(edited by J. Stiles)

Module: Cell Cycle
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V. Exercises
Open MPF.ode in XPP or WinPP. A set of parameter values and initial conditions have been provided.

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

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

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

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? What are the bounds on the rate of cyclin synthesis to produce sustained oscillations in MPF activity?

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? Why?

b. What is the minimal threshold concentration of cyclin required to activate MPF?

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?

3. Solomon et al. measured a cyclin threshold for MPF activation. 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?

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

b. Using your results in Exercises 2 and 3, plot the steady state concentration of MPF as a function of total cyclin concentration for the two cases: (i) initial MPF =

0 and total_cyclin increasing, and (ii) initial MPF = total_cyclin decreasing. Notice that the system has two stable steady states for total_cyclin between 8 and 16.

4. Using the original set of parameter values, plot MPF vs. total cyclin. What is the interpretation of the oval-shaped curve you obtain?

a. Compare this oval to your graph of steady states in Exercise 3b.

b. How are MPF oscillations related to the *activation* and *inactivation* thresholds investigated in Exercises 2 and 3?

c. What advantages does bistability confer to the physiology of mitosis?

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?

b. Plot MPF vs. time under these conditions. Describe the simulations.

c. What does this experiment tell us about the feedback loops that affect MPF phosphorylation?

d. How would you simulate the original conditions of the experiment, in which endogenous Cdk1 is supplemented with an equal amount of Cdk1AF?

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.

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

c. To engage a checkpoint, is it sufficient for Chk1 to phosphorylate only Wee1 or only 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?