Apoptosis

The process of programmed cell death –cellular suicide– is called apoptosis (from the Greek for "a falling off"). Apoptosis is a necessary part of the development of many multicellular organisms. Some cells play only a transient role during development; when they are no longer needed, they receive signals that induce apoptosis. (A commonly cited example is the tail of a tadpole, which is not needed by the adult frog.) Compared with death caused by stress or injury, apoptosis is a tidy process; rather than spill their contents into the environment, apoptotic cells quietly implode, thus ensuring there are no detrimental effects on the surrounding tissue.

Apoptosis is invoked by caspase proteins, which are always present in the cell, but lie dormant until activated. The family of caspase proteins is split into two categories:

• Initiator caspases respond to apoptosis-inducing stimuli. They can be triggered externally (via transmembrane receptors) or internally, by stress signals from the mitochondria (e.g., starvation signals).

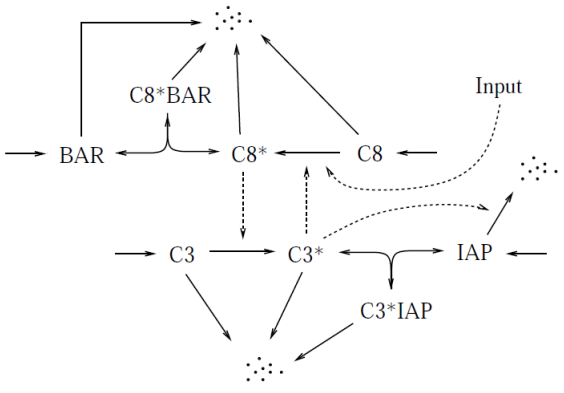
• Executioner caspases are activated by initiator caspases. They carry out the task of cellular destruction by cleaving a number of key proteins and activating DNases that degrade the cell's DNA.

We will consider a model published in:

Eissing,T., Conzelmann,H., Gilles, E.D., Allgöwer, F., Bullinger,E., and Scheurich,P., Bistability analyses of a caspase activation model for receptor-induced apoptosis. Journal of Biological Chemistry, 279(2004), 36892-36897.

The model focuses on caspase-8, an initiator, and caspase-3, an executioner. Caspase-8 is triggered by external stimuli. When active, it activates caspase-3. Caspase proteins are activated by the removal of a masking domain, revealing a catalytic site. This cleavage is irreversible; the protein can only be inactivated by degradation. Consequently, to describe steady-state behavior, the model includes both production and degradation processes for each protein.

To guarantee that the decision to undergo apoptosis is irreversible, a feedback mechanism is in place: active caspase-3 activates caspase-8. This positive feedback ensures that caspase activity is self-perpetuating. The feedback scheme in the figure shows the core reaction network for the Eissing model. In addition to the caspases, the model incorporates two proteins, IAP and BAR, which inhibit apoptosis by binding active caspases, forming inert complexes.



Eissing apoptosis model (shown not as a chemical reaction network, but using a different graphical representation). An extracellular signal triggers activation of caspase-8 (C8) from its inactive form (C8). Once active, caspase-8 activates caspase-3 (C3 to C3*). Active caspase-3 activates caspase-8, forming a positive feedback loop. Because activation of caspases is irreversible, protein degradation and production are included in the model. Two apoptotic inhibitors are included: BAR and IAP. These proteins bind active caspase-8 and -3, respectively, thus inhibiting the progression to apoptosis. (Dots indicate degraded proteins.)*

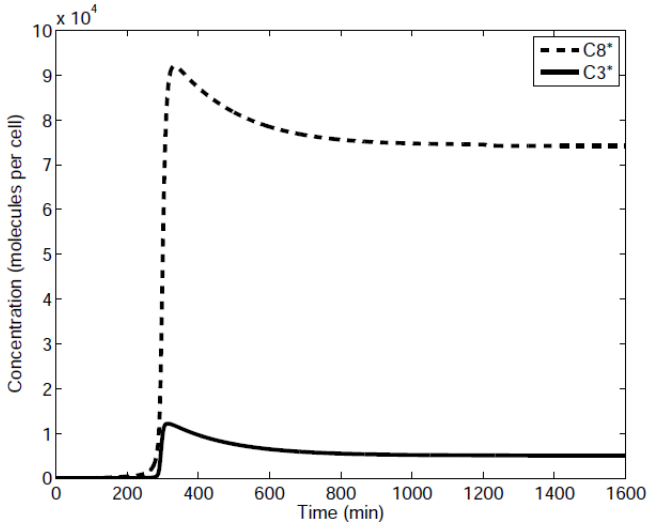
(Dashed lines represent enzymatic reactions like $C3^* + C8 \rightarrow C3^* + C8^*$ which do not consume the enzyme. The authors implicitly assume these occur fast enough that we ignore intermediate complexes.)

Treating all enzyme-catalyzed reactions as first-order, we can write the model as

$$\begin{aligned}
d/dt[\text{C8}] &= k_1 - k_2[\text{C8}] - k_3([\text{C3}^*] + [\text{Input}]) \cdot [\text{C8}] \\
d/dt[\text{C8}^*] &= k_3([\text{C3}^*] + [\text{Input}]) \cdot [\text{C8}] - k_4[\text{C8}^*] - k_5[\text{C8}^*] \cdot [\text{BAR}] + k_6[\text{C8}^*\text{BAR}] \\
d/dt[\text{C3}] &= k_7 - k_8[\text{C3}] - k_9[\text{C8}^*] \cdot [\text{C3}] \\
d/dt[\text{C3}^*] &= k_9[\text{C8}^*] \cdot [\text{C3}] - k_{10}[\text{C3}^*] - k_{11}[\text{C3}^*] \cdot [\text{IAP}] + k_{12}[\text{C3}^* IAP] \\
d/dt[\text{BAR}] &= k_{13} - k_5[\text{C8}^*] \cdot [\text{BAR}] + k_6[\text{C8}^*\text{BAR}] - k_{14}[\text{BAR}] \\
d/dt[\text{IAP}] &= k_{15} - k_{11}[\text{C3}^*] \cdot [\text{IAP}] + k_{12}[\text{C3}^*\text{IAP}] - (k_{16} + k_{17}[\text{C3}^*]) \cdot [\text{IAP}] \\
d/dt[\text{C8}^*\text{BAR}] &= k_5[\text{C8}^*] \cdot [\text{BAR}] - k_6[\text{C8}^*\text{BAR}] - k_{18}[\text{C8}^*\text{BAR}] \\
d/dt[\text{C3}^*\text{IAP}] &= k_{11}[\text{C3}^*] \cdot [\text{IAP}] - k_{12}[\text{C3}^*\text{IAP}] - k_{19}[\text{C3}^*\text{IAP}]
\end{aligned}$$

This system is bistable. At low levels of activated caspase, the system is at rest in a "life" state. Once caspase activity rises above a threshold, the positive feedback commits the system to reaching a steady state with high levels of active caspase – a "death" state. (Of course, the death state is transient - the cell is being dismantled. We are justified in calling it a steady state on the timescale of the signaling pathway.)

The simulation of the model shows the response of the system to an input signal. The system begins at rest with zero input and low caspase activity. At time t = 100 minutes an input is introduced, causing a slow increase in the activity level of caspase-8. This slow activation leads to a rapid rise in caspase activity at about t = 300 minutes. The system then settles into the "death" state with high caspase activity. When the input signal is removed (at time t = 1200 minutes), this self-perpetuating state persists, confirming that the system is bistable. Because complete removal of the input signal does not cause a return to the initial state, this life-to-death transition is irreversible.



Behavior of the Eissing apoptotic pathway model. This simulation begins in the low caspase activity "life" state. At time t =100 minutes, an input signal (Input=200) is introduced, causing an increase in caspase-8 activity. This triggers a positive feedback loop that results in a rapid increase in activated caspase-8 and caspase-3 abundance at about t = 300. The system then settles to the high caspase-activity "death" state. The input stimulus is removed at time t =1200 minutes, but there is no effect: the apoptotic switch is irreversible. Parameter values: (in mpc/min) $k_1 = 507, k_7 = 81.9, k_{13} = 40, k_{15} = 464$*; (in min-1)* $k_2 = 3.9 \times 10^{-3}, k_4 = 5.8 \times 10^{-3}, k_6 = 0.21, k_8 = 3.9 \times 10^{-3}, k_{10} = 5.8 \times 10^{-3}, k_{12} = 0.21, k_{14} = 1 \times 10^{-3}, k_{16} = 1.16 \times 10^{-2}, k_{18} = 1.16 \times 10^{-2}, k_{19} = 1.73 \times 10^{-2}$*; in 1/(mpc.min)* $k_3 = 1 \times 10^{-5}, k_5 = 5 \times 10^{-4}, k_9 = 5.8 \times 10^{-6}, k_{11} = 5 \times 10^{-4}, k_{17} = 3 \times 10^{-4}$ *(mpc =molecules per cell).*

Problems

1. Write the chemical reaction network (e.g., $BAR + C8^* \leftrightarrow C8^*BAR$, etc.) that gives rise to the equations that were given.

2. For this CRN, write the vector of species, the stoichiometric matrix, and the vector of reaction rates, and verify that, multiplying out, one gets the equations that were given.

3. (Duration of triggering signal.) The simulation shown in the figure is done using the MATLAB program *apoptosis_eissing_model.m*. The input signal was maintained for 1100 minutes (until after the system had settled to the caspase-active "death" state.) Re-run this simulation many times, with different durations, until you determine what is the shortest input pulse can trigger the irreversible life-to-death transition. Use the same input size as in the figure (Input=200). Your answer should be an integer, and you should print out a plot showing the behavior with this duration and a plot showing the behavior with 1 minute less. (For example, if you find that 323 minutes is the smallest duration that works, show the plots for 322 and for 323.)

   Be smart about how you do the search - a bisection method worked quite well for me.

   ANSWER: I get roughly 125 minutes as the shortest that works. At 125, I get a graph very much as the one in the figure, but at 124, I get a flat line at zero, basically.

4. Explain what one means by this statement: "In the model, active caspase-3 promotes degradation of IAP. This interaction can be seen as an additional positive feedback of $C3^*$ on itself." Your answer should be a couple of sentences explaining why the degradation of IAP implies that $C3^*$ gets to stay around longer than if IAP was not degraded.

   ANSWER: IAP binds active caspase-3, removing it from the pathway. By enhancing degradation of IAP, active caspase-3 increases its own concentration, because there is less IAP to bind it.

   Therefore, this degradation helps self-sustained caspase activity, hence also helping the positive feedback that makes caspase activation irreversible.

5. Which k_i should be set to zero to get rid of the effect of caspase-3 promoting degradation of IAP?

   ANSWER: k_{17}

6. Simulate (with an input of length 1100 and magnitude 200) what happens when the k_i from the previous problem is set to zero. Plot the result and print it.

   ANSWER: