nullcline relationship to get a single relationship for v^*:

$$v^* \left[1 + \left(\frac{\alpha_1}{1 + v^\beta} \right)^\gamma \right] = \alpha_2 \tag{2.31}$$

Note that the lhs is a function of v^*. When $v^* \to 0$, then the function is approximately $v(1 + \alpha_1^\gamma)$. When $v^* \to \infty$ then the function is approximately v. In between, the function has a local maximum and minimum. Hence, depending on the value of α_2 there can be a regime in which there are three possible steady states (see Figure 2.11). However, if α_2 is either too small or too large, then there won't be multiple equilibrium points and the toggle switch will only have one potential state. Notice that the single states corresponding to monostability have either v^* quite high or quite low. Only one repressor wins! Note that as intuition suggests, for lower values of α_2 (corresponding to the maximum strength of v) then the repressor concentration v is forced into an Off state, and for sufficiently high values of α_2 then the repressor concentration v is forced into an On state. Whereas in the bistable regime then both repressors can win – in exact analogy to the Off and On states of the autocatalytic loop. Figure 2.11 illustrates that point, given $\beta = 3 = \gamma$. This relationship can be turned on its side and, in so doing, we can ask instead, what values of v^* are possible given a fixed value of α_2. There are many ways to do this, but one can think of this as turning the previous graphic on its side, and then finding the intersections of v^* as α_2 increases. Figure 2.12 shows the result of such a procedure, where the black points denote regions of bistability and the red points denote regimes of monostability. Hence, in theory, this toggle switch has a robust region of multistability for a broad range of promoter strengths – the precise feature that the genetic toggle switch was designed to replicate.

2.5.2 Synthetic biology - nonlinear dynamics in the real world

What did Gardner do? They built a genetic construct on a plasmid that had an antibiotic resistance cassette and then ran their experiments with antibiotics to ensure that the plasmid would be detained by the cells. The construct included two promoters, each of which turned on a different repressor. That is, X_1 represses P_2 and X_2 represses P_1. One would expect that either, but not both X_1 or X_2 could be turned on. One such construct included the lac repressor

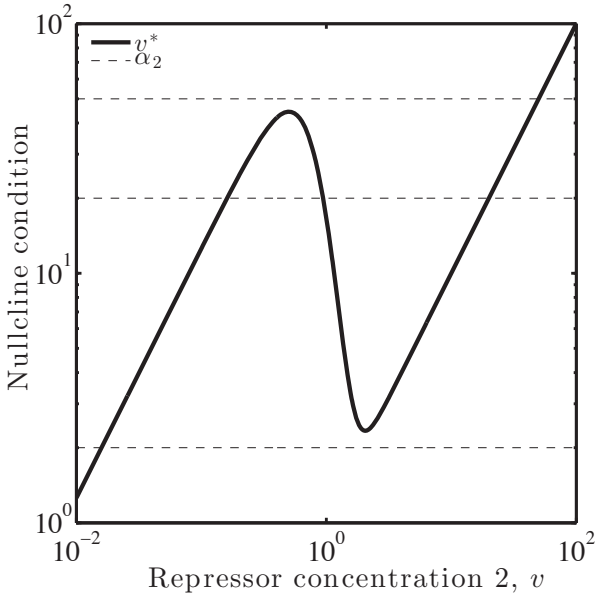


Figure 2.11: A potential bifurcation given variation α_2 for the toggle switch. Here, $\beta = 3$, $\gamma = 3$, and $\alpha_1 = 5$. The solid lines denotes the lhs of Eq. (2.31) and the dashed line denotes the value $\alpha_2 = 2, 20$ and 50.

lacI and the temperature sensitive phage λ cI repressor *cIts*. In *E. coli*, the lac repressor controls the levels of enzyme expression to process sugars. But, the lac repressor can bind to galactose or a galactose mimic, like the molecular inducer IPTG. Similarly, in phage λ, the repressor *cI* can bind to a core operator site at the center of the phage λ genetic circuit, enhancing the likelihood that phage will integrate into the bacterial host rather than killing it. But *cIts* is a mutant repressor that loses its ability to bind at high temperatures. This setup, as depicted in Figure 2.2, underlies the tension of the toggle switch.

Let's say experimentalists add IPTG, in that case lacI proteins will bind to IPTG and not repress expression of *cIts*. In which case the GFP protein associated with the P2 direction of the construct will be expressed and cells will turn green. Similarly, the cIts protein will bind to the P1 promoter, keeping the system 'on' even IPTG is removed (see Figure 2.2). Next, when the system temperature increases, then the temperature sensitive repressor will no longer function, and then expression via *lacI* will subsequently repress expression at the P2 promoter. The green GFP protein will dilute away, and the cell will remain off, even after temperature is returned to normal.

This construct and dynamic results illustrate the core findings: that the system does have bistability and that the bistability has a 'memory' like feature,

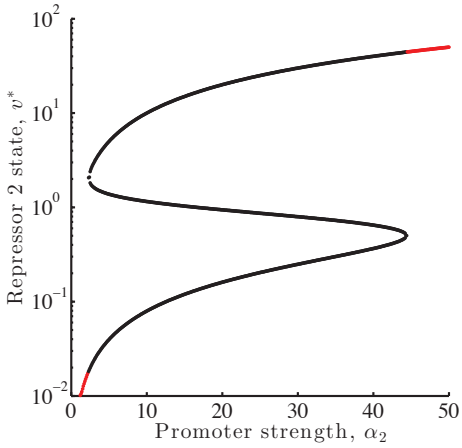


Figure 2.12: Equilibrium state of repressor 2, v^*, as a function of the input promoter strength α_2. For intermediate values of α_2 there are multiple solutions, corresponding to a bistable regime where v^* is either low or high (note the log axes). In contrast, when $\alpha_2 \to 0$, then there is only a single stable state corresponding to low v^* and when $\alpha_2 \to \infty$, then there is only a single stable state corresponding to high v^*. The equation for this condition is found in Eq. (2.31).

retaining an imprint of past environmental changes due to the mutual inhibition and feedback embedded into the design of the plasmid and, indeed, of the nonlinear dynamical system itself. Indeed, the genetic toggle switch is remarkable in many ways, not least of which because it leverages concepts of nonlinear dynamics to design and implement a toggle switch. The implementation are inside *E. coli* cells. The components of the toggle switch are themselves derived from organisms. The components are well characterized parts of the *E. coli* lactose utilization system and of the gene circuit of the bacteriophage λ. The advantages of using these parts is that their behavior is relatively well understood in isolation and in their original context - but the combination yields a reproducible genetic circuit that does an altogether new function in a different context.

That the genetic toggle switch can in fact exhibit bistability mutually reinforces the utility of quantitative approaches in characterizing and engineering living systems while also creating a pathway for a field of study: engineered cellular systems biology. The finding that adding a stimulus can induce a state and removal of that stimulus does not change the state implies that it should also be possible, in theory and in practice, to program cellular state. Notably, the process is reversible, in that applying a different stimulus (e.g., increasing temperature which deactivates a temperature-dependent repressor molecular)

can lead to a switch back to the other state. This example of robust switching between alternative stable states reveals that individual cells with the same genetic architecture need not have the same phenotype, but rather, their phenotypes may be dependent on past stimulus, precisely because intrinsic feedback in their regulatory circuits. In the next chapter, we will see that such variation extends even further given that stochastic expression of genes involved in regulation - whether for the toggle switch or other circuits - can lead to sustained differences in cells as a result of intrinsic noise.

2.6 TAKE-HOME MESSAGES

- Regulation of gene expression enables cells to respond to internal and external stimuli.
- Gene regulation is fundamentally nonlinear.
- Nonlinear gene regulation is the basis for bistability, i.e., in which vastly different outcomes can arise given small differences in input.
- Bistability can be recapitulated and engineered in synthetic switches, in which a stimulus can induce a change in gene expression that is retained over multiple cell generations.
- Memory and bistability in cells is the basis for differentiation in single- and multi-cellular organisms.
- The robustness of gene expression is also modulated by intrinsic and extrinsic stochasticity, a topic explored in the next chapter.

2.7 PROBLEM SET

These problems are intende to deepen your understanding of bistability both in theory and in practice, i.e., as part of *in silico* experiments. The overall objective of this problem set is to explore the principles by which a system may exhibit bistability, with an emphasis of synthetically designed bistable gene regulatory networks. The problem sets builds upon a set of methods developed in the corresponding computational laboratory associated with this chatper including:

- Simulating coupled systems of differential equations.
- Phase plan visualization.
- Qualitative analysis of nonlinear dynamics systems (including nullcline analysis).
- Linear stability analysis, *in silico*.
- Bistability and bifurcation diagrams

Problem 1. Characterizing a "Bifurcation" Given Bistability

Write a program to simulate an autoregulatory, positive feedback loop X→X. Assume that the protein dilution rate is α, the max production rate is β_+, the basal level is β_-, and the half-saturation concentration is K. Here you will explore the consequences of adding in proteases that may specifically target and degrade the protein.

- First, explore the theoretical limits to bistability given Boolean logic, i.e., is there a range of a values for which the system should be bistable?
- Assume that β_- = 5 nM/hr, β_+ = 50 nM/hr and K = 25 nM. Find a range of α values in which the long-term dynamics exhibit monostability and bistability - keep in mind that cannot go below 0.5 /hr if cells divide at this rate. Explain and contrast your results to theory.
- Relax the "Boolean" assumption and explore whether or not your conclusions guide similar results when the cooperativity of the feedback is of order 1, 2, 3, and 4.

Problem 2. Simulating the idealized "Toggle Switch"

Write a program to simulate the toggle switch - as described in an idealized fashion in Box 1 of Gardner et al. Nature 2000 (call this Model A):

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^\beta} - u \tag{2.32}$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^\gamma} - v. \tag{2.33}$$

Here you will explore the consequences of changes in activation, degradation and cooperativity in modulating bistability.

- Using 'Model A', and using the following values $\gamma = 3$, $\beta = 3$, and $\alpha_1 = 5$, $\alpha_2 = 5$, then (i) numerically identify nullclines and visualize them; (ii) numerically identify fixed points; (iii) confirm the stability of each of the identified fixed points.
- Again, using 'Model A', fix the value of $\alpha_1 = 5$ and modulate α_2 across a range of values relative to that of α_1, simulating each and identifying the relevant steady state(s). Visualize and describe your findings.
- Given the results of the prior analysis, either confirm/refute the claims of Gardner et al with respect to the role of degradation timescales in driving bistability?

Problem 3 - Toggle Switch in Practice

In this problem, write a program to simulate the toggle switch as described in the caption to Figure 5 of Gardner et al. Nature 2000 (call this Model B):

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^\beta} - u \tag{2.34}$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+\left(\frac{u}{1+IPTG/K)^\eta}\right)^\gamma} - v. \tag{2.35}$$

Using Model B and parameters from Gardner's Figure 5 (α_1 = 156.25, α_2 = 15.6, β = 2.5, γ = 1, η = 2.0015, and $K = 2.9618 \times 10^{-5}$), try to recapitulate the following predictions, providing visual evidence and supporting analysis (when feasible):

- Cells can be put into the high GFP and low GFP states by the use of inducers
- Cells remain in such states even when the inducers are removed
- There exist up to 3 steady states of the cell's GFP expression as a function of changing IPTG concentrations.
- Once you have identified the steady states, then describe each and identify the regime of bistability.

Additional Problems

- What features are required for bistability in networks with only one or two components?
- How robust is Model B switch to variations in the cooperativity?
- How long is a typical residence time in an On or Off state for the reporter in Model B?
- For growing cells, how long would it take for the daughters of an On cell to lose the memory of their parental state?

2.8 TECHNICAL APPENDICES

Diffusive contact between TF-s and DNA: A randomly diffusing protein will travel a characteristic squared distance x^2 in a period of time $t \approx x^2/D$ where D is the diffusion constant. For globular proteins of approximately 5 nm in effective size, then the diffusion rate is approximately $10-40\ \mu\text{m}^2/\text{sec}$. Hence given a characteristic distance of $x = 1\mu\text{m}$, then it should take $t \approx 0.025$–0.1 seconds for a TF to traverse a cell (though longer to find a particular target location). Note that the value of D was derived by Einstein, in his theory of Brownian motion (Einstein 1956), i.e.,:

$$D = \frac{kT}{6\pi\eta a} \approx \frac{4\times 10^{-14} g\cdot cm^2/sec}{20\times 10^{-2} g/cm\cdot sec\times 5nm} \approx 40\mu m^2/sec \tag{2.36}$$

where k is Boltzmann's constant, η is viscosity of water, and a is the effective hydrodynamic radius. Note that cytoplasm is more viscous than water and that larger molecules may be 'caged' and unable to move freely through a cellular cytoplasm.

Analytical solution of constant on and off dynamics: Consider the dynamics in which a protein is turned on at a rate β and turned off at a per-capita rate α:

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \beta - \alpha y. \tag{2.37}$$

The dynamics can be solved explicitly via integration by parts, i.e.:

$$\frac{\mathrm{d}y}{\beta-\alpha y} = \mathrm{d}t \tag{2.38}$$

$$\frac{\log\beta-\alpha y}{-\alpha} = t + \text{const} \tag{2.39}$$

$$\beta-\alpha y = \text{const}\times e^{-\alpha t} \tag{2.40}$$

or

$$y(t) = \frac{\beta}{\alpha} - Ce^{-\alpha t} \tag{2.41}$$

where the value of C must be determined from initial conditions. In the event that $y(t=0)=0$, then $C=\beta/\alpha$ such that

$$y(t) = \frac{\beta}{\alpha}\left(1-e^{-\alpha t}\right). \tag{2.42}$$

Cooperativity in gene regulation The standard nonlinear functions of activation by a gene X whose concentration is x can be written as

$$f(x) = \frac{\beta x^n}{K^n + x^n} \tag{2.43}$$

whereas if X is a repressor then

$$f(x) = \frac{\beta}{1 + x^n/K^n}. \tag{2.44}$$

Where do these functions come from? At some level, we can think of them as phenomenological. The activator starts at $f(0) = 0$ and increases toward saturation at $f(x \gg K) \to \beta$. Likewise, the repressor starts at $f(0) = \beta$ and decreases as $f(x \to 0) \to 0$. The transitions happen close to the value $x = K$. The sharpness of the transition from low to high, or high to low, depends on n. This exponent characterizes the degree of nonlinearity in the system, but it also has a biophysical interpretation.

To understand how, consider the case of the activator, in which the expected expression is β when the promoter is occupied and 0 when it is not. In that event, the average expression is

$$f(x) = p_0 \times 0 + p_1 \times \beta \tag{2.45}$$

where p_0 and p_1 denote the probability the promoter is unoccupied and occupied respectively. Yet promoter occupancy probabilities depend on interactions with transcription factors, e.g., X. Hence, consider the concentrations of unoccupied and occupied promoters as c_0 and c_1, respectively. In that case, the system can be represented in terms of a series of forward and backwards chemical reactions:

$$X + C_0 \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C_1. \tag{2.46}$$

This set of chemical reactions representing binding and unbinding can be written as a dynamical equation for the concentration c_0, i.e.,

$$\frac{\mathrm{d}c_0}{\mathrm{d}t} = -k_+ c_0 x + k_- c_1. \tag{2.47}$$

The solution is identified by noting that $c_0 + c_1 = c_T$, i.e., a fixed total concentration representing the density of promoter binding sites in the cell. Hence, reducing this one equation-two unknown problem into that of one equation and one unknown renders it solvable. The solution can be derived as follows:

$$\begin{aligned} k_+ c_0 x &= k_-(c_t - c_0) \\ (k_+ x + k_-) c_0 x &= k_- c_t \\ c_0 &= c_T \left(\frac{k_-}{k_- + k_+ x} \right). \end{aligned} \tag{2.48}$$

Given that $p_0 = c_0/c_T$, then

$$p_0 = \frac{1}{1 + x/K_D} \tag{2.49}$$

and

$$p_1 = \frac{x}{K_D + x} \tag{2.50}$$

where $K_D = k_+/k_-$ has units of a concentration. Even at this stage, this relatively minimal model of gene regulation leads to a nonlinear expression:

$$f(x) = \frac{\beta x}{K_D + x} \tag{2.51}$$

Similar logic leads to the conclusion that for repressors, then

$$f(x) = \frac{\beta}{1 + x/K_D} \tag{2.52}$$

Yet, why are the sigmoidal functions so sharp, that is to say: why is $n > 1$?

One answer lies in cooperativity. Consider for example a transcription factor that does not bind to promoters in the form of a monomer, but instead in the form of a dimer. In that event, the concentration of dimers, x_2 should be in equilibrium with the concentration of monomers, x, i.e.,

$$X + X \underset{k_-}{\overset{k_+}{\rightleftharpoons}} X_2 \tag{2.53}$$

which can be written in terms of the dynamical system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = -k_+ x^2 + 2k_- x_2 \tag{2.54}$$

such that at equilibrium then

$$x_2 = k_+ x^2/(2k_-). \tag{2.55}$$

The rules for activation and repression apply as before, except that dimers (with concentration x_2) bind to promoters rather than having monomers bind to promoters (with concentration x). We can rewrite the activation function as

$$f(x) = \frac{\beta x_2}{K_D + x_2} \tag{2.56}$$

or

$$f(x) = \frac{\beta k_+ x^2/(2k_-)}{K_D + k_+ x^2/(2k_-)}. \tag{2.57}$$

This shows that dimerization imposes sharper thresholds for activation. In practice, the value of n represents the effective degree of cooperativity and can be mapped explicitly to the number of monomers required for binding and regulation of expression downstream of a target promoter.

Linear stability analysis of a nonlinear dynamical system Most of the

dynamic models in this book are *nonlinear*. A full analysis of nonlinear dynamical systems is outside the scope of this text and can be found in introductory texts such as: "Nonlinear Dynamics and Chaos" (Strogatz 1994) or "Population Biology: Models and Concepts" (Hastings 1997). In practice, there is a standard approach to diagnose potential dynamics of a nonlinear system. This approach includes identifying fixed points, approximating the dynamics near the fixed point via a process termed 'linearization', and then solving for the expected qualitative dynamics near the fixed point by taking advantage of standard approaches to solve linear systems. The following sections demonstrate these in practice in the case of one-dimensional and two-dimensional systems. *Note that the technical details below are reproduced from Quantitative Viral Ecology (Weitz 2015).*

Linear stability analysis in 1D: Microbial population dynamics with implicit resources and a fixed washout rate can be written as:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = rN\left(1 - \frac{N}{K}\right) - \omega N \tag{2.58}$$

where N is the density and r is the maximal growth rate, ω is the density-independent death rate due to washout, and K is the density at which net growth goes to zero in the limit of vanishing washout. Formally, this system can be analyzed for any non-negative value of N. As was shown before, the dynamics of this system lead to either the death of the population or its persistence at an equilibrium. Here, the system is used as an example to show how the stability of these equilibria can be studied (in one-dimensional systems), in part as a prelude to analysis of stability of higher-dimensional dynamical systems. In this example, the description will utilize the convention to suppress the time-dependency of time-varying populations, e.g., writing x instead of $x(t)$, but noting the (constant) equilibrium values of such populations with an *, e.g., x^*.

Recall that the term "steady state" refers to those population densities (in this case the density N) such that there is *no time-rate of change*. Formally, this means that for a dynamical system that tracks changes in some population $x(t)$ of the form:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x) \tag{2.59}$$

the equilibria correspond to values of $x = x^*$ such that $f(x^*) = 0$. There are two equilibria in the case of the model above:

$$N^* = 0 \quad \text{Death} \tag{2.60}$$

$$N^* = K\left(1 - \frac{\omega}{r}\right) \quad \text{Persistence} \tag{2.61}$$

The local stability of a given equilibria can be thought of as a classification of the response of the system to small perturbations. The system is termed "unstable" if the size of the perturbation increases with time. Whereas, the system is termed "stable" if the size of the perturbation decreases with time.

In the biological example, consider the stability of the equilibrium $N^* = 0$. This equilibrium has no individuals of the microbial population - one can think of it as a test tube with sterile media. Then, the "perturbation" can be thought of as the addition of a small inoculum of a microbial population, $N(t = 0) = n \ll K$. What happens in such a case? If the media is conducive to growth, then the microbial population will grow in number. If the media is not conducive to growth, then the microbial population will decrease in number. In the former case, density increases from a small perturbation and so the fixed point is deemed unstable. Whereas, in the latter case, density decreases from a small perturbation and so the fixed point is deemed stable. This intuition can be formalized, at least for nonlinear dynamical systems of a single variable, using a graphical, perturbative and a linearization approach.

A useful way to assess the stability of a one-dimensional nonlinear dynamical system is to plot the rate of change of the population as a function of the population. In this case, this corresponds to visualizing $\mathrm{d}N/\mathrm{d}t$ as a function of N (see solid line in Figure 2.13). On the same plot, it is useful to draw a horizontal line corresponding to the value of $\mathrm{d}N/\mathrm{d}t = 0$ (see dashed line in Figure 2.13). In this way, one can rapidly identify those regions of N for which the population rate of change is positive – the solid line should lie above the dashed line. Similarly, regions for which the population rate of change is negative correspond to when the solid line lies below the dashed line. Finally, the values for which the population rate of change is zero are identified by finding the intersection of the solid and dashed lines.

With this graphical scheme in place, the following procedure can then be used to determine the dynamics of $N(t)$ given some initial value of $N(t = 0) = N_0$. First, determine whether the population is in an increasing or decreasing region (as above). If increasing, then the population will continue to increase until it approaches a point where there is no net rate of change. If decreasing, then the population will continue to decrease until it approaches a point where there is no net rate of change. The two possible equilibrium points are at $N^* = 0$ and at $N^* = K(1 - \omega/r)$. In the example, all values between $0 < N < K(1 - \omega/r)$ correspond to increases in the population and all values that satisfy $N > K(1 - \omega/r)$ correspond to decreases in the population. It is evident that the equilibrium point $N^* = 0$ is unstable, as small increases in the population away from this point lead to even further increases. Similarly, it is evident that the equilibrium point $N^* = K(1-\omega/r)$ is stable, as small decreases or increases in the population away from this point lead to subsequent increases or decreases back toward the equilibrium. The classification of one-dimensional nonlinear dynamical system can be completed in this way. However, this graphical approach is difficult to extend to higher dimensional dynamical systems. Moreover, it does not specify the quantitative rate of change of the population away from or toward an equilibrium.

Tracking the dynamics of perturbations represents an alternative approach to characterizing the equilibria in a population model. As noted above, there are two equilibria in this model. Consider first a small perturbation $N(t = 0) =$

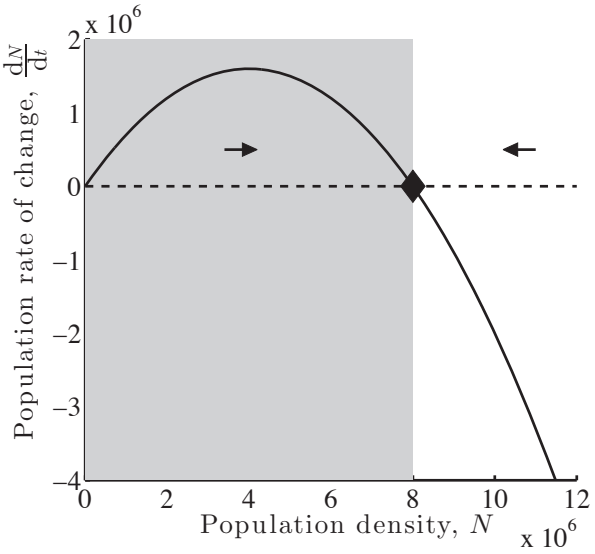


Figure 2.13: Population dynamics, $\mathrm{d}N/dt$ as a function of population density for the chemostat growth model where $r = 1$, $K = 10^7$ and $\omega = 0.2$. Dashed line denotes $\mathrm{d}N/\mathrm{d}t = 0$, diamond denotes equilibrium $N^* = K(1 - \omega/r)$, and shaded region denotes values of N where population growth rate is positive.

$n \ll K$, equivalent to adding a small inoculum of a bacterial population to an otherwise sterile flask. The dynamics of the population follow:

$$\frac{\mathrm{d}n}{\mathrm{d}t} = rn\left(1 - \frac{n}{K}\right) - \omega n \tag{2.62}$$

$$= rn - rn\frac{n}{K} - \omega n \tag{2.63}$$

Because $n \ll K$, any term of the form n/K is much smaller than terms with only a density of n. The dynamics of the perturbation $n(t)$ in this limit can be approximated as:

$$\frac{\mathrm{d}n}{\mathrm{d}t} \approx rn - \omega n = (r - \omega)n \tag{2.64}$$

In other words, for very small perturbations, the population should grow exponentially so long as $r > \omega$ and decrease exponentially so long as $r < \omega$. The stability of the $N^* = 0$ equilibria is determined by the ratio: r/ω,

$$\frac{r}{\omega} > 1 \qquad \text{unstable} \tag{2.65}$$

$$\frac{r}{\omega} < 1 \qquad \text{stable} \tag{2.66}$$

When $r = \omega$, this borderline case is term "neutrally stable", in that small perturbations are expected neither to grow or decay over time.

Alternatively, consider the situation in which a system is perturbed away from the equilibrium $N^* = K\left(1-\frac{\omega}{r}\right)$? What are the dynamics of n in the case when $n = N^* - N$? Replacing $N(t) = N^* - n(t)$ yields:

$$\frac{\mathrm{d}(N^*-n)}{\mathrm{d}t} = r\,(N^*-n)\left(1-\frac{N^*-n}{K}\right) - \omega(N^*-n) \tag{2.67}$$

which can be rewritten as:

$$-\frac{\mathrm{d}n}{\mathrm{d}t} = rN^*\left(1-\frac{N^*}{K}\right) - \omega N^* + \frac{rnN^*}{K} - rn\left(1-\frac{N^*-n}{K}\right) + \omega n \tag{2.68}$$

However, the definition of the equilibrium N^* is that $rN^*\left(1-\frac{N^*}{K}\right) - \omega N^* = 0$, so that:

$$\begin{aligned}
\frac{\mathrm{d}n}{\mathrm{d}t} &= -\frac{rnN^*}{K} + rn\left(1-\frac{N^*-n}{K}\right) - \omega n && (2.69)\\
&= -\frac{rnN^*}{K} + rn\left(1-\frac{N^*}{K}\right) - \omega n + \frac{rn^2}{K} && (2.70)\\
&\approx (r-\omega)n - 2\frac{rnN^*}{K} && (2.71)
\end{aligned}$$

where the final line is derived, as before, by retaining terms to order n. Substituting the value of N^* yields:

$$\frac{\mathrm{d}n}{\mathrm{d}t} = -(r-\omega)n. \tag{2.72}$$

In other words, a small perturbation will decrease whenever $r > \omega$ and increase when $r < \omega$. Because the perturbation is a reduction in population density below that of the steady state, biologically this can be interpreted as implying that the persistence equilibrium is stable whenever the density-independent growth rate r is greater than the density-independent death rate ω. Notice that the two conditions coincide. Moreover, the equilibrium $N^* = K\left(1-\frac{\omega}{r}\right)$ only exists (i.e. is positive) when $r > \omega$. The overall conclusion from this analysis is that a population in which density-independent birth exceeds death has two fixed points. Of the two fixed points, one is unstable, corresponding to the absence of the population, and one is stable, corresponding to the persistence of the population.

The analysis above is somewhat cumbersome as it depends on expanding the nonlinear dynamics, and setting all higher order terms to zero. Formally, this is equivalent to "linearizing" the nonlinear dynamics near the equilibria points - a method that scales up to more complex systems with more than one variable. For a system whose dynamics are: $\frac{\mathrm{d}x}{\mathrm{d}t} = f(x)$, the behavior of the system near $x = x^*$ can be described in terms of some perturbation $x(t) = x^* + u(t)$. The dynamics near the fixed point $x = x^*$ can be approximated by a

Taylor approximation of the function $f(x)$ in terms of the perturbation $u = x - x^*$ such that:

$$\frac{\mathrm{d}x^*}{\mathrm{d}t} + \frac{\mathrm{d}u(t)}{\mathrm{d}t} = f(x) \tag{2.73}$$

$$\cancel{\frac{\mathrm{d}x^*}{\mathrm{d}t}} + \frac{\mathrm{d}u}{\mathrm{d}t} = \cancel{f(x^*)} + \left.\frac{\mathrm{d}f}{\mathrm{d}x}\right|_{x=x^*} u + \mathcal{O}\left(u^2\right) \tag{2.74}$$

$$\frac{\mathrm{d}u}{\mathrm{d}t} \approx \left.\frac{\mathrm{d}f}{\mathrm{d}x}\right|_{x=x^*} u \tag{2.75}$$

because $f(x^*) = 0$ and higher order terms are ignored when u is small. The perturbation will increase or decrease depending on the change of the system dynamics with respect to changes in the population near the fixed point: $\frac{\mathrm{d}f}{\mathrm{d}x}|_{x=x^*}$. When local increases in the population correspond to increases in the rate of change, the system is unstable and vice-versa. This is the algebraic equivalent of the graphical approach illustrated earlier in this section. In the case of the microbial population growth model, then

$$\frac{\mathrm{d}u}{\mathrm{d}t} = (r-\omega)u - 2\frac{ruN^*}{K} \tag{2.76}$$

exactly as in Eq. 2.72 (albeit with the variable n standing in for the perturbation rather than u). Substituting in N^*, yields

$$\frac{\mathrm{d}u}{\mathrm{d}t} = -(r-\omega)u \tag{2.77}$$

for dynamics near $N = K\left(1 - \frac{\omega}{r}\right)$ and

$$\frac{\mathrm{d}u}{\mathrm{d}t} = (r-\omega)u \tag{2.78}$$

for dynamics near $N = 0$. As before, the linearization method predicts that persistence is stable when $r > \omega$ and population extinction is stable when $r < \omega$. The linearization approach can be extended to analysis of multi-variable systems.

Linear stability analysis in 2D: Consider the following example model, in this case of virus-host dynamics:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \overbrace{rN\left(1 - N/K\right)}^{\text{host growth}} - \overbrace{\phi NV}^{\text{lysis}} - \overbrace{\omega N}^{\text{cell death}} \tag{2.79}$$

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \overbrace{\tilde{\beta}\phi NV}^{\text{virus burst}} - \overbrace{\omega V}^{\text{virus decay}} \tag{2.80}$$

where $\tilde{\beta} = \beta - 1$. There are three equilibria of the form (N^*, V^*): $(0,0)$, $\left(K(1-\frac{\omega}{r}), 0\right)$ and $\left(\frac{\omega}{\tilde{\beta}\phi}, \frac{r}{\phi}\left(1 - \frac{\omega}{\tilde{\beta}\phi K}\right) - \frac{\omega}{\phi}\right)$. The local stability of each of these

equilibria can be evaluated using a linearization approach, analogous to that used in the analysis of the 1-D microbial population growth model.

The key insight in conducting a linear stability analysis is to recall that the rate of change of populations can be expressed, near an equilibria, by approximating the nonlinear response as a linear response in terms of two (or more) variables. Formally, consider the generic dynamical system:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x,y), \tag{2.81}$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = g(x,y). \tag{2.82}$$

The population state (x^*, y^*) is an equilibrium if $f(x^*, y^*) = 0 = g(x^*, y^*)$. The local stability of an equilibria can be evaluated by consider the perturbations u and v such that $x(t) = x^* + u(t)$ and $y(t) = y^* + v(t)$. The dynamics of these perturbations can be written as:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = f(x^*,y^*) + \left.\frac{\partial f}{\partial x}\right|_{x^*,y^*} u + \left.\frac{\partial f}{\partial y}\right|_{x^*,y^*} v + \mathcal{O}(u^2, uv, v^2), \tag{2.83}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t} = g(x^*,y^*) + \left.\frac{\partial g}{\partial x}\right|_{x^*,y^*} u + \left.\frac{\partial g}{\partial y}\right|_{x^*,y^*} v + \mathcal{O}(u^2, uv, v^2). \tag{2.84}$$

Because the local stability analysis is near the equilibrium, these two equations can be approximated as:

$$\frac{\mathrm{d}u}{\mathrm{d}t} \approx \left.\frac{\partial f}{\partial x}\right|_{x^*,y^*} u + \left.\frac{\partial f}{\partial y}\right|_{x^*,y^*} v \tag{2.85}$$

$$\frac{\mathrm{d}v}{\mathrm{d}t} \approx \left.\frac{\partial g}{\partial x}\right|_{x^*,y^*} u + \left.\frac{\partial g}{\partial y}\right|_{x^*,y^*} v \tag{2.86}$$

The local dynamics of the perturbation (u, v) can be written as:

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} u \\ v \end{bmatrix} = J\begin{bmatrix} u \\ v \end{bmatrix} \tag{2.87}$$

where

$$J = \begin{bmatrix} \frac{\partial f}{\partial x} & \frac{\partial f}{\partial y} \\ \frac{\partial g}{\partial x} & \frac{\partial g}{\partial y} \end{bmatrix} \tag{2.88}$$

is termed the Jacobian. The Jacobian is evaluated at a specific point, corresponding to the equilibrium under investigation: (x^*, y^*).

The Jacobian encodes information on the rate of change of population dynamics with respect to changes in the populations. The Jacobian can be evaluated at a given equilibrium yielding, in this case, four numbers - each corresponding to a given element of a matrix. In this way, a nonlinear dynamical system in two-dimensions can be linearized yielding a linear matrix equation with coefficients

that depend on the equilibria (which themselves depend on parameter values). The solution to a linear system of the form $\mathrm{d}x/dt = Ax$ for a single variable is one of exponential growth or decay, i.e., $x(t) = x_0 e^{\lambda t}$. The same holds for two and higher-dimensional systems. For example, consider the candidate solution $(u(t), v(t)) = (u_0, v_0)e^{\lambda t}$, in which case $\mathrm{d}u/\mathrm{d}t = \lambda u$ and $\mathrm{d}v/\mathrm{d}t = \lambda v$, such that Eq. 2.87 can be re-written as:

$$\lambda \begin{bmatrix} u \\ v \end{bmatrix} = J \begin{bmatrix} u \\ v \end{bmatrix} \tag{2.89}$$

For $u_0 \neq 0$ and $v_0 \neq 0$, then the only feasible values of λ are those that satisfy:

$$\det(J - \lambda I) = 0 \tag{2.90}$$

where I is the 2×2 identity matrix and det refers to the determinant of the matrix.

Consider the motivating example: that of viral-host dynamics. The Jacobian can be expressed in terms of the variables N and V as:

$$J = \begin{bmatrix} r - 2rN/K - \phi V - \omega & -\phi N \\ \tilde{\beta}\phi V & \tilde{\beta}\phi N - \omega \end{bmatrix} \tag{2.91}$$

For the $(0, 0)$ equilibrium, the Jacobian is:

$$J = \begin{bmatrix} r - \omega & 0 \\ 0 & -\omega \end{bmatrix} \tag{2.92}$$

such that the eigenvalues are $\lambda_1 = r - \omega$ and $\lambda_2 = -\omega$. Observe that $\lambda_1 > 0$ when $r > \omega$, such that the trivial steady state is unstable to perturbations so long as the density-independent growth rate of hosts exceeds the density-independent death rate of hosts. The fact that $\lambda_2 < 0$ can be interpreted to mean that if only viruses are added, then the viral population will decrease exponentially with a decay rate ω. The $(0, 0)$ equilibrium is a saddle point on the boundary of the phase space.

For the equilibrium in which hosts are present but viruses are absent (i.e., $(K(1 - \omega/r), 0)$), the Jacobian is:

$$J = \begin{bmatrix} -(r - \omega) & -\phi K(1 - \omega/r) \\ 0 & \tilde{\beta}\phi K(1 - \omega/r) - \omega \end{bmatrix} \tag{2.93}$$

Because this is an upper-triangular matrix, the eigenvalues are equivalent to the diagonal elements of J: $\lambda_1 = -(r - \omega)$ and $\lambda_2 = \tilde{\beta}\phi K(1 - \omega/r) - \omega$. As before, $\lambda_1 < 0$ when $r > \omega$, so that the host-only equilibrium is stable so long as as the density-independent growth rate of hosts exceeds the density-independent death rate of hosts. The sign of λ_2 depends on whether the coexistence steady state is feasible. When feasible, $\lambda_2 > 0$, suggesting that addition of viruses destabilizes the host-only equilibrium. When infeasible, $\lambda_2 <$, so that a perturbation of a

small population of viruses would decay to extinction. Stability analysis of the coexistence equilibrium confirms this result.

In summary, local analysis of stability predicts that the washout equilibrium and the host-only equilibrium are unstable, while the coexistence equilibrium is stable, whenever it exists. The global dynamics of this particular system agree with this local stability analysis.

More generally, the stability of a fixed point can be classified based on the sign of the real components. If one of the eigenvalues has a positive real component, then the fixed point is unstable. If none of the eigenvalues has a non-negative real component, then the fixed point is stable. The type of stability can be further classified based on whether or not the eigenvalues of a non-zero imaginary component. When the eigenvalues are complex, this suggests that dynamics will oscillate. For example, dynamics may oscillate away from or toward a fixed point, given instability or stability respectively.