Quantitative Bootcamp Dynamical Systems Tutorial

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Contents

0.1 Dynamical analysis of cell cycle progression

In this tutorial, we will explore how one can turn simple biochemical circuit diagrams into mathematical equations and then analyse those equations, both graphically and numerically, to get insights into circuit's dynamics.

We will focus on one of the most intensively studied biochemical circuits in all of biology, the circuit that controls cell cycle progression in eukaryotic cells. The state of the cell cycle is encoded by the activity of cyclin-dependent kinases (CDKs). CDKs complex with proteins called Cyclins, which undergo cycles of synthesis and destruction over time. When complexed with Cyclin, Cdk can become active and phosphorylate different target proteins to regulate diverse cell cycle functions.

In some cells, such as early embryonic cells,the cell cycle is a freely running oscillator, in which Cyclin levels, and CDK activity, go up and down with clock-like regularity. Other cells progress through the cell cycle in a series of steps, involving switch-like transitions in CDK activity (low $\rightarrow$ high or high $\rightarrow$ low), that are controlled by external inputs like nutrient availability or by internal checkpoint controls that monitor the completion of key events like DNA replication or chromosome alignment. As you will see, switch-like transitions and oscillatory dynamics are intimately connected.

0.2 A biochemical circuit for cell cycle control.

Figure 1 summarizes a set of basic facts about the core biochemical circuit that is thought to generate cell cycle oscillations. Cyclin protein is synthesized at a constant rate. It forms a complex with Cdk1 (Cdk1-Cyclin) which is rapidly and constitutively activated by the Cdk-activating kinase (CAK, not shown).

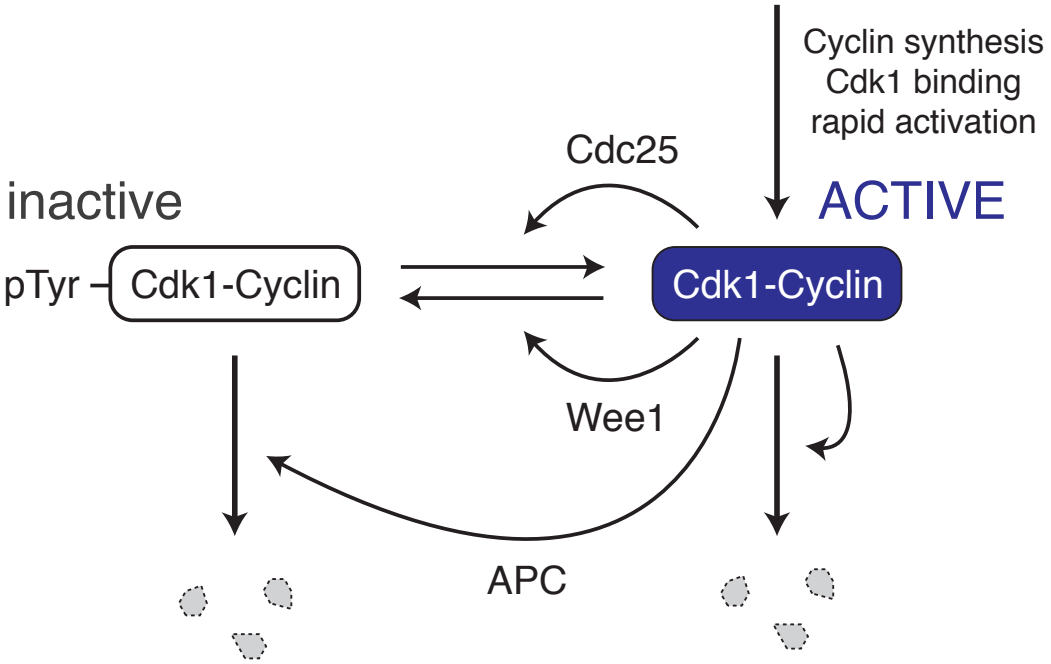

Figure 0.1 Biochemical circuit view of the core cell cycle oscillator

Active Cdk1-Cyclin then participates in three feedback loops: (1) It inhibits a tyrosine kinase called Wee1, which phosphorylates and inactivates Cdk1; (2) It activates a protein phosphatase called Cdc25, which dephosphorylates and reactivates Cdk1 and (3) It promotes the activity of the APC complex which in turn promotes the destruction of Cdk1-Cyclin complexes (both active and inactive).

Question: A feedback loop is called positive if it is self-reinforcing (e.g. active Cdk1-Cyclin promotes more active Cdk1-Cyclin) and negative if it is self-antagonizing. Which of these feedback loops is positive and which is negative?

In this tutorial, you will explore whether and how these feedback loops can account for two key properties of cell cycle control observed in experiments:

- The ability to convert slow changes in Cyclin levels into switch-like (all-or-none)

changes in Cdk1 activity.

- The ability to produce free-running oscillations in the absence of any additional inputs.

Session 1

Analysis of a very simple circuit

Lets start by analyzing a very simple circuit in which the Cdk1-Cyclin complex undergoes rapid conversion between inactive and active states at constant rates.

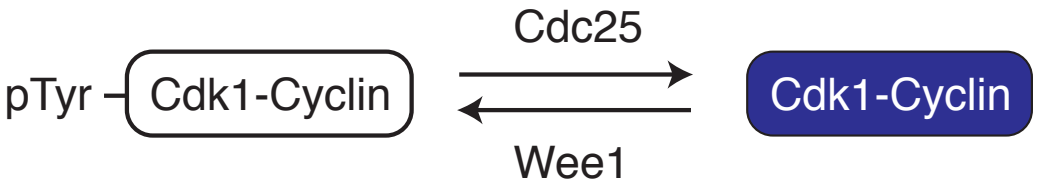

Figure 1.1 Simple interconversion of active and inactive Cdk1 at constant rates

From experimental studies, we know that Cyclin binds Cdk1 with very high affinity and that Cdk1 is always much more abundant than Cyclin. We will assume throughout this tutorial that all available Cyclin is complexed with Cdk1, and therefore that the total amount of the complex is the same as the total amount of Cyclin. From now on, we will refer to the Cdk1-Cyclin complex simply as Cdk1.

We want to write an equation that describes how the rate of change in active Cdk1 depends on the current amounts of active and inactive Cdk1. If we let C represent the total amount of Cyclin protein, and A represent the amount of active Cdk1, then the amount of inactive Cdk1 is $(C - A)$.

The total rate of change in active Cdk1 is equal to the rate at which inactive Cdk1 becomes active minus the rate at which active Cdk1 becomes inactive. If each molecule of inactive Cdk1 becomes active with a fixed probability per unit time described by k_{act}, then the total activation rate is $k_{act}(C - A)$. Likewise, if each molecule of active Cdk1 becomes

inactive with a probability per unit time described by k_{inact}, then the inactivation rate is $k_{inact}A$.

Putting these together, we can write the following differential equation:

$$\frac{dA}{dt} = k_{act}(C - A) - k_{inact}A \tag{1.1}$$

Equation 1.1 specifies how the rate of change in active Cdk1 depends on its current level and on the three parameters: C, k_{act} and k_{inact}. You could solve this equation with pencil and paper if you knew the values of all three parameters. However, this is almost never true for more complicated circuits. Here you will learn approaches to solving such equations that do not require pencil and paper. We will focus on two complementary approaches: graphical and computational.

1.1 Graphical approach: Flux balance analysis

Lets start with a simple graphical approach called flux balance analysis. The basic idea is to divide the right hand side of Equation 1 into a positive contribution, the positive flux and a negative contribution, the negative flux. As you will see, plotting the positive and negative fluxes as a function of A can tell us almost everything we want to know about the dynamics of this circuit. Lets start by defining a list of parameters in R and specifying provisional values:

Here and throughout the tutorial, when you encounter a piece of R code, your response should be to type (or copy and paste) the code into an R terminal window and execute:

```
params <- list (k_act=0.1 , k_inact=0.1 , C=100 )
```

Now we define R functions for the positive and negative flux contributions:

```
posflux <- function(A, params) {
    return (params\$k_act * (params\$C-A) )
}

negflux <- function(A, params) {
    return (params\$k_inact * A )
}
```

Now plot the positive and negative fluxes as a function of A:

```
A = seq (0 , 100 , 1 )
plot(A,posflux(A,params), type="l", col="red", main="Flux balance", xlim
    =c(0, 100), ylim=c(0, 10))

lines(A,negflux(A,params), col="blue")
```

The interpretation of this plot is very simple: Every possible state of the system is represented by a point on the horizontal axis, corresponding to a particular amount of A. The sum of the positive and negative flux equals dA/dt, the total rate of change in A. So dA/dt is positive when the positive flux is greater than the negative flux; it is negative when the positive flux is less than the negative flux and it is 0 (i.e. the system is at steady state) when the two fluxes are equal. This means that from the flux balance plot, for any current value of A, we can read off immediately how A will change in the next instant of time!

Note that there is a unique steady state point at which the positive and negative curves cross one another, and therefore where $dA/dt = 0$. The molecular meaning of this steady state point is that Cdk1 molecules are becoming activated exactly as fast as they are becoming inactivated, so the overall state of the system is constant.

Exercise 1.1

Question: Do you think this steady state is stable or unstable? That is, if you pushed the value of active Cdk1 up or down a little, would it return to the steady state value or move further away?

Here is a simple way to answer this question: Reproduce the above plot on a sheet of paper. Beneath the plot, at different points along the active Cdk1 (A) axis, draw arrows whose direction represents the sign of the difference between the positive and negative flux, and whose magnitude represents the magnitude of that difference. These arrows tell you, for a given value of active Cdk1, in what direction and how fast the amount of active Cdk1 is changing. Reading these arrows, is the steady state point stable or unstable?

1.2 Numerical approach

An complementary approach is to solve Equation 1 numerically, using a computer to solve for $A(t)$ which tells us how the state of the system changes as a function of time. To do so, we need to define a function that represents the right hand side of Equation 1; we need to supply particular values for the parameters k_{act}, k_{inact} and C; an initial value for A, and a sequence of time points at which to evaluate the solution.

Lets start by importing a package called desolve, which defines the relevant functions:

```
## Import the ‘ deSolve‘ package
2
library (deSolve)
```

We have already defined the list of parameters above. Now we define the right hand side function:

```
rhs <- function(t, A, params) {
    return (with (params, list (k_act * (C-A) - k_inact*A)))
}
```

We define a starting value for A:

```
A0 <- 0.0
```

and we define a sequence of time points at which to evaluate the solutions:

```
t <- seq (0 , 100 , 1 )
```

Now we call a numerical ODE solver function called ode to solve the equations, passing it the initial value A0, the time sequence t, the right hand side function rhs, and the list of parameters params:

```
out <- ode (A0, t, rhs, params)
```

The ode solver returns the solution in the form of an array of values of A at the specified time points contained in t.

We can plot this solution as follows:

```
plot(out, lwd=2, main="Simple Circuit", xlim=c(0, 100), ylim=c(0, 100))
```

Of course, it is very easy to evaluate the solution for a different initial value of A and add that solution to our plot:

```
A0=10
out <- ode (A0, t, rhs, params)
lines (out, lwd=2 )
```

Exercise 1.2

Try repeating this for a number of different initial conditions. How does the long term (steady state) behavior of this circuit depend on the initial conditions? Does this agree with what you learned from the graphical analysis?

1.3 Plotting the steady state level of Cdk1 as a function of Cyclin input

A key question that we would like to answer is: How does the steady state level of active Cdk1 vary as the amount of Cyclin changes? Again, there are two complementary ways to address this question: graphical and numerical.

Graphical construction of steady state response to cyclin levels:

The graphical approach is a simple extension of the flux balance analysis: For each value of C, the flux balance plot tells us what the steady state value of A will be. Note that in Equation 1, only the positive flux (the activation rate) depends on C. This suggests the following simple approach: Plot the negative flux once, and then plot the positive flux for a sequence of different values of C and observe how the position of the crossing (the steady state value of A) varies with C.

Here is a start:

```
A = seq (0 , 100 , 1 )
plot(A,negflux (A,params), type="l" , col="red" , main="Flux balance
    plot" ,   xlim=c (0 , 100 ), ylim=c (0 , 10 ))

params\$C=0
lines(A,posflux(A,params), col=blue)

params\$C=10
lines(A,posflux(A,params), col=blue)
```

Exercise 1.3

Now continue for a sequence of increasing values of C. On a separate sheet of paper, sketch a graph that shows (qualitatively) the relationship between the steady state level of Cdk1 and the input level of Cyclin.

Question: What is the shape of this graph?

Question: How does the fraction of active Cyclin/Cdk1 relative to the amount of Cyclin change as the total amount of Cyclin increases?

Actually, a slicker way to do this would be to use a simple for loop:

```
A = seq (0 , 100 , 1 )
plot (A,negflux (A,params), type="l" , col="red" , main="Flux balance
    plot" ,    xlim=c (0 , 100 ), ylim=c (0 , 10 ))

for (C in seq (0 ,200 ,10 )){
    params\$C=C
    lines (A,posflux (A,params), col="blue" )
}
```

1.4 Numerical construction of steady state response to cyclin levels:

Here is another way to construct the same graph numerically: Start with C = 0. Set the initial value of A to 0 and solve Equation 1 numerically to find the steady state level of A.

```
    plot(x=NULL,y=NULL,xlim=c(0,100),ylim=c(0,100),xlab="Cyclin level",
        ylab="Steady State Cdk1")
    params\$C=0
    A0 = 0
    out <- ode (A0, t, rhs, params)
    points(params\$C,out[101,2])
```

Now increase C by a small amount; use the previous steady state value for A as a new initial value and solve again for steady state.

```
    params\$C=10
    A0 = out[101,2]
    out <- ode (A0, t, rhs, params)
    points(params\$C,out[101,2])
```

Exercise 1.4

Repeat this process many times as you step params$C from 0 to some very high level (say params$C = 200), adding each steady state value to your plot.

Hint: try using a simple for loop to accomplish the this task.

Question: How does this plot compare to the qualitative plot you constructed on paper using flux balance analysis?

Important note: For this very simple scenario, we could have gotten this answer much more directly by setting $dA/dt = 0$ in Equation 1 and then solving algebraically for A in terms of C, k_{act} and k_{inact} to obtain:

$$0 = k_{act}(C - A) - k_{inact}A \implies A = \frac{k_{act}C}{k_{act} + k_{inact}} \tag{1.2}$$

However, this is only possible because the right hand side of Equation 1 is very simple. As soon as we add more complicated feedback, the equations we write down become impossible to solve this way!

Challenge: Above you constructed a response curve in which the input is the level of Cyclin and the response is the steady state level of Cdk1. Use the same approaches to construct a response curve in which the input is the rate of Cdk1 activation k_{act}. Now do the same for the rate of Cdk1 inactivation k_{inact}.

Session 2

How does positive feedback lead to switch-like activation of Cdk1?

For the simple circuit that you analyzed above, the steady state level of active Cdk1 deponds linearly on the level of Cyclin input. Now let's ask how this steady state response changes when we add the two feedback loops in which (a) active Cdk-1 activates Cdc-25 and Cdc-25 promotes activation of Cdk-1, (b) active Cdk-1 inhibits Wee-1 and Wee-1 promotes inactivation of Cdk-1.

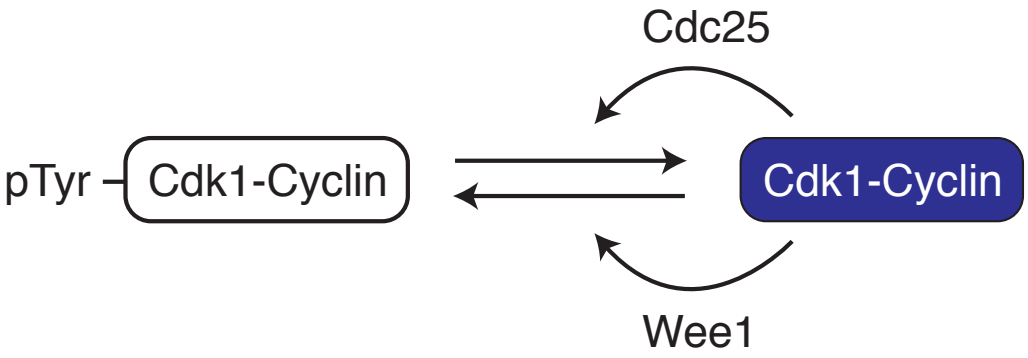

Figure 2.1 Interconversion of active and inactive Cdk1 with feedback control of activation and inactivation rates

Now the rate of Cdk1 activation is proportional to the product of Cdc25 enzyme activity and inactive Cdk1, and the rate of inactivation is proportional to the product of Wee1 enzyme activity and active Cdk1:

$$\frac{dA}{dt} = Cdc25(A) \cdot (C - A) - Wee1(A) \cdot A \tag{2.1}$$

We write Cdc25(A) and Wee1(A) in Equation 2 to reflect the fact that Cdc25 and Wee1 activities themselves depend on how phosphorylated these enzymes are (through the feedback loops involving Cdk1): Phosphorylated Cdc25 is active; phosphorylated Wee1 is inactive. To build our model, we will use empirical data showing what these functions look like. The dependencies of Cdc25 and Wee1 phosphorylation on Cdk1 activity have been measured experimentally in cytoplasmic extracts and they look like this:

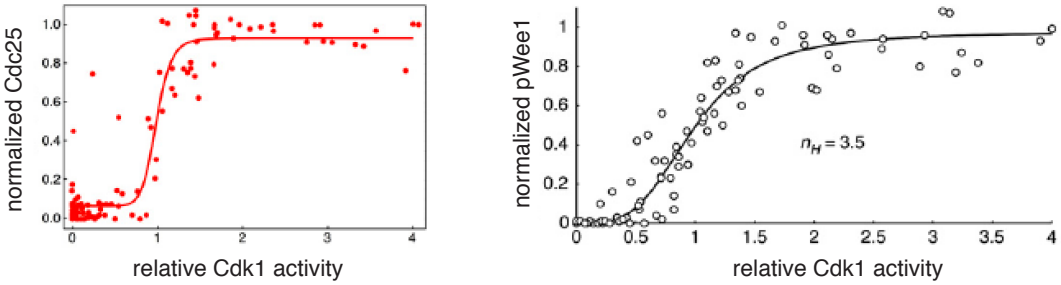

Figure 2.2 Normalized levels of phosphorylated (active) Cdc25 and phosphorylated (inactive) Wee1 as a function of Cdk1 activity

This kind of S-shaped, or sigmoidal dependence is very common in biology. The sharp rise in output level above some threshold value of the input is called ultrasensitivity. Ultrasensitive responses can arise in many ways in biochemical circuits, for example through cooperative binding, or multisite phosphorylation, or substrate competition (see Ferrell and Ha, 2013 for a nice discussion of this). The saturation of the response at high levels of input reflects the fact that there exists a maximum number of Cdc25 or Wee1 molecules that can become active.

Hill functions are a very useful way to represent sigmoidal responses in mathematical models. Originally invented by Archibald Hill to describe the sigmoidal binding curve for O_2 to hemoglobin, The equation for a Hill function looks like this:

$$\mathsf{Hill}(x, K, n) = \frac{x^n}{K^n + x^n} \tag{2.2}$$

where x is the input variable, and K and n are parameters.

Lets define the Hill function in R and graph it for a particular choice of K and n values.

```
hill <- function(X,K,n) {
    return(X^n/(K^n + X^n)
}

x = seq(0,4,0.1)
plot(x,hill(x,1,4),xlim=c(0,4),ylim=c(0,1),xlab=X,ylab=Hill(X))
```

How do the parameters K and n control the shape of the Hill function? Lets plot $\mathsf{Hill}(x, K, n)$ for a fixed value of K ($K = 1$) and a range of different n values:

```
    plot(x=NULL,y=NULL,xlim=c(0,4),ylim=c(0,1),xlab="X",ylab="Hill(X)")
    for (n in seq(1,10,1)) {
        lines(x,hill(x,2,n))
    }
```

Exercise 2.1

Now repeat the above, but this time plot the Hill function for a fixed value of n (say n = 4), and a range of K values.

Question: What key features of the Hill functions shape do K and n control?

The solid lines in Figure 4 above show the results of fitting Hill functions to the experimental measurements of phosphorylated (active) Cdc25 and Wee1 vs Cdk, yielding Hill coefficients n = 3.5 for Wee1 and n = 11 for Cdc25. These fits justify the use of Hill functions to represent Cdc25(A) and Wee1(A) in Equation 2 above. That is, we can write:

$$Cdc25(A) = a_{Cdc25} + f_{Cdc25}\frac{A^{11}}{K_{Cdc25}^{11} + A^{11}} \tag{2.3}$$

where a_{Cdc25} represents the basal level of Cdc25 activity, f_{Cdc25} is the strength of Cdk1 feedback, and K_{Cdc25} represents the threshold for this feedback.

Similarly, we can write:

$$Wee1(A) = a_{inact} + f_{inact}\frac{K_{inact}^{3.5}}{K_{inact}^{3.5} + A^{3.5}} \tag{2.4}$$

where a_{inact} represents the minimum level of Wee1 activity when fully inhibited, f_{inact} is the additional activity when uninhibited, and K_{inact} is the threshold for inhibitory feedback.

Importantly, the values of a_{Cdc25}, a_{inact}, f_{Cdc25}, f_{inact}, K_{Cdc25}, and K_{inact} have all been estimated by fitting equations 2.3 and 2.4 to experimental data (see Xiong and Ferrell, 2013).

Using these values, and substituting our expressions for $Cdc25(A)$ and $Wee1(A)$ into Equation 2 above gives:

$$\frac{dA}{dt} = \left(0.05 + 1.2\frac{A^{11}}{34^{11} + A^{11}}\right) \cdot (C - A) - \left(0.08 + 0.4\frac{A^{3.5}}{33^{3.5} + A^{3.5}}\right) \cdot A \tag{2.5}$$

This equation may look complicated, but it is built in straightforward way out of the pieces discussed above. If it looks overwhelming go back and see where each piece came from. Note here that we've suppressed the units for protein concentrations for simplicity. In Equation 4, the rate of change in A depends only on A itself, and on the level of Cyclin (C). However, unlike the simple circuit, we can no longer solve Equation 4 with pencil and paper. Here is where flux balance analysis and numerical solutions become really useful! Lets apply the same tools we used for the simple circuit above to analyze the more complicated dynamics of this feedback circuit. As before, we will first characterize active Cdk1 dynamics for a particular level of Cyclin; then well explore how the steady state Cdk1 levels vary with Cyclin levels.

2.1 Flux balance analysis of the positive feedback circuit

Lets start by defining a list of parameters and assigning their values from the published data:

```
feedback_params <- list(a_act = 0.16, f_act = 0.8, K_act = 34, n_act =
    11, a_inact = 0.08, f_inact = 0.4, K_inact = 33, n_inact = 3.5, C =
    60)
```

Note that we have assigned a nominal value for Cyclin ($C = 60$).

Exercise 2.2

Modify the flux balance analysis approach that we used in Section 1.1.1 to identify the steady states for this system and determine their stability: i.e. first define new positive and negative flux functions based on terms from the right hand side of Equation 4; then plot these functions vs A.

Question: How many steady state crossings are there? which of these do you think are stable and which are unstable?

As you did for the simpler case above, answer this question by reproducing the flux balance plot and drawing arrows below the Cdk1 axis that indicate the magnitude and direction of the rate of change in Cdk1 for different values of Cdk1.

Exercise 2.3

Numerical analysis of the positive feedback circuit

Now verify your conclusions from the flux balance analysis by solving Equation 4 numerically for different starting values of Cdk1. Following the approach we used in Section 1.1.2, define a new function for the right hand side of Equation 4, and define a sequence of time values at which to evaluate the solutions. Now choose a bunch of different starting values for A, and for each one, solve the equations numerically and plot the solutions.

Question: How many different stable steady state points are there?

Question: For what range of initial A values does A approach each of these stable steady states?

Question: What happens if you try to pick starting values closer and closer to an unstable steady state?

Question: Do you think the circuit could remain at this point for very long if it were operating in a real cell? Why or why not?

Summary: A key take-home message of this analysis is that, at least for some Cyclin

levels, this circuit can exhibit what we call bistable dynamics - it can home in on and remain stably at one of two possible states, depending on initial conditions. How do bistable dynamics shape the response of the circuit to changing levels of Cyclin?

Exercise 2.4

Map the steady steady response to changing levels of Cyclin.

We are particularly interested in asking how steady state levels of Cdk1 activity change as the levels of Cyclin slowly increase. Can positive feedback generate a switch-like response in Cdk1 activity to slow increases in Cyclin, as observed during cell cycle progression in living cells? Again we can answer these questions using a combination of graphical and numerical approaches:

Graphical construction of steady state response to cyclin levels: Lets start by adapting the graphical approach that we used for the simple circuit above. As for the simper circuit, only the positive flux depends on the value of C. So again, you can plot the negative flux vs A once, and then plot the positive flux vs A for a range of different values of C. Modify the code we used in Section 1.1.3 to do this.

Of course now, for some values of C, there are multiple stable and unstable steady states. As you vary C, these steady states move continuously along the A axis. This is more complicated than the simple circuit, but you can still make sense of whats happening:

As before, on a separate sheet of paper, construct a graph in which the horizontal axis represents the Cyclin level (C) and the vertical axis represents steady state levels of Cdk1(A). Draw a curve with a solid line to indicate the changing positions of stable steady states and curves with a dashed line to indicate the positions of unstable steady states.

We call this graph a bifurcation diagram.

Question: What does this bifurcation diagram tell you about the steady state response to changing Cyclin levels? How do you make sense of this when there are multiple possible steady state values for some levels of Cyclin?

Take a few moments to ponder these questions and discuss with your partners before going to the next step.

2.2 Numerical construction of steady state response to cyclin levels

Your analysis shows that for an intermediate range of C values, the circuit exhibits bistable dynamics: I.e. for some values of C, the circuit can adopt one of two possible stable steady

states, depending on initial conditions. This suggests that the steady state response of this circuit to changing Cyclin levels will depend on the history of prior Cyclin levels.

Exercise 2.5

During cell cycle progression, Cyclin and active Cdk1 levels both start out low, and steady state Cdk1 activity levels respond continuously to slow increases in Cyclin levels. Revise the numerical approach that we used for the simple circuit in Secrtion 1.1.4 above to determine the steady state Cdk1 activity responds to slowly increasing Cyclin levels.

Start with low values for C and A; solve to find a steady state value for C. Now increase C in a series of steps. At each step solve the equations numerically, using the previous steady state level of A as a new initial value, and then plot the steady state value of Cdk1 (A) vs the level of input Cyclin.

Question: How does this plot differ from the one you constructed earlier for the simple circuit lacking positive feedback? How does it relate to the bifurcation diagram you constructed in the last step?

Exercise 2.6

Now repeat the same exercise, but this time start with a very high level of Cyclin, and map out the steady state Cdk1 response as you decrease the Cyclin level stepwise towards zero.

Now, on the same graph, plot the steady state Cdk1 values that you obtained by increasing Cyclin slowly from a low value and by decreasing Cyclin slowly from a high value.

Question: How do these two plots differ and why? How do these two plots relate to the bifurcation diagram?

Optional Challenge: What is the point of having two feedback loops instead of just one?

One possibility is that two feedback loops make the switchlike behavior of the circuit more robust with respect to variation in parameter values. Here is a way to test this idea:

Looking at the bifurcation diagram you constructed above, choose an intermediate value of Cyclin for which the circuit exhibits bistable dynamics.

First, consider the parameter f_{inact}, which controls the strength of feedback through Wee1 and effectively controls the maximum rate of Cdk1 inactivation. Use flux balance analysis, ask: For what range of values of f_{inact} does the circuit continue to exhibit bistable dynamics?

Now consider a reduced circuit in which there is no feedback through Wee1, i.e the rate of Cdk1 inactivation is controlled by a constant parameter k_{inact}, as in Equation 1. Again use flux balance analysis to ask: For what range of values of k_{inact} does the circuit continue to exhibit bistable dynamics? What version of the circuit tolerates a larger range of variation in Cdk1 inactivation rates?

Session 3

From switch-like transitions to free-running oscillations: Adding negative feedback.

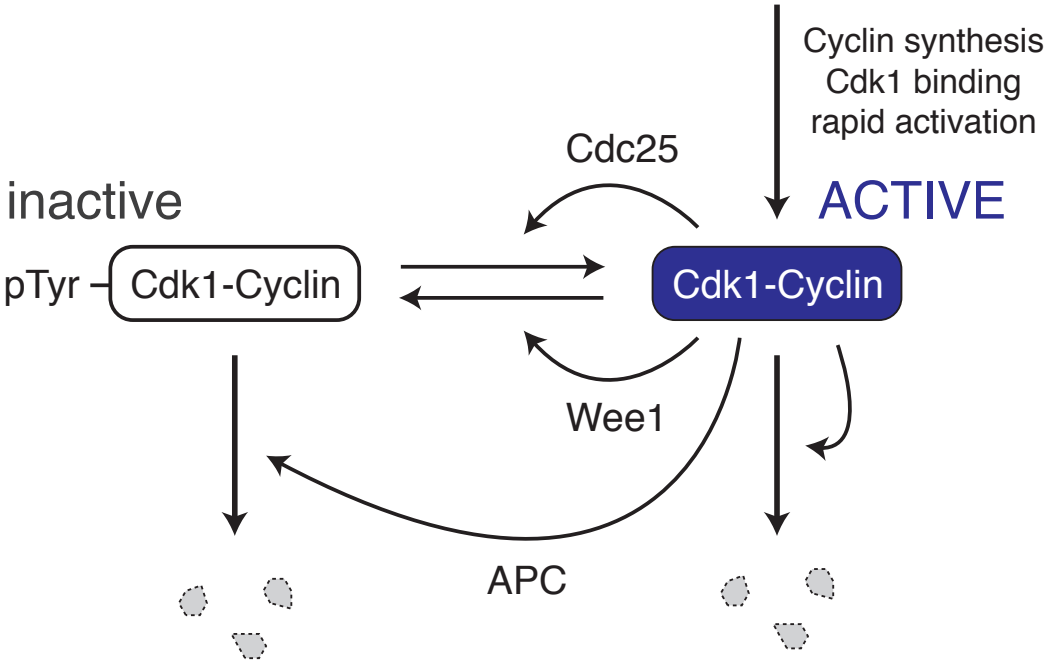

Figure 3.1 The full circuit with negative feedback

So far we have considered Cyclin levels as an external input or control variable. In real cells, Cyclin synthesis and degradation are coupled to Cdk1 activity: As Cyclin builds up above a threshold, Cdk1 becomes active; active Cdk1 in turn promotes APC-dependent degradation of Cyclin, causing Cyclin levels to fall rapidly again. Figure 3.1 again is a schematic view of the Cyclin/Cdk1 circuit with this additional negative feedback.

Could switch-like activation of Cdk1 and negative feedback in which active Cdk1 promotes APC-dependent Cyclin degradation lead to robust free-running oscillations in Cyclin and Cdk1 activity? Again, we can use simple mathematical models to explore how this works. Lets start with a simple thought experiment.

Exercise 3.1

A simple thought experiment: Consider the bifurcation diagram and the response curves for steady state Cdk1 vs Cyclin that you constructed in Part 2. Discuss with your partners how negative feedback in which Cdk1 activates APC and Cyclin degradation could drive the rise and fall of Cyclin levels and Cdk1 activity.

Now lets test your intuition by adding negative feedback to our circuit model, based on the arrows in Figure 5 above. We need to write an additional equation for the rate of change in Cyclin due to synthesis and degradation:

$$\frac{dC}{dt} = k_{syn} - k_{deg}(A) \cdot C \tag{3.1}$$

Here, we have written $k_{deg}(A)$ to reflect the fact that the degradation rate is a function of APC activity which in turn depends on the level of Cdk1 activity. Recent experimental measurements from the James Ferrells lab show that, like Cdc25 and Wee1, APC activity is a switch-like function of Cdk1 activity, which is well-fit by a Hill function with Hill coefficient $n = 17$:

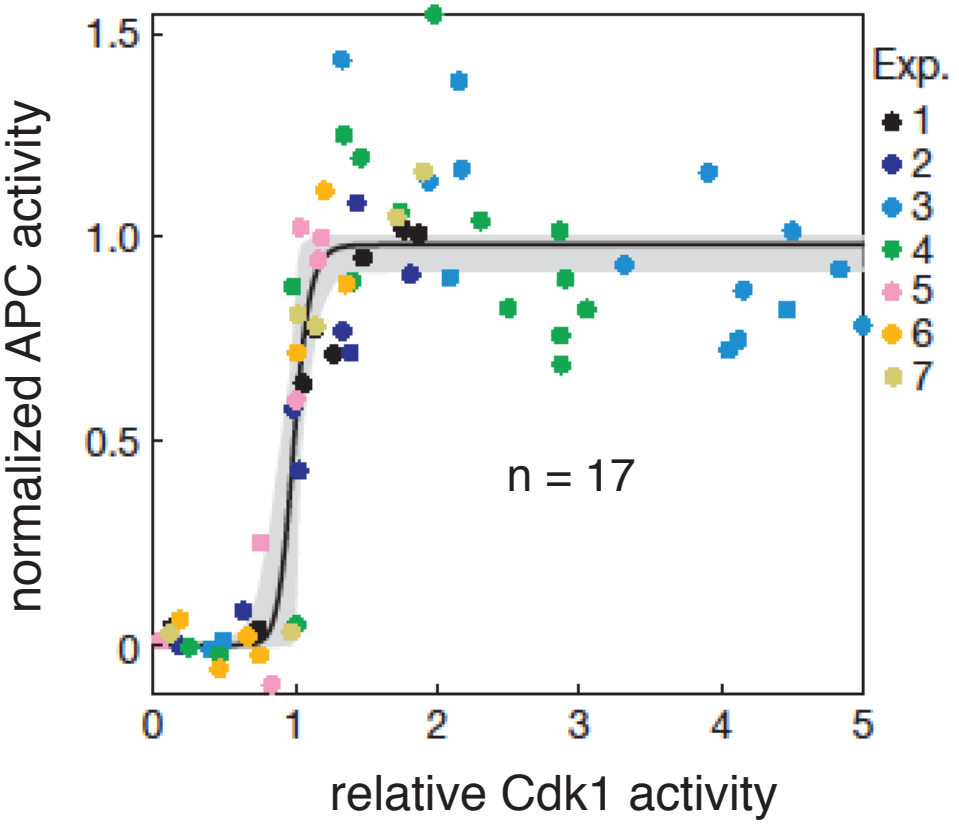

Figure 3.2 Dependence of APC activity on Cdk1

Again, we can represent the dependence of Cyclin degradation rates on Cdk1 using a function of the form:

$$k_{deg}(A) = a_{deg} + f_{deg}\frac{A^{n_{deg}}}{K_{deg}^{n_{deg}} + A^{n_{deg}}} \tag{3.2}$$

where $a_{deg} = 0.01$, $f_{deg} = 0.04$, $K_{deg} = 32$, and $n_{deg} = 17$ are estimated from experiments.

Plugging into Equation 3.1, and combining with our previous equation for active Cdk1 (A), we now have a pair of differential equations:

$$\frac{dA}{dt} = \left(0.16 + 0.8\frac{A^{11}}{35^{11} + A^{11}}\right) \cdot (C - A) - \left(0.08 + 0.4\frac{A^{3.5}}{30^{3.5} + A^{3.5}}\right) \cdot A$$
$$\frac{dC}{dt} = k_{synth} + \left(0.01 + 0.04\frac{A^{17}}{32^{17} + A^{17}}\right) A \qquad (3.3)$$

Again, we can analyze the dynamics this system using a combination of graphical and numerical approaches:

3.1 Graphical

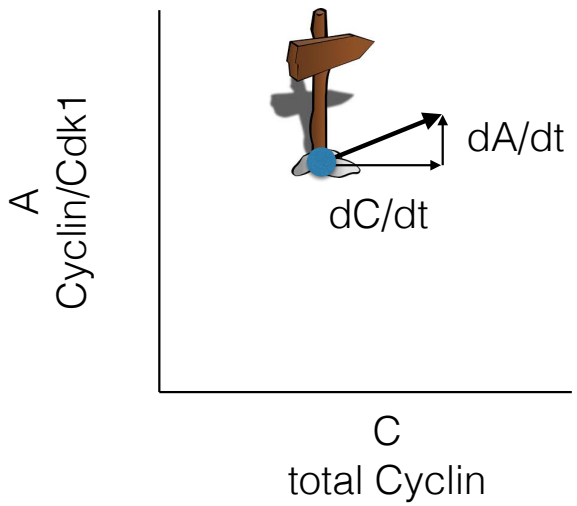

Figure 3.3 A phase plane

The state of this system is described by two values: the concentration of Cyclin and the concentration of active Cdk1. We can think of the state of the system, at any moment in time, as being defined by a point in a 2D "phase plane". The position of this point on the A-axis indicates how much active Cyclin/Cdk1 there is in the cell at that moment, and the position along the C-axis indicates the total amount of Cyclin. The differential equations for dC/dt and dA/dt above are rules that tell you, given the current values of A and C, how much the system will move in the C direction and A direction in the next instant in time. We can think of these rules as arrows attached to each point in the 2D plane, together constituting what we call a flow field. The flow along these arrows defines what will happen to the system.

Analyzing the behavior of a system with two dynamical variables in the phase plane is more difficult than the 1D examples from before. More kinds of behavior are possible, but we can still use graphical tools to think intuitively about what will happen.

First, we need to load a library that defines some useful tools for graphical "phase plane analysis"

```
## Loading the 'phaseR' library
library(phaseR)
```

Now lets define a function that computes the right hand sides of our pair of differential equations:

```
RHS_2D <- function(t, y, parameters) {
    with(as.list(parameters), {
        C = y[1]
        A = y[2]
        dc=k_syn-(a_deg + f_deg*A^n_deg/(K_deg^n_deg + A^n_deg))*C
        pda = (a_act+f_act*A^n_act/(K_act^n_act+A^n_act))*(C-A)
        nda = (a_inact+f_inact*A^n_inact/(K_inact^n_inact+A^n_inact))
            *A
        return(list(c(dc,pda-nda)))
    })
}
```

The arguments to this function are the current time t, a list y containing the current values of C (y[1]) and A (y[2]) and a list of paramater values. The function returns a list of two values corresponding to dC/dt and dA/dt

Finally, let's define the list of parameters and assign their values:

```

full_pars = list(k_syn=1.0,a_deg=0.01,f_deg=0.04,K_deg=32,n_deg=17,a_act
    =0.16,f_act=0.8,K_act=35,n_act=11,a_inact=0.08,f_inact=0.4,K_inact
    =30,n_inact=3.5)
```

OK, now we can use a predefined function (part of the phaseR library) that computes and displays the flow field:

```
flowField(RHS_2D, x.lim = c(0, 100),
y.lim = c(0, 100),
xlab="C", ylab="A",
parameters = full_pars,
points = 15, add = FALSE)
```

Question: What can you infer about the dynamics of this circuit by looking at the flow field?

Recall that for the 1D system, it was very useful to know where the steady states are. How do we accomplish this in 2D? Instead of plotting positive and negative fluxes and locating steady states where these two curves cross, we will instead plot two nullclines" and locate steady states where these cross. The A nullcline is the set of (A,C) value pairs for which dA/dt = 0, and the C nullcline is the set of (A,C) value pairs for which dC/dt = 0. Where these cross, both dA/dt and dC/dt must be 0. To think intuitively about the meaning of the nullclines, notice that when the system is on the A-nullcline, the next instantaneous step must be in the C direction (and vice versa). Thus, the arrows in the flow field must point along the C direction on the A-nullcline.

For this particular system of equations, you could find the A and C nullclines algebraically with pencil and paper. Feel free to do that now if you really want to show off your algebra chops. However, if you just want to get the nullclines quickly with minimal effort, you can use this built in R function to plot the nullclines over the flowfield you plotted a moment ago:

```
nullclines(FHN, x.lim = c(0, 100),
y.lim = c(0, 100),
parameters = full_pars,
points = 500)
```

Exercise 3.2

Question: How many steady states are there? Do you think they are stable or unstable? How could you tell?

3.2 Numerical

The short answer to the above question is that you cannot determine if a steady state is stable or unstable just by looking at the shapes of the nullclines (except in very special cases). A very straightforward (and quite powerful) way to investigate the dynamics of this circuit is to solve the equations numerically for different starting values of A and C and then plot the solutions in the A/C plane.

```
trajectory(FHN, y0 = c(10,10), t.end = 500,
parameters = full_pars)
```

Exercise 3.3

Repeat this for a series of different starting values. Does the long term behavior of this circuit depend on the initial conditions? If so, how?

3.3 A closer look at the limit cycle dynamics

Plot A(t) and C(t) vs time. Does the system go to a steady state? If a system does not approaches a steady state point, but instead stably cycles through the same states over and over, we say that the system is attracted to a "limit cycle". Relate the time series to the limit cycle view in the phase plane. Infer that there is slow movement along the upper and lower branches of the Cyc nullcline and fast jumps between branches.

A mathematical model of the cell cycle like this of course simplifies many molecular details and leaves out some of the biology. The power of a description like this is that it now allows to ask which features of the molecular circuit are crucial for the observed behavior. Now that you have assembled these tools, you can computationally manipulate the cell cycle circuit to see how the behavior changes.

3.4 Challenges

Challenge: What is the importance of switch-like dependence of Cyclin degradation on Cdk1?

Set n = 1 in the differential equation for Cyc and in the equation for the Cyc nullcline.

Again plot nullclines and plot solutions for a variety of different initial conditions. What is the behavior of this system?

Is it possible to get oscillatory dynamics to return by tuning e.g. the threshold or strength of negative feedback?

Challenge: How tolerant is the cell cycle oscillator to changes in the threshold for activation of Cyclin degradation by Cdk1? How does this tolerance change as you vary the steepness of the feedback (i.e. the exponent n)?

Challenge: Explore what happens as you vary Cyclin synthesis and degradation rates. How does the period of the oscillation change? Why?

Challenge: The original form of the circuit had two feedback loops on active Cycle/Cdk1: one acted on the phosphatase Cdc25, and one acted on the kinase Wee1. If you computationally remove the feedback loop involving Wee1, what happens to oscillations? Can you get a different answer if you change the parameters describing the feedback loop through Cdc25? It may help to go back to the single differential equation describing dA/dt and reanalyze it without the Wee1 feedback loop to look for multiple steady states.