# Identifying mechanisms for bistability in an apoptosis network

M Chaves, T Eissing & F Allgöwer

Institute for Systems Theory and Automatic Control, University of Stuttgart, Pfaffenwaldring 9, 70550 Stuttgart, Germany

## *Abstract*

The apoptosis network is a fundamental module in cellular signaling. Typically, two outcomes may be generated by this network, leading to the decision between two steady states: cell life or programmed cell death (a bistable response). A simple system consisting of three essential components is analyzed, which reproduces the main interactions among the apoptosis network and an anti-apoptotic pathway. To study how the bistability property emerges from the network structure, activation and inhibition functions are generally formulated, and the two network outcomes are associated with two disconnected invariant sets in the state space. Network links responsible for existence of each set, as well as sufficient conditions on the production and degradation rates are provided, which guarantee existence of both, or only one of the "life" or "death" sets.

## *1 Introduction*

The apoptosis network is responsible for programmed cell death, in response to certain stimuli. Apoptosis enables the organisms to eliminate unwanted cells and thus prevent, for instance, replication of damaged cells (see for example [1]). Cancer, as well as other diseases, may develop if the apoptosis network fails to respond in an appropriate manner. At the heart of the apoptosis network is a family of proteins (caspases, each existing in a pro-form and an active form), which are activated in a cascade ([1]; for more references see also [2]). Caspase 3 (C3) is a prominent downstream member of this cascade, and it is responsible for the cleavage (and destruction) of other proteins in the cell: thus high abundance of active C3 (C3a) typically leads to cell death.

Other pathways interact with the apoptosis network, in particular the well known Nuclear Factor $\kappa$B (NF$\kappa$B) pathway [1]. NF$\kappa$B is a transcription factor responsible for transcription of various genes, including one for its own inhibitor, and another for an inhibitor of C3a. Thus, the presence of NF$\kappa$B (or, more precisely, its transcription products) typically promotes survival of the cell. While the NF$\kappa$B pathway can be generally considered an anti-apoptotic pathway, it is often activated in parallel with the pro-apoptotic caspase cascade. For example, a common signal is stimulation of extrinsic death receptors with Tumor Necrosis Factor (TNF). The interaction among pro- and anti-apoptotic modules, will influence and fine tune the cellular decision to survive or undergo apoptosis.

Recently, mathematical models have been proposed for both the NF$\kappa$B pathway [3] and the caspase cascade [2, 4]. An interesting challenge is the identification of basic mechanisms that lead to the choice between a "living" and an "apoptotic" state. In [2, 4], this question is answered and discussed in detail, for the caspase cascade module. A complementary point of view is now given, with the analysis of a reduced model including only major players from both pathways. Representing the basic interactions among the pro- and anti-apoptotic modules, the model aims to study the importance of the network's various links and their contribution to bistability in the cellular decision.

## *2 The simplest model*

The model depicted in Fig. 1 reproduces the main crosstalking interactions among the NF$\kappa$B pathway and the apoptosis network. Both modules are triggered by TNF. In this model, C3a represents

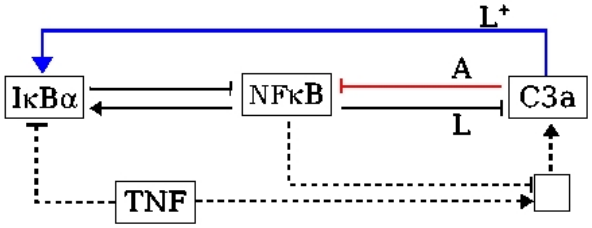


Figure 1: The network consisting of three nodes (NF$\kappa$B, I$\kappa$B, and C3a) and one input (TNF). A strong link A promotes apoptosis, while strong links L and $L^+$ support life.

the final result of caspase cascade activation, regulated by NF$\kappa$B. Because NF$\kappa$B is also inactivated by an element in its own pathway, two components from the anti-apoptotic pathway are needed.

NF$\kappa$B is a transcription factor responsible for, among others, transcription of I$\kappa$B mRNA and inhibitor of apoptosis proteins (IAP) mRNA [5]. So NF$\kappa$B leads to production of its own inhibitor I$\kappa$B and the C3a inhibitor IAP. Link L represents the latter interaction. C3a is thought to inactivate and thereby inhibit NF$\kappa$B (link A), and also indirectly contribute to the presence of I$\kappa$B (C3a inactivates an inhibitor of I$\kappa$B) [6]. This interaction is introduced as a direct activation link ($L^+$) in the network. Finally, TNF stimulation acts in two ways: it contributes to I$\kappa$B inhibition, and (through a longer pathway) activates C3a. The latter activation is also controlled by protein products of NF$\kappa$B mediated transcription.

The main objective is to model this network in a form as general as possible, retaining the structure of the interconnections without specifying particular kinetic laws. Thus we consider that each of the three nodes is freely degraded, and is produced according to the overall result of the several activation and inhibition links particular for that node. Let $\mathbb{R}_+ = (0, \infty)$. To each activation link ($x \to \cdot$) associate an *activation function* $\nu(x)$, defined as:

(i) $\nu : [0, \infty) \to [0, V]$ is strictly increasing, continuously differentiable ($V \in \mathbb{R}_+$);

(ii) $\lim_{x\to 0} \nu(x) = 0$ and $\lim_{x\to\infty} \nu(x) = V$;

(iii) There exists a threshold $0 < \phi < \infty$ and constants $0 < \varepsilon < \frac{1}{2}V$, $0 < \Delta < \frac{1}{2}\phi$ such that

$$x \in [0, \phi - \Delta] \;\Leftrightarrow\; \nu(x) \in [0, \varepsilon],$$
$$x \in [\phi + \Delta, \infty] \;\Leftrightarrow\; \nu(x) \in [V - \varepsilon, V].$$

Similarly, to each inhibition link ($x \dashv \cdot$) associate an *inhibition function* $\mu(x)$, defined as:

(i) $\mu : [0, \infty) \to [0, M]$ is strictly decreasing, continuously differentiable ($M \in \mathbb{R}_+$);

(ii) $\lim_{x\to 0} \mu(x) = M$ and $\lim_{x\to\infty} \mu(x) = 0$;

(iii) There exists a threshold $0 < \theta < \infty$ and constants $0 < \varepsilon < \frac{1}{2}M$, $0 < \Delta < \frac{1}{2}\theta$ such that

$$x \in [0, \theta - \Delta] \;\Leftrightarrow\; \mu(x) \in [M - \varepsilon, M],$$
$$x \in [\theta + \Delta, \infty] \;\Leftrightarrow\; \mu(x) \in [0, \varepsilon].$$

So, there is a "tube" inside which the functions must lie. Examples of such functions include Hill and other sigmoidal shaped functions. Observe that the limiting case $\varepsilon \equiv \Delta \equiv 0$ reduces essentially to the piecewise linear systems introduced first by Glass and Kauffman [7], and more recently used to study gene regulatory networks in [8], and [9].

The dynamical system for the network depicted in Fig. 1 can then be written:

$$\begin{aligned}
\frac{d[\text{NF}\kappa\text{B}]}{dt} &= -k_{\text{NF}\kappa\text{B}}[\text{NF}\kappa\text{B}] + \mu_1([\text{I}\kappa\text{B}])\,\mu_3([\text{C3a}]) \\
\frac{d[\text{I}\kappa\text{B}]}{dt} &= -k_{\text{I}\kappa\text{B}}[\text{I}\kappa\text{B}] + \nu_1([\text{NF}\kappa\text{B}])\,\nu_2([\text{C3a}])\,\mu_5([\text{TNF}]) \\
\frac{d[\text{C3a}]}{dt} &= -k_{\text{C3a}}[\text{C3a}] + \mu_2([\text{NF}\kappa\text{B}])\;(1 + [\text{TNF}]\mu_4([\text{NF}\kappa\text{B}]))\,.
\end{aligned} \tag{1}$$

The term $\mu_1(\cdot)\mu_3(\cdot)$ does not represent a reaction rate, but instead it should be interpreted as a total production rate for NF$\kappa$B, which depends only on how large the concentrations of I$\kappa$B and C3a are at each instant. Similar interpretation holds for the other production terms. TNF stimulation maybe assumed as a constant input [TNF] to the system. TNF activates first caspase 8, which in turn activates caspase 3, and NF$\kappa$B also functions as an inhibitor of this step (through the activity of FLIP, an inhibitor of caspase 8) [1]. In this model, we wish to study the existence of steady states, both with and without TNF stimulation. Thus, this stimulus is represented by the term $(1 + [\text{TNF}]\mu_4([\text{NF}\kappa\text{B}])$: in the absence of TNF the regulatory links between caspase 3 and NF$\kappa$B are unchanged, while the presence of TNF enhances the inhibition from C3a to NF$\kappa$B link.

## 3 Conditions for bistability

The apoptosis system must be able to respond in two distinct ways, which in previous models have been represented by two different stable steady states (eg. [2, 4]). Following the introductory discussion, in model (1) a "living" response corresponds to low concentration of C3a and high concentration of NF$\kappa$B, and conversely an "apoptotic" response corresponds to high concentration of C3a and low concentration of NF$\kappa$B. A more general approach is now proposed, where the "living" and "apoptotic" responses are represented by two appropriate sets, rather than two steady state points, in space. More precisely, in Propositions 3.1 and 3.2 below, we will establish the existence of *two disconnected forward-invariant sets* for system (1), one characterized by low C3a / high NF$\kappa$B and the other by high C3a / low NF$\kappa$B (as depicted in Fig. 3). From the biological point of view, this is a relevant notion: the two invariant sets allow the trajectories to have fluctuations around a given state, while maintaining their qualitative properties.

We will consider now system (1) without inputs (i.e., TNF $= 0$). To avoid cumbersome notation, let $X = (x, y, c)'$ with $x :=$ [NF$\kappa$B], $y :=$ [I$\kappa$B], $c :=$ [C3a], denote the three variables. Since the right-hand side of (1) is continuous, there exists a unique solution of the initial value problem (1) with $X(0) = X_0$, defined on some interval $\mathcal{T}$. Let $X(t, X_0)$ denote this solution, with $t \in \mathcal{T}$. We will say that a set $J$ is *forward-invariant* for system (1), if every trajectory $X(t, X_0)$ with $X_0 \in J$ remains in $J$ for all times, i.e., $X(t, X_0) \in J$ for all $t \in \mathcal{T}$. Consider system (1), with TNF $\equiv 0$. Let $\varepsilon > 0$, $\Delta > 0$, and define the functions $\mu_i$ and $\nu_i$ as above, so that $M_i, V_i > 2\varepsilon$ and $\theta_i, \phi_i > 2\Delta$ for all $i$. Consider the set:

$$J = \left[0, \frac{1}{k_{\text{NF}\kappa\text{B}}} M_1 M_3\right] \times \left[0, \frac{1}{k_{\text{I}\kappa\text{B}}} V_1 V_2\right] \times \left[0, \frac{1}{k_{\text{C3a}}} M_2\right]. \tag{2}$$

It is clear that $J$ is a forward-invariant set for the system: the vector field at the boundary of $J$ points toward its interior. Furthermore, since $J$ is compact, any trajectory $X(t, X_0)$, $X_0 \in J$ is in fact defined for all times, that is $\mathcal{T} \equiv [0, \infty)$. From now on, we consider only trajectories evolving in $J$.

Next we will establish conditions on the degradation and production rates (summarized in Table 1), that guarantee existence of both the "living" and "apoptotic" responses, or only one of

Table 1: Sufficient conditions for existence of one or more distinct steady states.

| Apoptosis | Apoptosis and Living | Living |
|---|---|---|
| $\varepsilon < M_2 - k_{\text{C3a}}(\theta_3 + \Delta)$ | $\varepsilon < \min\{\frac{k_{\text{NF}\kappa\text{B}}}{M_1}(\theta_2 - \Delta),$ $M_2 - k_{\text{C3a}}(\theta_3 + \Delta),$ $k_{\text{C3a}}(\theta_3 - \Delta),$ $M_3 - \frac{k_{\text{NF}\kappa\text{B}}}{m_1}(\theta_2 + \Delta)\}$ | $\varepsilon < M_3 - \frac{k_{\text{NF}\kappa\text{B}}}{m_1}(\theta_2 + \Delta)$ |
| $k_{\text{NF}\kappa\text{B}} > \frac{M_1 M_3}{\theta_2 - \Delta}$, $k_{\text{C3a}} < \frac{M_2}{\theta_3 + \Delta}$ | $k_{\text{NF}\kappa\text{B}} < \frac{m_1 M_3}{\theta_2 + \Delta}$, $k_{\text{C3a}} < \frac{M_2}{\theta_3 + \Delta}$ | $k_{\text{NF}\kappa\text{B}} < \frac{m_1 M_3}{\theta_2 + \Delta}$, $k_{\text{C3a}} > \frac{M_2}{\theta_3 - \Delta}$ |

them. Define also

$$m_1 := \mu_1\left(\frac{1}{k_{\text{I}\kappa\text{B}}}V_1V_2\right). \tag{3}$$

**Proposition 3.1** *Assume: (a)* $\frac{1}{k_{C3a}}\varepsilon < \theta_3 - \Delta$*, and (b)* $\frac{m_1}{k_{NF\kappa B}}(M_3 - \varepsilon) > \theta_2 + \Delta$*. Then the set:*

$$\mathcal{L} \;=\; \left[\frac{m_1}{k_{NF\kappa B}}(M_3 - \varepsilon), \frac{M_1}{k_{NF\kappa B}}M_3\right] \times \left[0, \frac{1}{k_{I\kappa B}}V_1V_2\right] \times \left[0, \frac{1}{k_{C3a}}\varepsilon\right],$$

*is forward-invariant for system (1).*

*Proof:* Pick any $X_0 \in \mathcal{L}$, and let $X(t) = (x(t), y(t), c(t))'$ denote the corresponding solution. First, note that $\mathcal{L} \subset J$, and that the interval for $y$ is unchanged (compare to (2)). So it is enough to show that $x(t) \geq m_1(M_3 - \varepsilon)/k_{\text{NF}\kappa\text{B}}$ and $c(t) \leq \varepsilon/k_{\text{C3a}}$ for all $t \geq 0$. To prove the statement by contradiction, consider the first instant when $X(t)$ leaves $\mathcal{L}$:

$$t_0 = \inf\{t \geq 0 :\; X(t) \notin \mathcal{L}\}.$$

Assume that coordinate $x$ is the first to leave the set (a similar argument holds if $c$ is the one). So: $x(t_0) \leq m_1(M_3 - \varepsilon)/k_{\text{NF}\kappa\text{B}}$ and $c(t_0) \leq \varepsilon/k_{\text{C3a}}$.

By assumption (a), $c(t_0) < \theta_3 - \Delta$ which implies $\mu_3(c(t_0)) > (M_3 - \varepsilon)$ (by definition of $\mu_3$). Therefore

$$f_{\text{NF}\kappa\text{B}}(X(t_0)) \;\;=\;\; -k_{\text{NF}\kappa\text{B}}x(t_0) + \mu_1(y(t_0))\mu_3(c(t_0)) > -k_{\text{NF}\kappa\text{B}}x + m_1(M_3 - \varepsilon) \geq 0,$$

and by continuity of solutions, there is an interval $(t_0 - \delta, t_0 + \delta)$, where $f(X(t)) > 0$. Then $x(t_0 - \delta) < x(t_0)$, contradicting the minimality of $t_0$. ∎

Following a similar reasoning, it can be shown that:

**Proposition 3.2** *Assume: (c)* $\frac{1}{k_{C3a}}(M_2 - \varepsilon) > \theta_3 + \Delta$*, and (d)* $\frac{M_1}{k_{NF\kappa B}}\varepsilon < \theta_2 - \Delta$*. Then the set:*

$$\mathcal{A} \;=\; \left[0, \frac{M_1}{k_{NF\kappa B}}\varepsilon\right] \times \left[0, \frac{1}{k_{I\kappa B}}V_1V_2\right] \times \left[\frac{1}{k_{C3a}}(M_2 - \varepsilon), \frac{1}{k_{C3a}}M_2\right],$$

*is forward-invariant for system (1).* □

Propositions 3.1 and 3.2 give conditions for the existence of "living" ($\mathcal{L}$) and "apoptotic" ($\mathcal{A}$) sets, respectively, both contained in the larger set $J$ (Fig. 3). Note that $\varepsilon < M_2/2$, and so these sets are disconnected if and only if $M_1\varepsilon < m_1(M_3 - \varepsilon)$. This is guaranteed, for instance, by sufficiently small $\varepsilon$. The next two Propositions provide stricter conditions, which guarantee that only one of the two possible responses may ultimately happen.

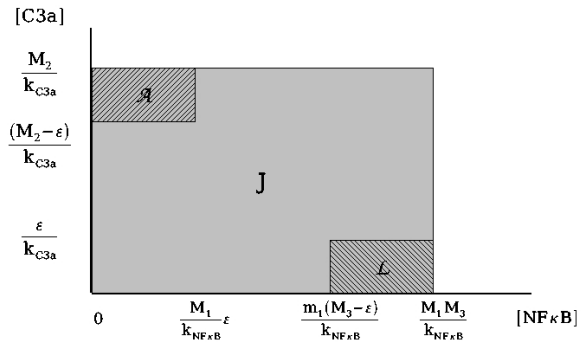


Figure 3: The "living" ($\mathcal{L}$) and "apoptosis" ($\mathcal{A}$) invariant sets.

**Proposition 3.3** *Assume: (e)* $\frac{1}{k_{C3a}}M_2 < \theta_3 - \Delta$. *Then the following set is an attractor of system (1):*

$$\mathcal{L}_* \;=\; \left[\frac{m_1}{k_{NF\kappa B}}(M_3-\varepsilon), \frac{M_1}{k_{NF\kappa B}}M_3\right] \times \left[0, \frac{1}{k_{I\kappa B}}V_1V_2\right] \times \left[0, \frac{1}{k_{C3a}}M_2\right].$$

*Proof.* Pick any $X_0 \in \mathcal{L}_*$, and let $X(t) = (x(t), y(t), c(t))'$ denote the corresponding solution. Again, note that $\mathcal{L}_* \subset J$, and that the intervals for both $y$ and $c$ are unchanged (see (2)). So it is enough to show that $x(t) \geq m_1(M_3-\varepsilon)/k_{\text{NF}\kappa\text{B}}$ for all $t$. To prove the statement by contradiction, assume that there exists $t_2 > 0$ such that $x(t_2) < \frac{m_1}{k_{\text{NF}\kappa\text{B}}}(M_3-\varepsilon)$. If this is so (and because solutions are continuous), then there exists a nonemtpy interval $[t_2-\delta, t_2]$ where $x(t)$ decreases, while in the interval $[0, \frac{m_1}{k_{\text{NF}\kappa\text{B}}}(M_3-\varepsilon))$. From assumption (e), the form of the invariant set (2), and from the definition of $\mu_3$ we have, for all $t$,

$$c(t) < \theta_3 - \Delta \;\Leftrightarrow\; \mu_3(c(t)) \geq M_3 - \varepsilon \;\Rightarrow\; f_{\text{NF}\kappa\text{B}}(X(t)) \geq -k_{\text{NF}\kappa\text{B}}x + m_1(M_3-\varepsilon).$$

It is immediate to see that $x < m_1(M_3-\varepsilon)/k_{\text{NF}\kappa\text{B}}$ implies $f_{\text{NF}\kappa\text{B}} > 0$, so on the interval $[0, \frac{m_1}{k_{\text{NF}\kappa\text{B}}}(M_3-\varepsilon))$, $x(t)$ is always an increasing function, contradicting the initial hypothesis. ∎

**Proposition 3.4** *Assume: (f)* $\frac{M_1M_3}{k_{NF\kappa B}} < \theta_2 - \Delta$. *Then the following set is an attractor of system (1):*

$$\mathcal{A}_* \;=\; \left[0, \frac{M_1}{k_{NF\kappa B}}M_3\right] \times \left[0, \frac{1}{k_{I\kappa B}}V_1V_2\right] \times \left[\frac{1}{k_{C3a}}(M_2-\varepsilon), \frac{1}{k_{C3a}}M_2\right].$$

□

The proof is quite similar to that of Proposition 3.3.

Observe that the conditions (a)-(d) are compatible, that is they can be simultaneously satisfied to guarantee existence of both sets $\mathcal{A}$ and $\mathcal{L}$. Alternatively, (a),(b) and (e) can be combined to produce a system whose trajectories eventually converge to set $\mathcal{L}$. Likewise (c), (d) and (f) can be combined to produce an apoptotic cell, that is, a system whose trajectories eventually converge to set $\mathcal{A}$. The results are summarized in Table 1, and can be written in the form of an upper bound on $\varepsilon$ – which is arbitrary, and can indeed be chosen as small as desired – and on the ratio of production to degradation rates of NF$\kappa$B and C3a. Indeed, $M_3$ denotes the maximal production rate of NF$\kappa$B (without loss of generality one may assume $M_1 = 1$) and $M_2$ denotes the maximal production rate of C3a. The conditions for existence of only the "living" set can be written as:

$$\left.\frac{\text{rate of production}}{\text{rate of degradation}}\right]_{\text{NF}\kappa\text{B}} > \frac{1}{m_1}\text{ inhibition threshold}_{\text{NF}\kappa\text{B}\dashv\text{C3a}} + \Delta,$$

$$\left.\frac{\text{rate of production}}{\text{rate of degradation}}\right]_{\text{C3a}} < \text{ inhibition threshold}_{\text{C3a}\dashv\text{NF}\kappa\text{B}} - \Delta,$$

that is, in a living cell there should be enough NF$\kappa$B available to inhibit C3a, but low amounts of C3a so that inhibition of NF$\kappa$B is weak, in agreement with the network pathways described in the introduction. Alternatively, a living cell may have a very high threshold value $\theta_3$, meaning that a very large concentration of C3a is required to inhibit NF$\kappa$B (link A in Fig. 1 is weak). Not surprisingly, the existence of only the "apoptotic" set is guaranteed by opposite inequalities. The life/death decision depends heavily on the relative strength of the mutual inhibition between NF$\kappa$B and C3a (links A and L in Fig. 1). Therefore, a bistable response emerges from the network's interconnections, rather than from specific reaction functions.

## *4 The role of TNF in the apoptosis/living decision*

To study the effect of TNF stimulation on the system, assume that TNF is a constant input to the system. As before, we adopt the notation $x := [\text{NF}\kappa\text{B}]$, $y := [\text{I}\kappa\text{B}]$, $c := [\text{C3a}]$, and also define $u := \text{TNF}$. An immediate observation is that the previous results are still valid, provided the value $M_2$ is updated:

$$M_2 \;\rightsquigarrow\; M_2(1 + M_4\text{TNF}).$$

Thus, if all other constants are fixed, as TNF increases there will be a point where condition (e) is no longer satisfied, and another point after which only condition (c) is satisfied:

$$\text{TNF} \geq \frac{1}{M_2 M_4}\,(k_{\text{C3a}}\,(\theta_3 - \Delta) - (M_2 - \varepsilon)).$$

Roughly, TNF stimulation weakens the activation link $\text{L}^+$, and strengthens the inhibition link A. For a numerical example, Fig. 4 indicates that, the intermediate, unstable, steady state shifts towards higher NF$\kappa$B values, as TNF strength increases. As a result, the basin of attraction of the "living" steady state (high NF$\kappa$B) decreases.

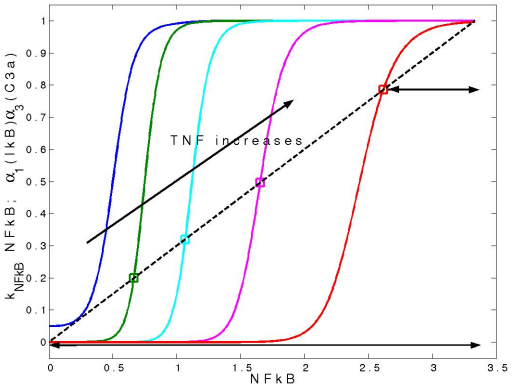


Figure 4: The steady states of the system as TNF strength increases. The dashed line represents $k_{\text{NF}\kappa\text{B}}[\text{NF}\kappa\text{B}]$, while the remaining functions represent $\mu_1(x)\,\mu_3(y)$, for increasing values of TNF (left to right) after numerically solving equation (4) for each constant $u = \text{TNF}$. The intermediate steady state (square) increases with TNF.

We will next make these remarks more precise. Fig. 4 indicates that steady states will vary continuously with TNF and, moreover, at an unstable steady state the NF$\kappa$B coordinate will increase with TNF while the C3a coordinate will decrease. To prove this, first note that the steady states of

system (1) can be determined by solving the system (with $u = \text{TNF}$) $F(x, y, c, u) = 0$:

$$F(x,y,c,u) = \begin{pmatrix} -k_{\text{NF}\kappa\text{B}}x + \mu_1(y)\,\mu_3(c) \\ -k_{\text{I}\kappa\text{B}}y + \nu_1(x)\,\nu_2(c)\,\mu_5(u) \\ -k_{\text{C3a}}\,c + \mu_2(x)\,(1 + u\mu_4(x)) \end{pmatrix} = 0. \tag{4}$$

The derivatives of $F$ are ("prime" denotes derivative with respect to a function's unique argument):

$$\frac{\partial F}{\partial X} = \begin{pmatrix} -k_{\text{NF}\kappa\text{B}} & \mu_1'\mu_3 & \mu_1\mu_3' \\ \nu_1'\nu_2\mu_5 & -k_{\text{I}\kappa\text{B}} & \nu_1\nu_2'\mu_5 \\ \mu_2'(1+u\mu_4) + u\mu_2\mu_4' & 0 & -k_{\text{C3a}} \end{pmatrix}, \quad \frac{\partial F}{\partial u} = \begin{pmatrix} 0 \\ \nu_1\nu_2\mu_5' \\ \mu_2\mu_4 \end{pmatrix},$$

and:

$$D(X,u) = \det\left(\frac{\partial F}{\partial X}\right) = k_{\text{I}\kappa\text{B}}k_{\text{C3a}}\left(R(X,u)P(X,u) - N(X,u)\right)$$

where (dropping dependencies on $X$ and $u$, for simplicity)

$$\begin{aligned} R &= -\frac{1}{k_{\text{I}\kappa\text{B}}k_{\text{C3a}}}(\mu_1'\mu_3\nu_1\nu_2'\mu_5 + k_{\text{I}\kappa\text{B}}\mu_1\mu_3') \\ N &= k_{\text{NF}\kappa\text{B}} - \frac{1}{k_{\text{I}\kappa\text{B}}}\mu_1'\mu_3\nu_1'\nu_2\mu_5 \\ P &= -\mu_2'(1+u\mu_4) - u\mu_2\mu_4'. \end{aligned}$$

Note that by definition of the functions $\mu_i$, $\nu_i$ it holds that $R, N, P > 0$ for any $X \in \mathbb{R}^3_{\geq 0}$. Now suppose that $X^* = (x^*, y^*, c^*)'$ is a steady state of the system, for $\text{TNF} = \text{TNF}^* = u^*$, and such that $D(X^*, u^*) \neq 0$. Then, by the Implicit Function Theorem, there exist neighbourhoods $\mathcal{V}_* \subset \mathbb{R}^3$ of $X^*$, and $\mathcal{U}_* \subset \mathbb{R}_+$ of $u^*$, and a function $f$ such that

$$f : \mathcal{U}_* \to \mathcal{V}_*, \;\; X = f(u) \;\; \text{and} \;\; \frac{df}{du} = -\left(\frac{\partial F}{\partial X}\right)^{-1}\frac{\partial F}{\partial u}.$$

Some simple algebra shows that (with $Q = -\frac{1}{k_{\text{I}\kappa\text{B}}}\mu_1'\mu_3 > 0$):

$$\left(\frac{\partial F}{\partial X}\right)^{-1} = \begin{pmatrix} \times\times & -\frac{Q}{RP-N} & -\frac{R}{RP-N} \\ \times\times & \times\times & \times\times \\ \times\times & \frac{1}{k_{\text{C3a}}}\frac{QP}{RP-N} & \frac{1}{k_{\text{C3a}}}\frac{N}{RP-N} \end{pmatrix}.$$

Then:

$$\begin{aligned} \frac{dx^*}{du} &= \frac{Q}{RP-N}\left(\nu_1\nu_2\mu_5' + \frac{R}{Q}\mu_2\mu_4\right) \\ \frac{dc^*}{du} &= -\frac{1}{k_{\text{C3a}}}\frac{PQ}{RP-N}\left(\nu_1\nu_2\mu_5' + \frac{N}{PQ}\mu_2\mu_4\right). \end{aligned}$$

Consider next funtions $\mu_i$ and $\nu_i$ and an input $\text{TNF}^*$ such that conditions (a)-(d) are satisfied, so both sets $\mathcal{L}$ and $\mathcal{A}$ exist. Then there must exist a (unstable) steady state of system (1), denoted $X^* = (x^*, y^*, c^*)'$, in $J \setminus (\mathcal{L} \cup \mathcal{A})$. The following result is now straightforward:

**Proposition 4.1** *Assume: (g)* $\lim_{u\to\infty}\mu_5'(u) = 0$*, and (h)* $R(X^*, \textit{TNF}^*)P(X^*, \textit{TNF}^*) > N(X^*, \textit{TNF}^*)$*. Then, the determinant* $D(X^*, \textit{TNF}^*)$ *is positive and steady state* $X^*$ *is unstable. Furthermore, for sufficiently large* $\textit{TNF} \in \mathcal{U}_*$*,* $x^*$ *(NF$\kappa$B) increases and* $c^*$ *(C3a) decreases with TNF.*

*Proof.* Assumption (h) immediately implies that the determinant is positive, and this means that at least one of the eigenvalues of $\partial F/\partial X$ has positive real part. Let $\mathcal{V}_*$ and $\mathcal{U}_*$ be the neighbourhoods provided by the Implicit Function Theorem. By assumption (g), and since functions $\nu_i$ and $\mu_i$ are bounded in these neighbourhoods, there exists $\bar{u}$ such that $|\nu_1\nu_2\mu_5'| < (R/Q)\mu_2\mu_4$, and $|\nu_1\nu_2\mu_5'| < (N/PQ)\mu_2\mu_4$ for all $u \geq \bar{u}$. If, in addition, $\bar{u} \in \mathcal{U}_*$, then

$$\frac{dx^*}{du} > 0 \text{ and } \frac{dc^*}{du} < 0, \ \forall\, u \in \{u \in \mathcal{U}_* : \ u \geq \bar{u}\}.$$

■

While this result is not very satisfying (if $\bar{u} \notin \mathcal{U}_*$, the second statement becomes empty), it indicates that strong TNF stimulation will shift the steady states in such a way that a larger set of initial conditions will evolve towards an apoptotic state.

## 5 Conclusions

A model of the network of interactions among pro- and an anti-apoptotic pathways was studied. One of the main features of the model is its general formulation, which allows freedom in the forms for activation and inhibition functions. The two possible outcomes of the apoptosis network, "life" and "death", are associated with two disconnected invariant sets, thus allowing the the model to capture cell-to-cell variability. Links which promote the existence of each invariant set are identified, and sufficient conditions for existence of both or only one of the sets are provided, relating the production-to-degradation ratios with the inhibition thresholds. The bistable response of the network thus emerges as a consequence of its links and structure, and does not depend on very specific reaction rates' functions. Under TNF stimulation, the model predicts that the "life" invariant set will vanish and the "death" invariant set becomes an attractor of the system: that is, the outcome of the network will more likely be cell death. This response to TNF stimulation is also in agreement with experimental observations.

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