Module: Cell Cycle
Authors: Jill C. Sible and John J. Tyson

Answers to Exercises

1. Using the Xi vs. T function, plot preMPF and MPF versus time. Also plot cyclin and total_cyclin versus time.

a. What biological behaviors regarding the cell cycle in frog egg extracts are reproduced by these simulations? *Spontaneous oscillations*

b. In what order do the peaks in preMPF and MPF occur in each cell cycle? *First preMPF, then MPF.*

c. What happens to the oscillations in MPF activity if you change the rate of cyclin synthesis to 1? to 0.2? to 0.05?

k1	Period	
1	54	Max Tot_Cyclin = 17.8
0.5	88	Max Tot_Cyclin = 22.4
0.2	198	Max Tot_Cyclin = 21.1
0.05	steady state	Tot_Cyclin = 10, MPF = 0.3, preMPF = 9.7

What are the bounds on the rate of cyclin synthesis to produce sustained oscillations in MPF activity? $0.085 < k1 < 2.9$

2. In 1990, Solomon et al. published a study in which extracts were treated with cycloheximide (to block protein synthesis) and supplemented with fixed amounts of a mutant, non-degradable form of cyclin B (Δcyclin B). Simulate this experiment.

a. What parameter values did you change? *k1 = V2' = V2" = 0*
Initial conditions: set all species = 0, except cyclin = 5, 10, 15, 20, …
Integrate equations and see if MPF stays close to zero or jumps up after a characteristic time lag.

b. What is the minimal threshold concentration of cyclin required to activate MPF? *Threshold is between 16 and 16.5.*

c. Why do you think this cyclin threshold exists?

d. What happens if you raise the concentration of cyclin incrementally just above the activation threshold?

Initial_cyclin	15	16	16.5	17	18	20	30
Time_lag	infinite	infinite	150	92	58	35	13

3. Solomon et al. measured a cyclin threshold for MPF activation. Now let's use the model to predict a new behavior: a cyclin threshold for MPF inactivation. As in Exercise 2, set k1 = V2' = V2" = 0, and set the following initial conditions: cyclin = 0, preMPF = 0, Cdc25P = 1, Wee1P = 1, IEP = 0, APC = 0, MPF = 20, 15, 10, … In this case, you are simulating an extract in which all the cyclin is initially in the form of active MPF. What is the cyclin threshold for MPF inactivation?

Threshold is between 7.9 and 8.0

How does the lag-time for MPF inactivation depend on total cyclin concentration?

Initial_MPF	7.5	7.6	7.7	7.8	7.9	8	9
Time_lag	110	125	160	220	580	infinite	infinite

4. Using the original set of parameter values, plot MPF vs. total cyclin. What is the interpretation of the oval-shaped curve you will see?
A plot of MPF vs. total cyclin is called a 'phase plane'. The closed, oval-shaped curve is a stable 'limit cycle'. It corresponds to periodic oscillations in all the variables of the control system.

a. Compare this oval to your graph of steady states in Exercise 3b.
The oval should sweep around the 'hysteresis' loop of steady states.

b. How are MPF oscillations related to the *activation* and *inactivation* thresholds investigated in Exercises 2 and 3?
First, with MPF low, total cyclin must increase enough to surpass the activation threshold, so that MPF can switch on. High MPF activity activates the APC and causes cyclin to be degraded. Now with MPF on, total cyclin must decrease enough to drop below the inactivation threshold. Then MPF switches off, the APC inactivates, and now cyclin can accumulate again.

c. What advantages does bistability confer to the physiology of mitosis?
Bistability helps to make progression through the mitotic cycle irreversible.

5. In 2005, Pomerening et al. published a study in which a frog egg extract was supplemented with a recombinant form of Cdk1 in which Thr 14 and Tyr 15 were mutated to Ala (A) and Phe (F), respectively (Pomerening et al., 2005). (We will refer to this mutant protein as Cdk1AF; for historical reasons, Pomerening et al. call it Cdc2AF.)

a. Assume that endogenous Cdk1 was removed from the extract and precisely replaced by Cdk1AF. What parameter value(s) would you change to simulate these conditions? Why? *Vwee' = Vwee" = 0*

b. Plot MPF vs. time under these conditions. Describe the simulations.
The oscillations have much lower amplitude and shorter period.

c. What does this experiment tell us about the feedback loops that affect MPF phosphorylation?
The negative feedback loop alone is sufficient for oscillations, but the oscillations are too fast and too wimpy (small amplitude) to drive irreversible progression through DNA synthesis and mitosis. The positive feedback loops are necessary for robust oscillations of MPF.

d. How would you simulate the original conditions of the experiment, in which endogenous Cdk1 is supplemented with an equal amount of Cdk1AF?
You will need to add a new variable, MPF_AF, to the differential equations, so that you can follow changes in both endogenous MPF and the recombinant MPF dimers.

6. In the presence of unreplicated DNA, a cell cycle checkpoint is activated. A protein kinase called Chk1 is activated. Chk1 phosphorylates both Wee1 and Cdc25 (on residues distinct from the MPF phosphorylation site), resulting in activation of Wee1 and inhibition of Cdc25.

a. Adjust parameters to represent the effect of unreplicated DNA on the mitotic control system. Describe the simulation of MPF vs. time.
V25" = V25' = 0.017, Vwee" = 5 (or larger).
Stable steady state with MPF activity very low.

b. By how much must V_{wee}" be raised to engage the checkpoint? *5 or larger*

c. To engage a checkpoint, is it sufficient for Chk1 to phosphorylate only Wee1 or only Cdc25?
Maybe so, maybe not. In either of these cases, MPF will eventually activate but only after a long delay. Certainly, to get a quick, strong, checkpoint response it is desirable both to activate Wee1 and to inactivate Cdc25..

d. If replicated chromosomes cannot be properly aligned on the mitotic spindle, then the cell engages a different checkpoint that prevents activation of the APC. How might you model cell cycle arrest at this checkpoint?
Try activating the phosphatase that converts IEP back to IE.