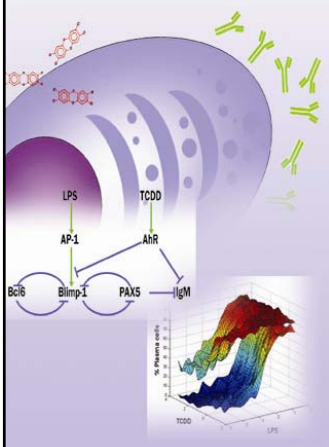

Tuesday Afternoon Exercise 2:

MAPK ultrasensitivity and bistability – modeling *Xenopus* oocyte maturation

September 23, 2008

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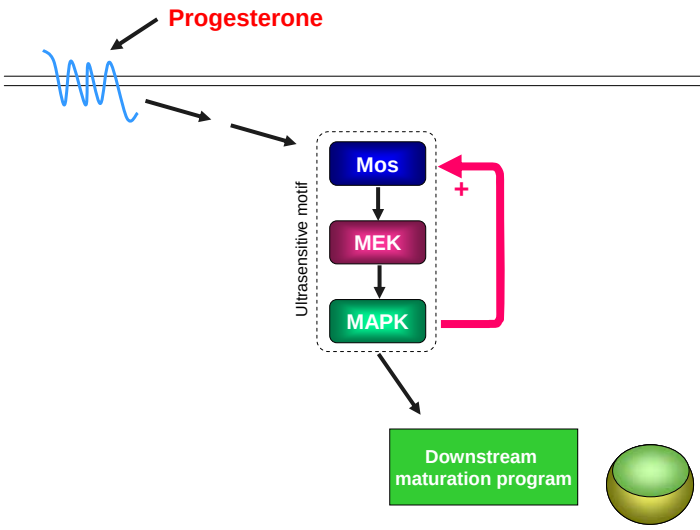
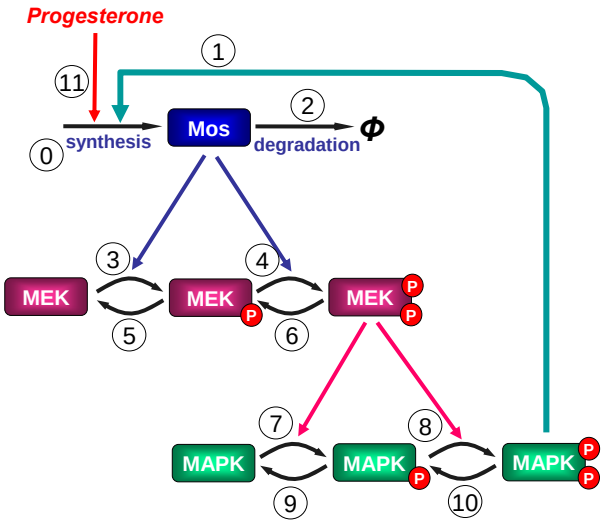
Goals of Exercise

- Understand the ultrasensitive response of the MAPK cascade and the sources of ultrasensitivity
- Understand the response behaviors of a MAPK-mediated bistable system underlying *Xenopus* oocyte maturation

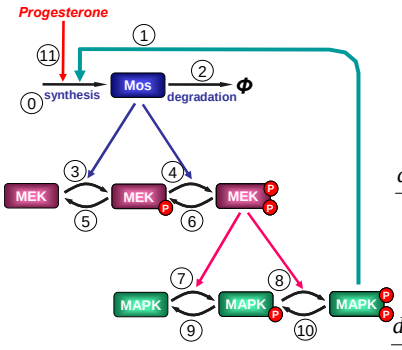
Xenopus oocyte maturation is mediated by a MAPK positive feedback loop



MAPK model structure



Differential equations of the MAPK model



$$\frac{d[Mos]}{dt} = k_0 + k_{11}\frac{PR_{tot}\cdot P}{Kd_{11}+P} + k_1[MAPK_{pp}] - k_2[Mos]$$

$$\frac{d[MEK]}{dt} = \frac{k_5[MEK_p]}{K_{m5}+[MEK_p]} - \frac{k_3[Mos][MEK]}{K_{m3}+[MEK]}$$

$$\frac{d[MEK_p]}{dt} = \frac{k_3[Mos][MEK]}{K_{m3}+[MEK]} + \frac{k_6[MEK_{pp}]}{K_{m6}+[MEK_{pp}]} - \frac{k_4[Mos][MEK_p]}{K_{m4}+[MEK_p]} - \frac{k_5[MEK_p]}{K_{m5}+[MEK_p]}$$

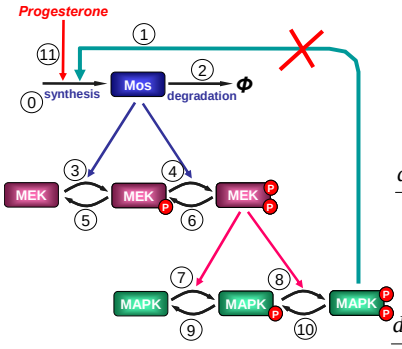
$$\frac{d[MEK_{pp}]}{dt} = \frac{k_4[Mos][MEK_p]}{K_{m4}+[MEK_p]} - \frac{k_6[MEK_{pp}]}{K_{m6}+[MEK_{pp}]}$$

$$\frac{d[MAPK]}{dt} = \frac{k_9[MAPK_p]}{K_{m9}+[MAPK_p]} - \frac{k_7[MEK_{pp}][MAPK]}{K_{m7}+[MAPK]}$$

$$\frac{d[MAPK_p]}{dt} = \frac{k_7[MEK_{pp}][MAPK]}{K_{m7}+[MAPK]} + \frac{k_{10}[MAPK_{pp}]}{K_{m10}+[MAPK_{pp}]} - \frac{k_8[MEK_{pp}][MAPK_p]}{K_{m8}+[MAPK_p]} - \frac{k_9[MAPK_p]}{K_{m9}+[MAPK_p]}$$

$$\frac{d[MAPK_p]}{dt} = \frac{k_8[MEK_{pp}][MAPK_p]}{K_{m8}+[MAPK_p]} - \frac{k_{10}[MAPK_{pp}]}{K_{m10}+[MAPK_{pp}]}$$

MAPK ultrasensitivity (w/o positive feedback)



$$\frac{d[Mos]}{dt} = k_0 + k_{11}\frac{PR_{tot}\cdot P}{Kd_{11}+P} + \cancel{k_1[MAPK_{pp}]} - k_2[Mos]$$

$$\frac{d[MEK]}{dt} = \frac{k_5[MEK_p]}{K_{m5}+[MEK_p]} - \frac{k_3[Mos][MEK]}{K_{m3}+[MEK]}$$

$$\frac{d[MEK_p]}{dt} = \frac{k_3[Mos][MEK]}{K_{m3}+[MEK]} + \frac{k_6[MEK_{pp}]}{K_{m6}+[MEK_{pp}]} - \frac{k_4[Mos][MEK_p]}{K_{m4}+[MEK_p]} - \frac{k_5[MEK_p]}{K_{m5}+[MEK_p]}$$

$$\frac{d[MEK_{pp}]}{dt} = \frac{k_4[Mos][MEK_p]}{K_{m4}+[MEK_p]} - \frac{k_6[MEK_{pp}]}{K_{m6}+[MEK_{pp}]}$$

$$\frac{d[MAPK]}{dt} = \frac{k_9[MAPK_p]}{K_{m9}+[MAPK_p]} - \frac{k_7[MEK_{pp}][MAPK]}{K_{m7}+[MAPK]}$$

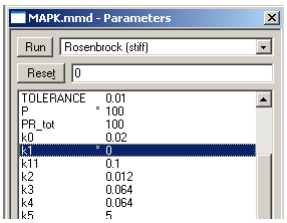
$$\frac{d[MAPK_p]}{dt} = \frac{k_7[MEK_{pp}][MAPK]}{K_{m7}+[MAPK]} + \frac{k_{10}[MAPK_{pp}]}{K_{m10}+[MAPK_{pp}]} - \frac{k_8[MEK_{pp}][MAPK_p]}{K_{m8}+[MAPK_p]} - \frac{k_9[MAPK_p]}{K_{m9}+[MAPK_p]}$$

$$\frac{d[MAPK_p]}{dt} = \frac{k_8[MEK_{pp}][MAPK_p]}{K_{m8}+[MAPK_p]} - \frac{k_{10}[MAPK_{pp}]}{K_{m10}+[MAPK_{pp}]}$$

MAPK ultrasensitivity

First Run of MAPK model without positive feedback

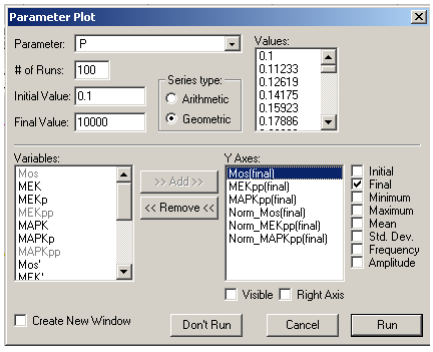
1. Open MAPK model (Desktop/Exercises/Monday/MAPK.mmd)
2. Examine model code
3. Open parameter window
   (Menu "Parameters" → "Parameter Window")
4. Disable positive feedback (Set k1 to 0)
5. Give some dose of progesterone (Set P to 100)
6. Hit button "Run", examine time evolution of Mos, MEKpp and MAPKpp, etc.



MAPK ultrasensitivity

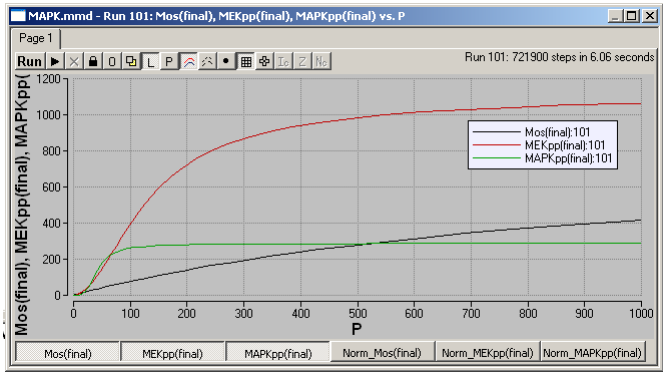
Plot steady-state dose response for Mos, MEKpp, MAPKpp vs. P (progesterone)

1. Menu "Parameters" → "Parameter Plot"
2. Set the pop-up "Parameter Plot" window as shown on the right:
   Check "Final" for each added variable (This reads the final steady-state value)
3. Hit button "Run"



MAPK ultrasensitivity

4. You should see dose response curves as shown below. Adjust the x-axis scale if needed by double-clicking on the x-axis.



5. Compare the steepness of Mos, MEKpp, MAPKpp dose response curves. Which one is steeper (more sigmoid)? Use normalized curves (Norm_Mos, Norm_MEKpp, and Norm_MAPKpp for better comparison.

MAPK ultrasensitivity

Revealing the multi-step signaling effect

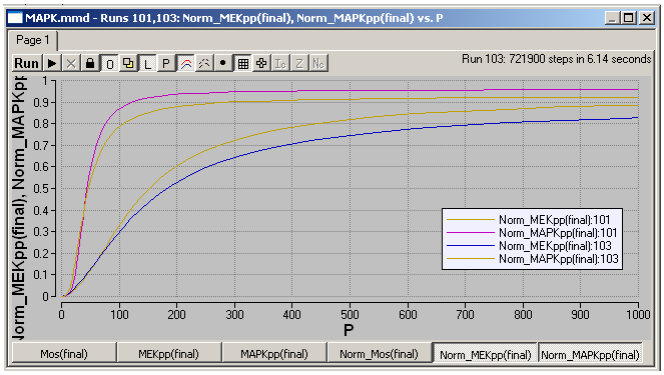
One ultrasensitive source for the MAPK cascade is dual-phosphorylation associated multi-step signaling. This source of ultrasensitivity can be revealed by minimizing the zero-order ultrasensitivity effect. This can be done by setting the Michaelis-Menten constants far above the total amounts of the substrates.

1. Menu “Parameters” → “Parameter Window”
2. Increase Km3 through Km6 from 1200 to 12000, and increase Km7 through Km10 from 300 to 3000. Compare these values with the total amount of MEK and MAPK.
3. Toggle back to the dose response window, click on button “O” for overlay, then hit button “Run”.
4. Examine the change in the steepness of MAPKpp and especially MEKpp response. Are they still sigmoid? If yes, why?

MAPK ultrasensitivity

Revealing the multi-step signaling effect (continued)

5. This is what you should see: (adjust x-axis scale if needed)



6. Reset Km3 through Km10 after you are done.

MAPK ultrasensitivity

Effect of zero-order ultrasensitivity

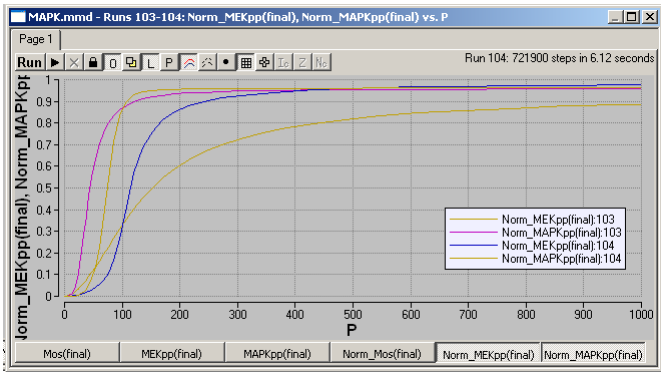
By making enzymes working closer to saturation, the effect of zero-order ultrasensitivity on the steepness of the response curves can be further enhanced. This is done by decreasing the Michaelis-Menten constants

1. Menu “Parameters” → “Parameter Window”
2. Set Km3 through Km6 to 120. Compare this number with the total amount of MEK.
3. Toggle back to the dose response window, click on button “O” for overlay if not depressed, then hit button “Run”.
4. Examine the change in the steepness of MEKpp and MAPKpp response. Is the steepness increased? Why?

MAPK ultrasensitivity

Effect of zero-order ultrasensitivity (continued)

5. This is what you should see: (adjust x-axis scale if needed)

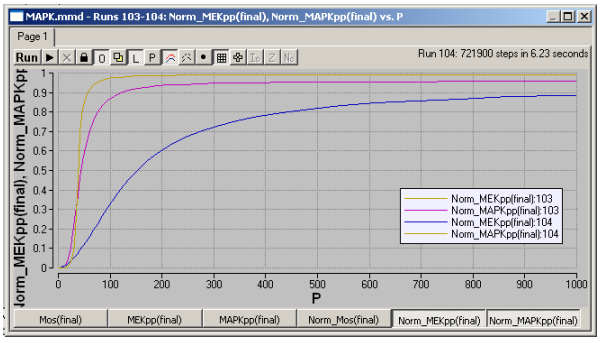


6. Reset Km3 through Km6 after you are done.

MAPK ultrasensitivity

Effect of zero-order ultrasensitivity (continued)

7. In parameter window, set Km7 through Km10 to 30. Compare this number with the total amount of MAPK.
8. Toggle back to the dose response window, then hit button “Run”.
9. Examine the change in the steepness of MEKpp and MAPKpp response. Is the steepness increased for MAPKpp? Any change to MEKpp?



10. Reset Km7 through Km10 after you are done.

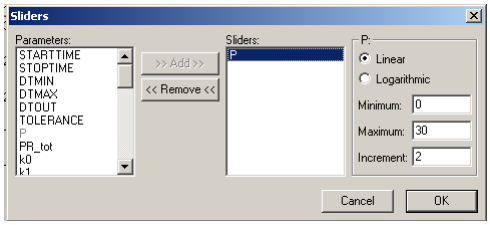
MAPK Bistability

Enable the positive feedback of MAPK model

1. Menu "Parameters" → "Parameter Window"
2. Reset k1 to 0.0035 (this enables the positive feedback)

Find the threshold concentration of progesterone required to trigger the MAPK bistable switch

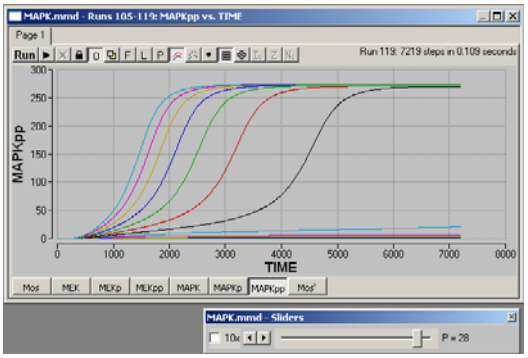
1. Menu "Parameters" → "Define Sliders"
2. Add "P" to Sliders. Set the slider as shown below:



MAPK Bistability

Find the threshold concentration of progesterone required to trigger the MAPK bistable switch (continued)

3. Using the slider to increase dose of P. Observe the response. What is the dose of P at which there is a sudden jump of MAPKpp response?



4. Try to generate a MAPKpp vs. P dose response curve using Parameter Plot. Is the dose response switch-like?

MAPK Bistability

Testing whether the system is bistable or not using a pulse of progesterone

1. Go to Equation window, scroll to “Parameter values” section.
2. Put a semicolon “;” before P = 0.
3. Remove the semicolons at the beginning of each of the 4 lines responsible for generating a pulse of progesterone. Save file.
4. After step 2 and 3, the code should look like the following:

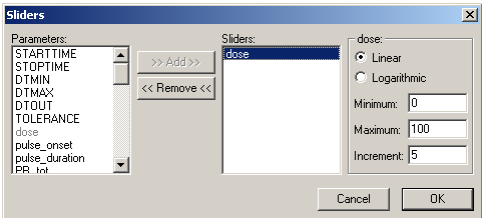
```
;<------------------Parameter values ---------------------------------->
;P      = 0             ;               Progesterone

;<-------The following 4 lines generate a pulse of progesterone
dose                   = 0     ; nM
pulse_onset= 0     ; s
pulse_duration     = 600  ;s
P = dose*SQUAREPULSE(pulse_onset, pulse_duration)
```


MAPK Bistability

Testing whether the system is bistable or not using a pulse of progesterone (continued)

5. Go to Menu “Parameters” → “Define Sliders”. Click “Yes” in the pop-up window.
6. Add “dose” to Sliders. Set the slider as show below:

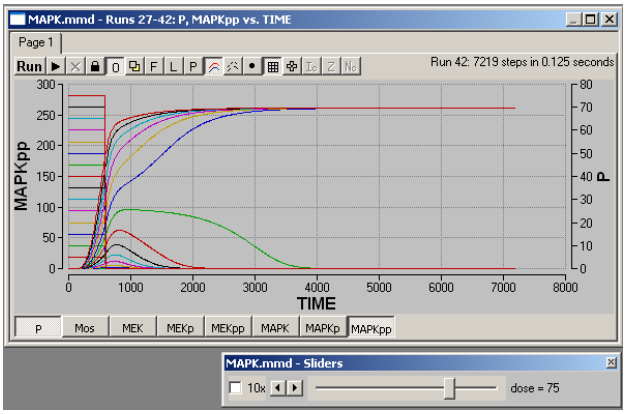


7. Go to Menu “Graph” → “New Window”. Click on button “O” for overlay in the new pop-up window.

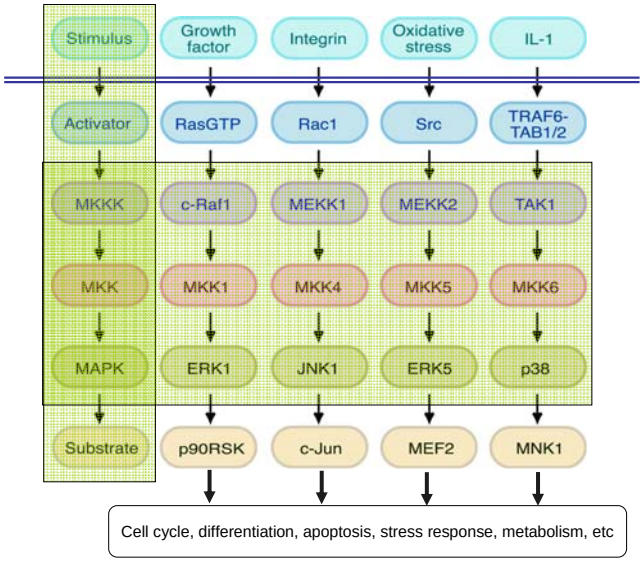
MAPK Bistability

Testing whether the system is bistable or not using a pulse of progesterone (continued)

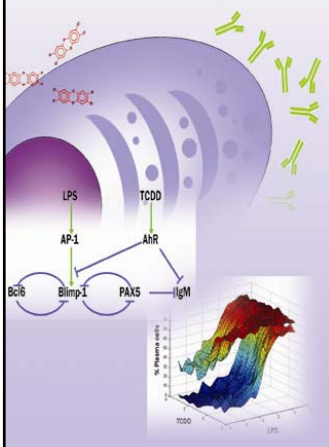
8. Using the slider to increase dose of P. You should see the following. Why does this demonstrate bistability of the system?



MAPK cascade – conserved motif mediating a variety of cellular responses



Adapted from Johnson and Lapadat, Science 2002



MAPK Modeling - Summary

- MAPK ultrasensitivity arises from
  (1) dual-phosphorylation
  (2) zero-order ultrasensitivity
  (3) layered arrangement
- When situated in a positive feedback circuit, MAPK provides a basis for bistability and switch-like response.