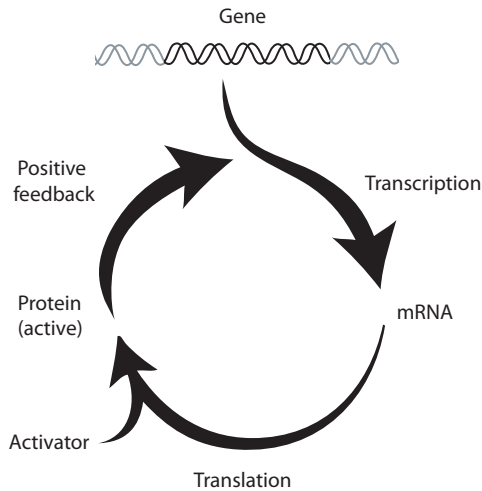


Chapter 2

Variation: Boolean Representations

Problem Key

Problem 2.1: Boolean analysis of a positive feedback network



First, let's practice with a system (shown above) that is very similar to the system in Figure 2.1, except that this system includes positive feedback instead of negative feedback. Just as before, transcription produces mRNA, translation yields protein, and an activator must be present for translation to occur. The gene and the protein must both be present in order for transcription to occur. The inputs to this system are the gene and the activator. You are interested in the changes in the mRNA and protein concentrations over time.

(a) Write out Boolean equations for each of the reactions and mRNA and protein.

Transcription = IF Gene AND Protein
Translation = IF mRNA AND Activator
mRNA = IF Transcription AFTER SOME TIME
Protein = IF Translation AFTER SOME TIME

(b) Construct the state diagram of your system. Circle all of the stable states.

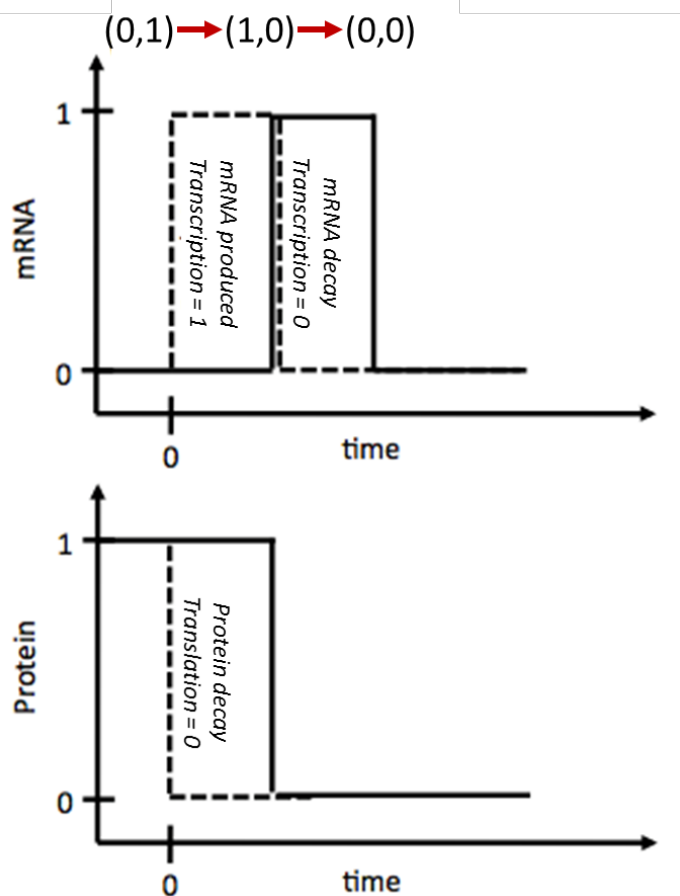
Answer:

		G A		G A		G A		G A	
		0 0		0 1		1 0		1 1	
M	P	trc	trl	trc	trl	trc	trl	trc	trl
0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	1	0	1	0
1	0	0	0	0	1	0	0	0	1
1	1	0	0	0	1	1	0	1	1

where G = Gene, A = Activator, M = mRNA, P = protein, trc = Transcription, and trl = Translation. Yellow, stable states; blue, oscillatory state.

- (c) Beginning with initial conditions Protein = 1, mRNA = 0, Gene = 1, and Activator = 0, plot the dynamics of the system. Label the changes that occur (for example, the onset of transcription).

Answer:



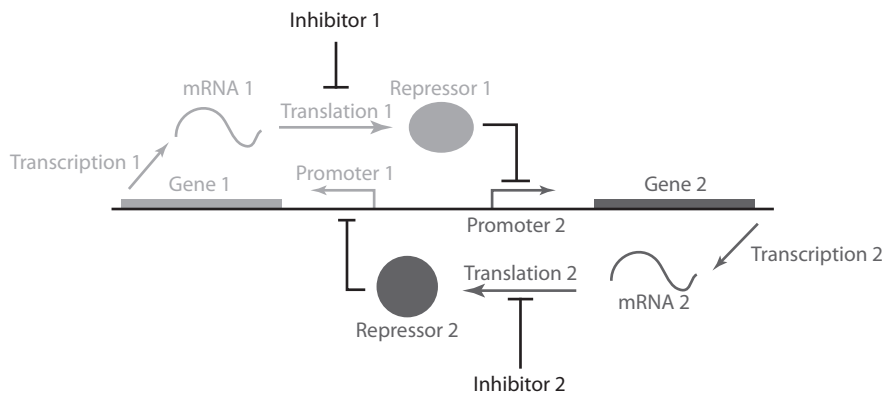
With the presence of protein, mRNA is transcribed, but the protein itself is lost over time, which in turn leads to mRNA loss.

- (d) Imagine that the system starts with mRNA but no protein; both the gene and the activator are present. Describe the progression of the system. How does this progression change if you take the relative timing of protein and mRNA decay into account?

mRNA is translated to protein, but there is no further transcription and the mRNA is degraded. Once protein is present, transcription is initiated and mRNA is produced, but in the meantime there has been no translation, leading to protein dilution and an oscillation.

If the slow degradation rate of the protein is taken into account, then this oscillation breaks when protein is produced; the mRNA is formed and translated before the protein degrades, and thus the system ends up in a stable state in which both mRNA and protein are present.

Problem 2.2: Two interlocking regulatory circuits



Now we will study a regulatory system where we have two proteins, each of which negatively regulates the expression of the other. In this case, there are also two inhibitors, each of which blocks translation for one of the two mRNA types. A diagram is shown above.

Interestingly, this type of circuit, called the “toggle switch,” was the first **synthetic circuit** (see Gardner et al. in Recommended Reading).

To pursue a Boolean analysis of this system, we will assume that the promoters and genes are always present and therefore need not be included in our equations or state matrix. Inhibitor 1 and Inhibitor 2 will be our inputs. There can be no translation of the first repressor if Inhibitor 1 is present, and no translation of Repressor 2 if Inhibitor 2 is present. The molecules of interest will be mRNA 1, Repressor 1, mRNA 2, and

Repressor 2. The processes we will track will be Transcription 1, Translation 1, Transcription 2, and Translation 2.

- (a) Write out Boolean rules for all of the molecules of interest and processes for this system.

We will abbreviate transcription as TRS/trs and translation as TRL/trl.

TRS 1: IF NOT (Repressor 2)
 TRL 1: IF (mRNA 1) and NOT (Inhibitor 1)
 TRS 2: IF NOT (Repressor 1)
 TRL 2: IF (mRNA 2) and NOT (Inhibitor 2)
 mRNA 1: IF (TRS 1) AFTER SOME TIME
 Repressor 1: IF (TRL 1) AFTER SOME TIME
 mRNA 2: IF (TRS 2) AFTER SOME TIME
 Repressor 2: IF (TRL 2) AFTER SOME TIME

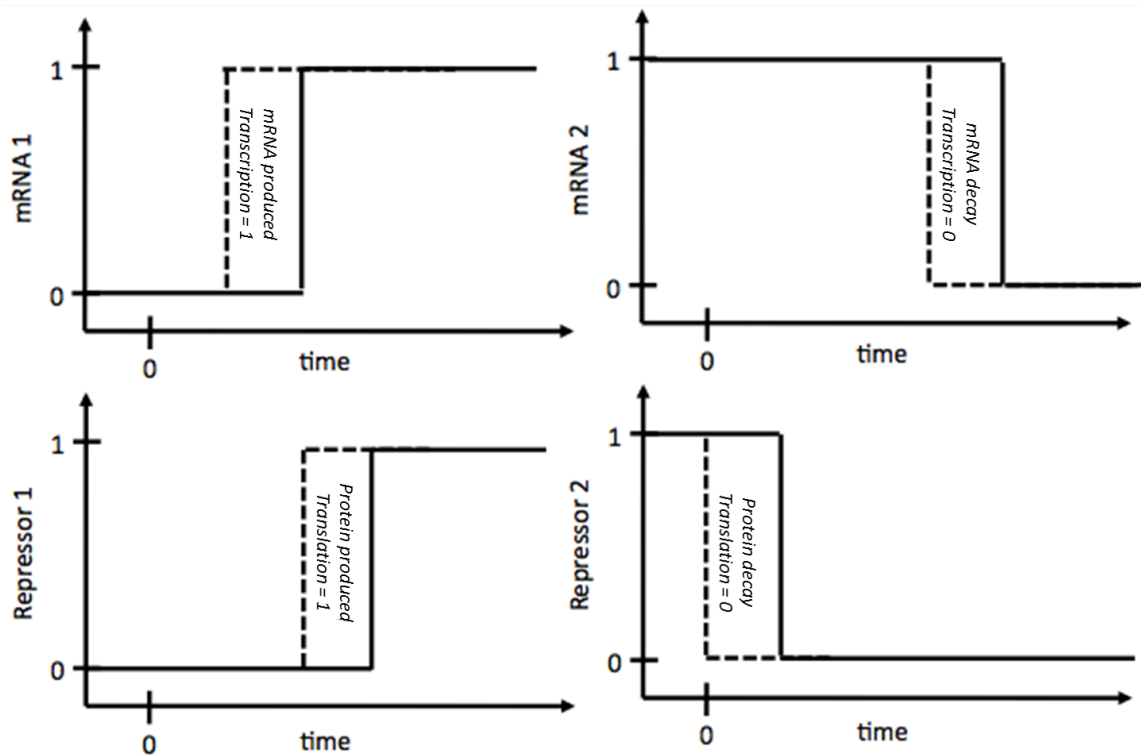
- (b) Calculate the state matrix. Circle or highlight any stable states.

Answer: The stable states are highlighted in yellow.

				Inhibitor 1 1		Inhibitor 2 0		Inhibitor 1 0		Inhibitor 2 1	
mRNA 1	R1	mRNA 2	R2	trs 1	trl 1	trs 2	trl 2	trs 1	trl 1	trs2	trl 2
0	0	1	0	1	0	1	1	1	0	1	0
0	0	1	1	0	0	1	1	0	0	1	0
1	0	1	0	1	0	1	1	1	1	1	0
1	1	0	0	1	0	0	0	1	1	0	0
1	0	1	1	0	0	1	1	0	1	1	0
1	1	1	0	1	0	0	1	1	1	0	0

- (c) Consider the stable state in which Inhibitor 1 is present and Inhibitor 2 is not. At time = 0, the culture medium is suddenly changed such that Inhibitor 2 is now present and Inhibitor 1 is not. Draw a time plot, and describe the progression of the system in terms of state transitions.

$(0,0,1,1) \rightarrow (0,0,1,0) \rightarrow (1,0,1,0) \rightarrow (1,1,1,0) \rightarrow (1,1,0,0)$



Changes in the inhibitors lead to degradation of Repressor 2 and production of mRNA 1, which is translated to Repressor 1, which finally represses the production of mRNA 2. This ability to switch from stable expression of one protein to another is why the circuit is called the “toggle switch.”

- (d) Using some of the typical time scales we discussed in this chapter, how long do you think the experiment described in (c) will take to reach a new stable state?

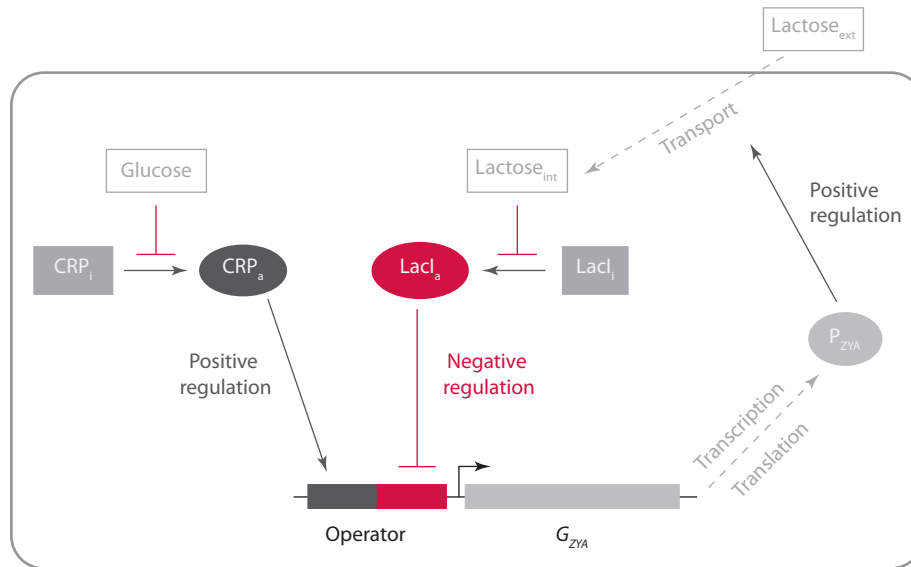
It would take several hours; the rate-limiting step would be the dilution/degradation of Repressor 2.

Problem 2.3: The lac operon regulatory network using Boolean logic

Now let's apply our Boolean methods to the analysis of a considerably more complicated bacterial regulatory network. The *lac operon* is required for the transport and metabolism of lactose in several enteric bacteria, including *E. coli*. The *lac* operon is still used as a powerful model for investigating aspects of gene regulation and provides the basis for many synthetic gene circuits. For more information on the pioneering work of Francois Jacob and Jacques Monod, who received the 1965 Nobel Prize in Medicine or Physiology for their work on gene regulation, see:

<http://symposium.cshlp.org/content/26/193.full.pdf+html>

The *lac* operon encodes three genes: *lacZ* (encoding the enzyme β -galactosidase, which cleaves lactose into glucose and galactose), *lacY* (the permease that transports lactose into the cell), and *lacA* (a transacetylase). The operon is controlled primarily by the *lac* repressor (LacI), which prevents transcription in the absence of lactose, and the glucose-regulated activator CRP. You will learn more about CRP in Chapters 7 and 10; for now, it's sufficient to know that CRP induces expression of the *lac* genes and is inactivated by glucose.



In the simplified version of the *lac* network illustrated above, there is one gene (G_{ZYA}) that is transcribed and translated to produce protein (P_{ZYA}) in the presence of an activator (CRP_a ; CRP is active in the absence of glucose) and the absence of a negative regulator ($LacI_a$; LacI is active in the absence of lactose). P_{ZYA} transports lactose into the cell if external lactose ($Lactose_{ext}$) is present.

The two inputs into this system are glucose and external lactose.

The four quantities that we wish to track are CRP_a , and $LacI_a$, internal lactose ($Lactose_{int}$), and P_{ZYA} . The four processes that we care about are the transport of external lactose into the cell, the synthesis of P_{ZYA} (we will lump transcription and translation into one process in this model), and the activation of LacI ($LacI_i$ to $LacI_a$) and CRP (CRP_i to CRP_a).

Assume that the inactive regulators, CRP_i and $LacI_i$, are present at stable levels inside the cell, and that G_{ZYA} is always present. These assumptions allow you to remove these quantities from your equations.

- (a) Initially, assume that activation of CRP and LacI happens much faster than transcription or transport. These assumptions allow you to eliminate two quantities and two processes from the system of equations. Write your equations for this simplified model.

$\text{Transport} = \text{IF Lactose}_{\text{ext}} \text{ AND } P_{ZYA}$
 $\text{Transcription} = \text{IF Lactose}_{\text{int}} \text{ AND (NOT Glucose)}$
 $\text{Lactose}_{\text{int}} = \text{Transport AFTER SOME TIME}$
 $P_{ZYA} = \text{Transcription AFTER SOME TIME}$

- (b) Build and fill in your state diagram. You may do this by hand or in Excel or MATLAB (Excel may be more useful in this particular case). Highlight the stable states of the system.

Answer:

LE	G	0	0	0	1	1	0	1	1
LI	P	Trp	Trc	Trp	Trc	Trp	Trc	Trp	Trc
0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	1	0	1	0
1	1	0	1	0	0	1	1	1	0
1	0	0	1	0	0	0	1	0	0

where LE = Lactose_{ext}, G = Gene, LI = Lactose_{int}, P = P_{ZYA}, Trp = Transport, and Trc = Transcription.

- (c) Is the system capable of oscillating? Identify any cyclic behavior and explain the logic behind it in biological terms.

Answer: Yes, with inputs LE = 1 and G = 0. If you start either with only P_{ZYA} or only Lactose_{int}, the system will oscillate. If you start with protein, you get transport (Lactose_{ext} is present). Now with Lactose_{int} present after some time and still no glucose, CRP is active and LacI is inactive, leading to transcription of P_{ZYX}. Similarly, if you start with Lactose_{int} but no P_{ZYX}, transcription leads to protein accumulation while Lactose_{int} decays, and the cycle continues.

- (d) Assume now that lactose transport across the membrane is slightly leaky. What will happen to the oscillations from (c) when external lactose is present in the absence of glucose? Could this leakiness be a useful attribute of the real system? Explain why or why not in biological terms.

Answer: With leaky transport, there will always be some Lactose_{int}. In the presence of Lactose_{ext} and no glucose, the system will stay active instead of oscillating. Thus, the *lac* operon would be expressed as long as Lactose_{ext} is present and glucose is absent.

- (e) Now consider the network without assuming instantaneous activation of CRP and LacI. What is your new set of equations?

Answer:

$Act_{CRP} = \text{IF NOT Glucose}$
 $Act_{LacI} = \text{IF NOT Lactose}_{int}$
 $Transport = \text{IF Lactose}_{ext} \text{ AND } P_{ZYA}$
 $Transcription = \text{IF (NOT LacI}_a) \text{ AND CRP}_a$
 $Lactose_{int} = \text{Transport AFTER SOME TIME}$
 $P_{ZYA} = \text{Transcription AFTER SOME TIME}$
 $LacI_a = Act_{LacI} \text{ AFTER SOME TIME}$
 $CRP_a = Act_{CRP} \text{ AFTER SOME TIME}$

(f) Build and show your new complete state diagram for the system in (e).

Answer:

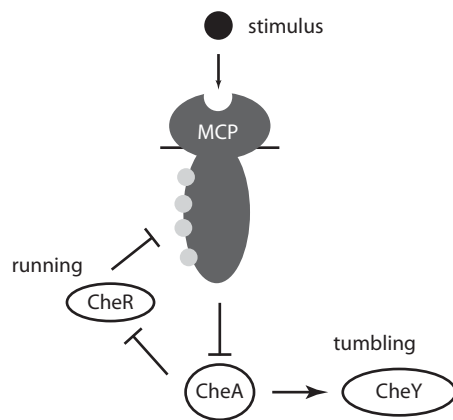
LE G				0 0				1 0				0 1				1 1			
C	L	LI	P	AC	AL	Trp	Trc	AC	AL	Trp	Trc	AC	AL	Trp	Trc	AC	AL	Trp	Trc
0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0
0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	1	1	0
0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0
0	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0
0	1	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	1	1	0
0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0
1	0	0	0	1	1	0	1	1	1	0	1	0	1	0	1	0	1	0	1
1	0	0	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1	1	1
1	0	1	0	1	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1
1	0	1	1	1	0	0	1	1	0	1	1	0	0	0	1	0	0	1	1
1	1	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0
1	1	0	1	1	1	0	0	1	1	1	0	0	1	0	0	0	1	1	0
1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0

where LE = Lactose_{ext}, G = Gene, C = CRP_a, L = LacI, LI = Lactose_{int}, P = P_{ZYA}, AC = Act_{CRP}, AL = Act_{LacI}, Trp = Transport, and Trc = Transcription.

(g) Highlight the stable states of the system in (e). Can this new system oscillate? Explain why this network behaves differently from the system in (c) under conditions of abundant external lactose and no glucose. Was the initial assumption to separate the timescales a reasonable one? Justify your answer.

Answer: In the matrix for (f), stable states appear in yellow (the two different shades separate the two stable states in the LE = 1, G = 0 case) and two different oscillatory loops appear in in blue and green. Changes in system behavior come from the time lag between the increase in Lactose_{int} through transport and the inhibition of LacI. The initial assumption was reasonable: protein synthesis/transport on the order of minutes, activation on the order of seconds. The simpler network, which takes this time-scale difference into account, may therefore better reflect the dynamics of the real system.

Problem 2.4. Bacterial chemotaxis



So far, we have focused on the regulation of gene expression, but the Boolean approach can also be used to analyze the dynamics of other biological circuits such as signaling networks, protein kinase cascades, and metabolic pathways. Here we will analyze a well-studied phenomenon: bacterial chemotaxis. In the lab, bacteria are most often cultured in homogenous environments that provide all of the nutrients and energy sources that the microorganisms need to grow and **proliferate**. However, in nature the environment in which the bacteria reside can be quite heterogeneous and dynamic. Bacteria need to be able to quickly sense the conditions of the surrounding environment and move toward food sources and/or away from dangerous chemicals or toxins. This process is called “chemotaxis.” This video gives a straightforward overview of the process:

<http://www.youtube.com/watch?v=1wW2CZz6nM4&feature=related>

Another interesting video tracks the motion of *E. coli* during chemotaxis:

<http://www.youtube.com/watch?v=EZ5ATNJfuCs>

Here, we will investigate the dynamics of bacterial motion using Boolean modeling. The above figure is a simplified representation of the chemotaxis network in *E. coli*. The methyl-accepting chemotaxis protein (MCP) is a transmembrane protein that binds potential food sources in the environment (stimulus). In the absence of these signals, the CheA protein autophosphorylates itself and transfers this phosphate group to the CheY and CheR proteins. The phosphorylated “active” form of CheY stimulates clockwise motion of the *E. coli* flagellum, which leads to tumbling. In contrast, when CheR is phosphorylated, it is inactive. When MCP is bound to a stimulus, it inhibits the kinase activity of CheA, leaving CheY in the inactive form and CheR in the active form; the bacterium therefore “runs.” However, CheR can also methylate the MCP **receptor**, which can cause CheA to regain its phosphorylation activity. This system therefore exhibits a form of negative feedback.

Assume that all of the activation and repression steps happen after some time. Also assume that CheA and CheR have a default active state (they are activated in the absence of the repressor) and that the repression of MCP by CheR overcomes activation by a stimulus.

We will model four processes of this system: activation of MCP (ActMCP), activation of CheA (ActCheA), activation of CheY (ActCheY), and activation of CheR (ActCheR). You are interested in the active forms of MCP, CheA, CheY, and CheR. We have one constant input into the system: the stimulus.

(a) Write out equations for each of the reactions and molecules of interest.

Answer:

ActMCP = IF stim AND NOT CheR

ActCheA = IF NOT MCP

ActCheY = IF CheA

ActCheR = IF NOT CheA

MCP = IF ActMCP AFTER SOME TIME

CheA = IF ActCheA AFTER SOME TIME

CheY = IF ActCheY AFTER SOME TIME

CheR = IF ActCheR AFTER SOME TIME

(b) Calculate the state matrix, and highlight any stable states.

Answer:

				Stimulus = 0				Stimulus =1			
MCP	CheA	CheY	CheR	actMCP	actCheA	actCheY	actCheR	actMCP	actCheA	actCheY	actCheR
0	0	0	0	0	1	0	1	1	1	0	1
0	0	0	1	0	1	0	1	0	1	0	1
0	0	1	0	0	1	0	1	1	1	0	1
0	1	0	0	0	1	1	0	1	1	1	0
1	0	0	0	0	0	0	1	1	0	0	1
0	0	1	1	0	1	0	1	0	1	0	1
0	1	0	1	0	1	1	0	0	1	1	0
1	0	0	1	0	0	0	1	0	0	0	1
0	1	1	0	0	1	1	0	1	1	1	0
1	0	1	0	0	0	0	1	1	0	0	1
1	1	0	0	0	0	1	0	1	0	1	0
0	1	1	1	0	1	1	0	0	1	1	0
1	0	1	1	0	0	0	1	0	0	0	1
1	1	0	1	0	0	1	0	0	0	1	0
1	1	1	0	0	0	1	0	1	0	1	0
1	1	1	1	0	0	1	0	0	0	1	0

- (c) Assume that your system starts from a state where no stimulus is present and none of the proteins of interest are activated. Trace out the dynamics of the system on your state matrix as the system moves from state to state. What happens to the system? What does your observation imply about bacterial chemotaxis in the absence of a stimulus?

Answer:

				Stimulus = 0			
MCP	CheA	CheY	CheR	actMCP	actCheA	actCheY	actCheR
0	0	0	0	0	1	0	1
0	0	0	1	0	1	0	1
0	0	1	0	0	1	0	1
0	1	0	0	0	1	1	0
1	0	0	0	0	0	0	1
0	0	1	1	0	1	0	1
0	1	0	1	0	1	1	0
1	0	0	1	0	0	0	1
0	1	1	0	0	1	1	0
1	0	1	0	0	0	0	1
1	1	0	0	0	0	1	0
0	1	1	1	0	1	1	0
1	0	1	1	0	0	0	1
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1	1	1	0	0	0	1	0
1	1	1	1	0	0	1	0

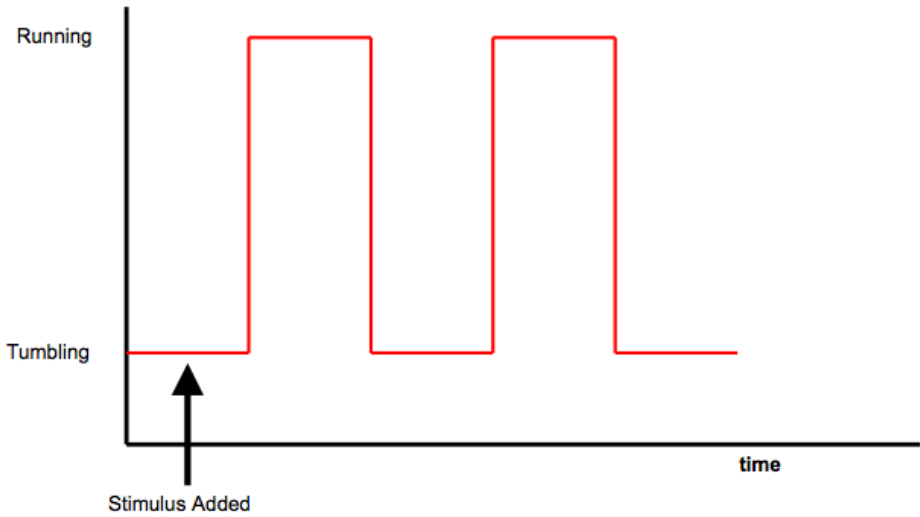
In this sample trace of the dynamics, (1) the absence of all active proteins leads to the activation of CheA and CheR, and (2) CheA then represses CheR and activates CheY, which is a stable state that promotes tumbling. Therefore, without stimulus this model predicts tumbling.

- (d) Starting from the state that you ended with in (c), assume that a stimulus is suddenly added to the environment. What happens to the network? Trace the new dynamics on a state matrix, and graph the behavior we would expect the bacterium to exhibit (running or tumbling) over time.

Answer:

				Stimulus =1			
MCP	CheA	CheY	CheR	actMCP	actCheA	actCheY	actCheR
0	0	0	0	1	1	0	1
0	0	0	1	0	1	0	1
0	0	1	0	1	1	0	1
0	1	0	0	1	1	1	0
1	0	0	0	1	0	0	1
0	0	1	1	0	1	0	1
0	1	0	1	0	1	1	0
1	0	0	1	0	0	0	1
0	1	1	0	1	1	1	0
1	0	1	0	1	0	0	1
1	1	0	0	1	0	1	0
0	1	1	1	0	1	1	0
1	0	1	1	0	0	0	1
1	1	0	1	0	0	1	0
1	1	1	0	1	0	1	0
1	1	1	1	0	0	1	0

Again, the dynamics are traced out as a sequence of arrows labeled 1-6. The system cycles between activated CheY (tumbling) and activated CheR (running).



(e) Now assume that the bacterium has a mutation in MCP that prevents its negative regulation by CheR, such that:

$$\text{ActMCP} = \text{IF Stimulus}$$

Draw a new state matrix corresponding to this new set of rules, determine the stable state of the new system with no stimulus present, and then trace the dynamics of the system upon addition of stimulus using a state matrix. How is your answer different from (d)? What is the purpose of CheR's negative regulation of MCP activity?

Answer: The state matrix in the absence of stimulus remains the same. Adding stimulus results in the following state matrix:

				Stimulus =1			
MCP	CheA	CheY	CheR	actMCP	actCheA	actChe	actCheR
0	0	0	0	1	1	0	1
0	0	0	1	1	1	0	1
0	0	1	0	1	1	0	1
0	1	0	0	1	1	1	0
1	0	0	0	1	0	0	1
0	0	1	1	1	1	0	1
0	1	0	1	1	1	1	0
1	0	0	1	1	0	0	1
0	1	1	0	1	1	1	0
1	0	1	0	1	0	0	1
1	1	0	0	1	0	1	0
0	1	1	1	1	1	1	0
1	0	1	1	1	0	0	1
1	1	0	1	1	0	1	0
1	1	1	0	1	0	1	0
1	1	1	1	1	0	1	0

The diagram shows three arrows tracing a path through the state matrix. Arrow 1. points from the state (MCP=1, CheA=0, CheY=0, CheR=1) to the state (MCP=0, CheA=1, CheY=1, CheR=0). Arrow 2. points from that state to the state (MCP=1, CheA=0, CheY=1, CheR=0). Arrow 3. points from that state back to the state (MCP=1, CheA=0, CheY=0, CheR=1), completing a cycle.

The arrows trace out the dynamics. (1) Initially, MCP is activated, which leads to (2) inactivation of CheA and (3) subsequent activation of CheR and inactivation of CheY. The system is therefore stably in a “running” state. The regulation of MCP by CheR ensures that some tumbling will occur, which allows the bacterium to change direction.