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A simple boolean model of extrinsic and intrinsic apoptosis pathway in mammalian cells.

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Abstract

Apoptosis is the process of programmed death cell. It is vital to various processes in a cell such as maintaining homeostasis (controlling tissue size and cell turnover), embryonic development, functionality of the immune system, etc. It is a tightly regulated process that is comprised of several signaling pathways, mainly: the extrinsic (death receptor) path and intrinsic (mitochondrial) pathway. All signaling pathways converge on the same terminal execution path which is initiated by the cleavage (activation) of caspase-3, and goes on to execute apoptosis by DNA fragmentation, cytoskeleton degradation, etc. until initiating phagocytosis process.

In this project I propose a simple boolean model of the intrinsic and extrinsic pathways until the initiation of the execution pathway, including a known cross talk between the two pathways. In addition to initiating the execution pathway, the model shows the amplification obtained by the cross talk and by triggering both pathways.

Introduction

Apoptosis, or programmed death cell (PDC) is a key process in multicellular organisms, as vital as its counterpart: mitosis – cell proliferation. As opposed to necrosis which is a passive process initiated due to cell injury (toxins, trauma, infection, etc.), apoptosis is normally an active process and beneficial to the organism. For example, apoptosis occurs during embryonic development of the nematode c elegans during specific stages in the development. Another example is its key role in maintaining homeostasis and tissue size by participating in cell turnover.

Several oncogenes as well as the p53 tumor suppressor gene are key in apoptosis regulation, therefore, it is also related to cancer transformations. In addition, abnormal amplification or suppression of apoptosis may lead to various diseases such as immune deficiency, ischemic diseases etc. For that and other reasons, apoptosis is a significant therapeutic research candidate.

There are various stimuli initiating apoptosis and pathways: intrinsic, extrinsic, perforin, etc. However not all cell respond in a similar way and result in apoptosis. In this project I chose to focus on two key pathways:

The **extrinsic** pathway is triggered by a death ligand binding to a death receptor on the cell's plasma membrane. There are numerous members of ligands and receptors in this family, and I chose to focus on the most notable: FasL and TNF ligands binding to Fas and TNF-R receptors respectively. An activated receptor binds in its cytoplasmic domain to FADD by recruiting various adaptor proteins. FADD then activates caspase-8 (via DISC formation and procaspase-8 cleavage). Caspase-8 goes on to activate caspase-3 which initiates the converged execution pathway of apoptosis. The c-FLIP protein inhibits this pathway by binding to FADD adaptor protein and suppressing the activation of caspase-8.

The **intrinsic** pathway is mediated by the mitochondria. Pro-apoptotic signals target the mitochondria, which is essential to cell respiration. Assistance by the intrinsic pathway is needed for weak death ligands or for high IAP (inhibitor of apoptosis) barrier. The pro-apoptotic signals "battle" with the anti-apoptotic signals on the decision to trigger this pathway. Triggering this pathway results in a formation of pores and release of mitochondria content into the cytoplasm, specifically: Smac (DIABLO) and cytochrome-C. Cytochrome-C activates caspase-9 (via apoptosome formation) which in turn, activates caspase-3 and triggers the apoptosis execution path. Smac protein inhibits IAP (inhibitor of apoptosis proteins) which inhibits caspase-9, therefore, but releasing Smac the inhibition of caspase-9 is removed.

Cross talk between the extrinsic and intrinsic pathways is accomplished via Bid, a pro-apoptotic protein. Caspase-8, activated in the extrinsic pathway activates Bid (cleavage) and Bid triggers the mitochondria pathway, i.e. releasing mitochondria content into the cytoplasm.
This integration of pathways results in accumulation of caspase-3 and thus to the amplification of apoptosis execution pathway.

Methods

I created a simplified model of apoptosis pathways described above. A graphic view of the network implemented (state graph) is shown in Figure 1.

As described above, I chose to focus on Fas and TNFR from the death receptors family for asserting the extrinsic pathway. Also, triggering of the intrinsic pathway is a balance between pro-apoptotic and anti-apoptotic proteins. In this model I chose the accumulation of p53 TSG as a pro-apoptotic agent, and survival factors as anti-apoptotic agent. The two compete with equal weight, canceling each other's effect if both are asserted. In addition, Bid is modeled to trigger the intrinsic pathway via the extrinsic pathway.

Caspase-3, the trigger of apoptosis execution path is the main output of this model.

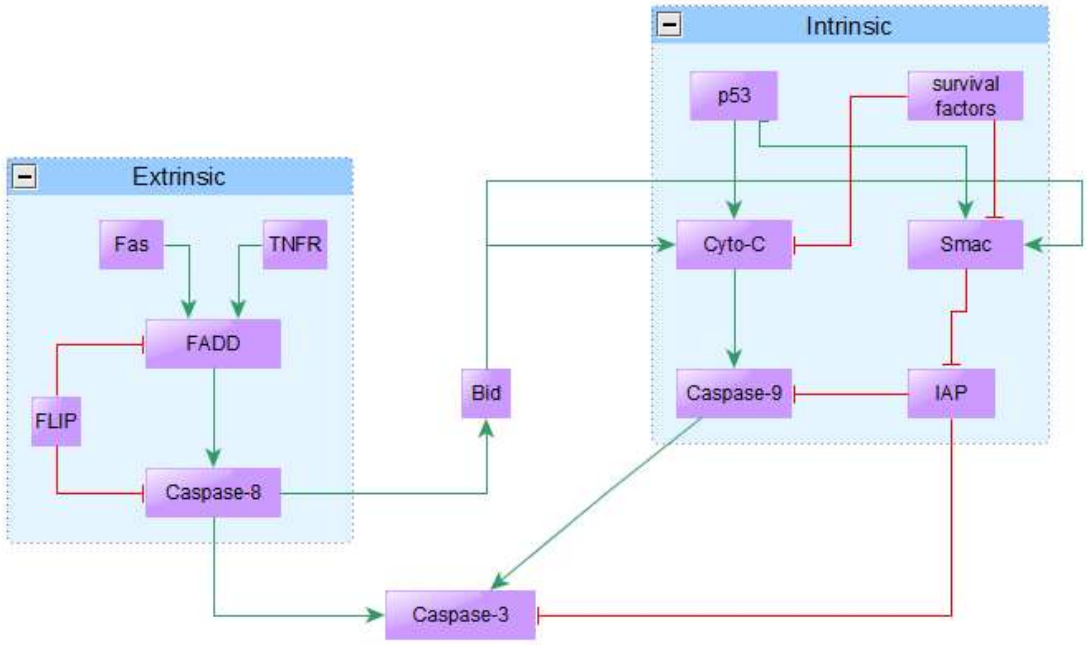

Figure 1. Apoptosis pathway network as implemented in this project.
Extrinsic and intrinsic pathways components are grouped to show cross-talk via Bid and the convergence on the execution pathway via caspase-3 triggering.

The model is based on python code presented in the course with several changes.

The state graph is implemented as list of lists, as in class. The transition function (Figure 2) was changed to show the accumulation of agents in the pathways using increasing integer values, while zero value represents an inactive state.

$$s_i(t+1) = \begin{cases} \min\left(MAX, s_i(t) + \sum weight(j,i) * s_j(t)\right) & if \sum weight(j,i) * s_j(t) > 0 \\ 0 & if \sum weight(j,i) * s_j(t) < 0 \\ s_i(t) & otherwise \end{cases}$$

Figure 2. A synchronous transition function for this boolean model.

I chose a different discrete space (MAX value) for caspase-3 and for the other agents. This was done to capture how triggering different pathways effect the behavior of apoptosis, i.e., to allow showing different patterns for accumulation of caspase-3.

I ran a simulation for all possible values of the "real" initial states (this is coded in function gen_init_states). A real initial state is defined as a state with no entering edge. Internal states where given zero as start value.

Results

I have written a function to print all the simulations, print_run. Its execution is commented out in code and was used to explore and debug model behavior.

I have also written a function – print_casp_3_summary – which is the focus of my results.

This function groups initial states based on the simulation run, according to caspase-3 pattern, i.e. triggering execution pathway. The results of caspase-3 modeling for each group/series are presented in Figure 3 and the groups are presented in Figure 4.

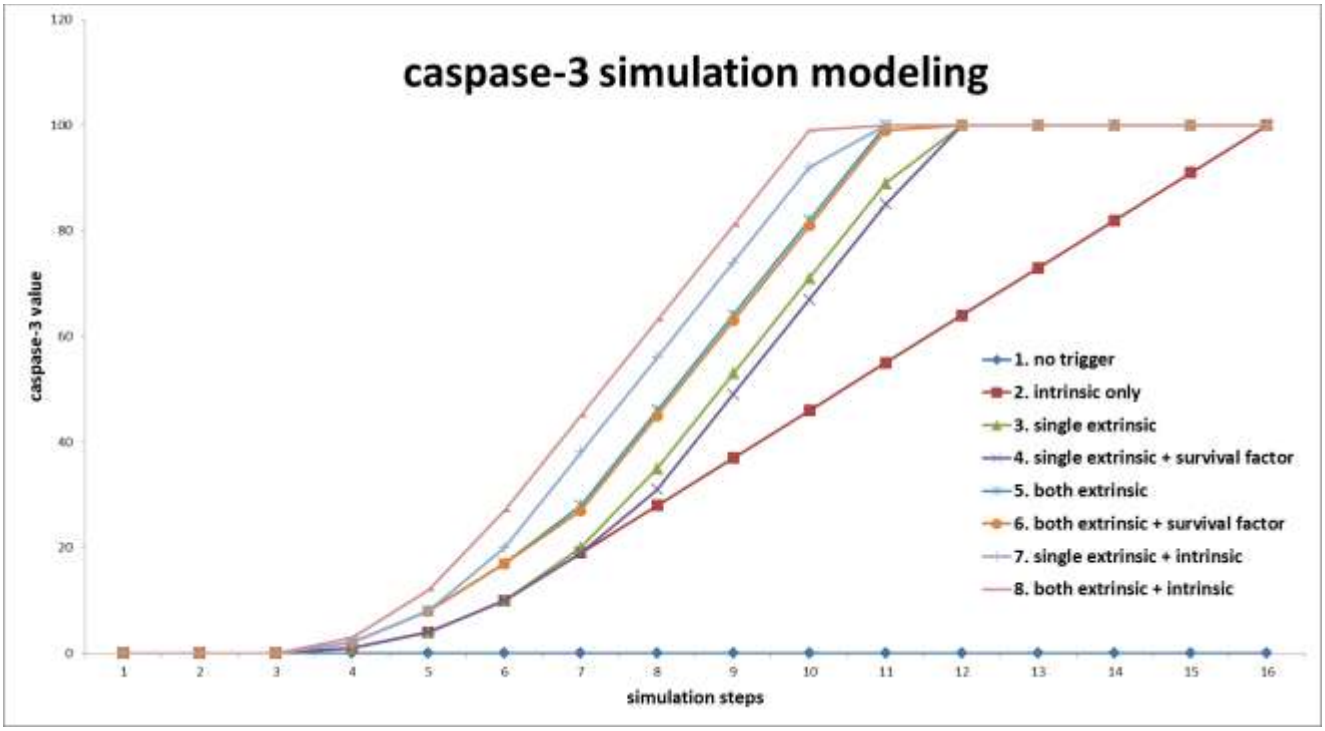

Figure 3. Caspase-3 simulation modeling for various groups. Each group represents a set of initial states described in Figure 4.

series 1: no trigger	series 2: intrinsic pathway only	series 3: single extrinsic pathway	series 4: single extrinsic pathway + survival factors
[0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0]	[0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0]	[0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0]	[0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1]
[0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1]	[0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0]	[0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1]	[1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1]
[0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1]	[0, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0]	[1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0]	
[0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0]	[1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0]	[1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1]	
[0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1]	[1, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0]		
[0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 1]			
[0, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0]			
[0, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1]			
[0, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 1]			
[1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0]			
[1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1]			
[1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 1]			
[1, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0]			
[1, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1]			
[1, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 1]			

series 5: both extrinsic pathways	series 6: both extrinsic pathways + survival factors	series 7: single extrinsic + intrinsic pathways	series 8: both extrinsic and intrinsic pathways
[1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0]	[1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1]	[0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0]	[1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0]
[1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1]		[1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0]	

Figure 4. groups of initial states showing similar apoptosis (caspase-3) pattern. Order of states (left to right): Fas*, TNFR*, FADD, Caspase-8, Caspase-3, c-FLIP*, Bid, Cytochrome-C, Smac, IAP, Caspase-9, p53*, Survival factors*
(* marks a real initial state, i.e. a state with no entering edges, whose value is generated and covered in simulation)

Discussion

Several observations on the model simulation can be made from figure 3 and 4 which correlate with what is known and expected.

Triggering apoptosis. Series 1 represents a set of initial states in which the three pathways are either not triggered or suppressed. For example: c-FLIP suppresses the death receptors Fas and TNFR or p53 is suppressed by the survival factors. As expected from the model, if none of the pathways is triggered as in series 1, then the execution pathway (caspase-3) is not triggered at all. In all other cases, caspase-3 is activated and amplified to its maximum simulation value.

Generally, the simulation analysis shows the impact of amplifying the apoptosis signaling cascade by triggering several pathways. First it shows that triggering three pathways (series 8) is the most significant: quickest rise in fewer steps to reach maximum value. This correlation between the number of triggered pathways and the amplification pattern of caspase-3 is also seen when triggering the two extrinsic pathways (series 5,6) compare to a single pathway (series 2,3,4).

It is also evident that triggering the intrinsic pathway explicitly (series 7) leads to amplified caspase-3 pattern compared to triggering the intrinsic pathway only via cross-talk by the extrinsic pathway (series 3). In other words, series 7 shows the importance of explicitly asserting the intrinsic pathway in amplifying the cascade (and not just through Bid). It is seen when comparing it to series 5, in which two extrinsic pathways are triggered initially and the intrinsic pathway is asserted indirectly.

Cross talk effects. In series 2, only the intrinsic pathway is triggered (as modeled by accumulation of p53) – the extrinsic pathway is either not triggered or the death receptors are suppressed by c-FLIP. This series clearly shows the most moderate activation of caspase-3. This can be explained by the absence of intrinsic → extrinsic communication. In contrast, in series 3, where only one of Fas or TNFR pathways is

triggered, the activation of caspase-3 is more significant. This can be attributed to the cross talk between extrinsic and intrinsic pathways via Bid protein. In other words, the extrinsic pathway triggers the intrinsic pathway and contributing to the activation of apoptosis cascade.

Another example of cross talk, though with smaller impact, is seen when anti-apoptotic signal is asserted in series 4 and 6, comparing to series 3 and 5 respectively. In both cases, caspase-3 amplification pattern is slightly suppressed when asserting the survival factor input (anti-apoptosis model) as expected with in-vivo apoptosis: a "battle" between pro- and anti-apoptosis agents. To increase this effect in the model it's possibly required to change the model, either by changing the weights or the transition function.

References

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Appendix

Code submitted in apop.py file.

Running the code outputs the data for figure 3 and figure 4.