

A Mathematical Model of Fas Signaling Induced Apoptosis*

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Abstract The ability for living cells to respond properly to apoptosis signals is crucial for cell development. Bistability (i.e., all-or-none behavior) of the caspase-3 activity has been proposed by some researchers to model the bimodal cell fate decision upon stimulation of the apoptosis signaling pathway. In this study, we investigate Fas-induced apoptosis, and establish a mathematical model without the requirement of bistability. In our model, the rapid degradation of the active forms of caspase-8 and -3 are highlighted. The model simulation reveals a pulse increasing of caspase-3 activation following FasL stimulation to trigger the irreversible death program.

Keywords Mathematical Model; Caspase-3; Caspase-8; Bistability

1 Introduction

Apoptosis is a type of genetic programmed cell death event that is crucial for development, tissue homeostasis and immune response in multicellular organisms [11]. Defects in apoptosis can result in a number of serious diseases such as cancer, autoimmunity, and neurodegeneration. The cell displays ‘bistability’ (i.e., all-or-none behavior) in response to death signaling [4]. On the one hand, the viable ‘alive’ state must be stable and resistant toward minor apoptosis signals. On the other hand, when apoptosis signals extend beyond a certain threshold, the cell must initiate the irreversible signaling pathways to trigger the cell death program. Nevertheless, the means by which cells determine their fate (survival or death) according to signaling pathways is not well understood. This paper will study

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the extracellular signaling induced apoptosis using a mathematical model without the prerequisite of bistability. With this model, we were able to study the temporal behavior of caspase-3 activity upon apoptosis stimulation.

Many efforts have been made to quantitatively study the process of cell fate determination upon death signal stimulation [1, 2, 4, 5, 8, 10, 12, 15]. In many studies, bistability was proposed as one of the reasons for the existence of such a threshold [4]. Several models have been proposed to explain the bistability properties associated with apoptosis signaling pathways [1, 4, 10]. In these models, coexistence of steady states with low or high caspase-3 activity was presumed to be an “essential condition” [4]. The implicit positive feedback loop involving caspase-3 activation by the inhibitors of apoptosis (IAPs) was considered to be essential for such bistability. However, to the best of our knowledge, no experimental evidence supporting such an assumption is known. Instead, the high caspase-3 activity is not necessary the steady state. A pulse increasing of caspase-3 activity could trigger apoptosis as well. In this paper, we will study Fas signaling induce apoptosis and establish a mathematical model with pulse increasing a caspase-3 activity.

Fas(CD95/Apo-1) is a member of the tumor necrosis factor receptor (TNF-R) family that is involved in death transduction signaling and it plays a significant role in immune functions [9]. The mathematical model developed in this paper was based on the Fas-signaling pathway model proposed by Hua et al. [8] (hereinafter abbreviated as the HCCSL model), which was optimized for human Jurkat T cells (also refer [12]). The main drawback in the HCCSL model is that the degradations of active caspase-8 and -3 are not considered. As result, the HCCSL model predicts that the caspase activities should be saturated after prolonged stimulation even when Fas signaling is very weak. These assumptions are not in agreement with experimental observations that the indicated caspase activities decrease after reaching a maximal value [2, 7, 13, 16]. The rapid degradation of active caspase-3 was reported in Tawa et al. [16], and might be particularly relevant in long-live cells [15].

2 Model and result

2.1 Model structure

Figure 1 shows a cartoon of the Fas-signaling associated apoptosis pathway. We adapted the main constituents of the pathway from the model studied by Hua et al. [8] and Okazaki et al. [12]. Fas is a member of tumor necrosis factor receptor (TNF-R) family. Upon the stimulation of Fas ligand (FasL), the Fas-associated protein with death domain (FADD) recruits procaspase-8 to form the death inducing signaling complex (DISC). These complexes cleave procaspase-8 and release the active caspase-8, which in turn induces the cleavage of procaspase-3, either directly (type I cells) or indirectly through mitochondria (type II cells)[14]. In type I cells, a large amount of caspase-8 is released from DISC and cleave caspase-3 directly to trigger the downstream apoptosis pathway. In type II cells, the caspase-8 signal is amplified via the mitochondria and procaspase-9 to result in cleaved procaspase-3. The amplification pathway is complicated, including the following main steps: Active caspase-8 enzymatically cleaves Bid to generate tBid. The tBid associated with two Bax proteins (tBid:Bax₂) ‘activates’ the mitochondria which then releases pro-apoptotic molecules, such as *cytochrome c* and Smac/DIABLO. Once released, *cytochrome c* associates with Apaf-1 (Apaf) and procaspase-9 to form apopto-

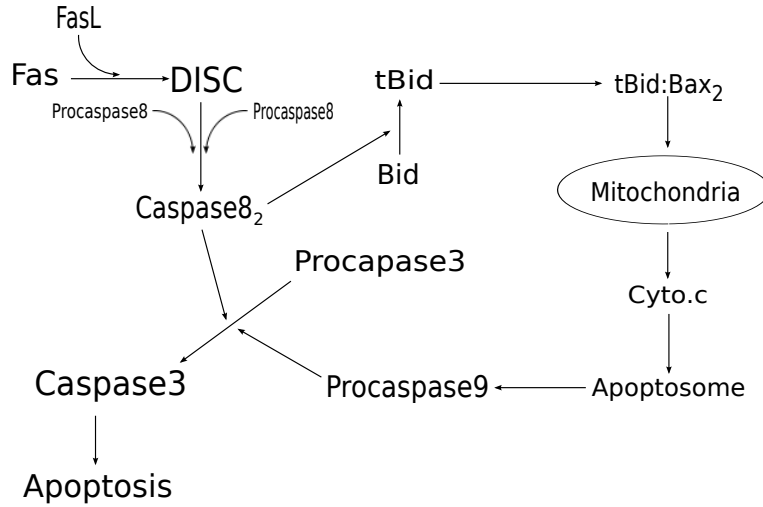


Figure 1: Cartoon of the model for Fas signaling induced apoptosis.

some, the second initiator complex of apoptosis, to generate active caspase-9 which then cleaves procaspase-3 to produce active caspase-3.

2.2 Mathematical model

In the following mathematical model, we will avoid modeling every reaction in the whole pathway. Instead, we will focus on the time courses of the initiator protein concentrations (Fas receptor, caspase-8) and executor concentrations (caspase-3) in the apoptosis pathway. There are five main components in our model, [FasC], [Casp8], [Casp3], [Casp8*] and [Casp3*], representing respectively the concentrations of bound Fas receptor, procaspase-8, procaspase-3, active caspase-8 and caspase-3, respectively. The concentrations of procaspases and caspases are expected to exist in a steady state prior to the onset of the apoptosis signal. When the Fas ligands are released and bind to their cognate receptors, the downstream signals will change due to the stimulus, synthesis, degradation, and cleavage of the procaspases.

The time course of caspase-3 activity includes association of FasL to the receptor Fas, dissociation of Fas, activation of caspase-8 through DISC, activation of caspase-3 directly and through mitochondria, synthesis of procaspases, and degradation of both inactive and active forms of the caspases. This process can be modeled by the following set of

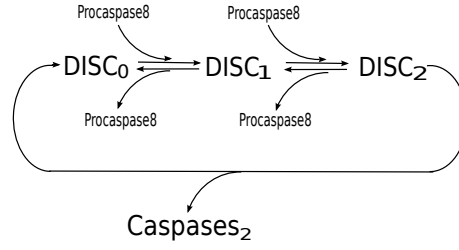


Figure 2: A simplified model of caspase-8 activation through DISC. DISC_{*i*} represents DISC with *i* procaspase-8.

differential equations

$$\frac{d[\text{FasC}]}{dt} = k_{\text{on}}[\text{FasL}]([\text{Fas}]_0 - [\text{FasC}]) - k_{\text{off}}[\text{FasC}] \quad (1)$$

$$\frac{d[\text{Casp8}]}{dt} = v_{\text{Casp8}} - 2 \frac{v_1 [\text{FasC}] [\text{Casp8}]^2}{K_1^2 + K_2 [\text{Casp8}] + [\text{Casp8}]^2} - d_{\text{Casp8}} \quad (2)$$

$$\frac{d[\text{Casp8}_2^*]}{dt} = \frac{v_1 [\text{FasC}] [\text{Casp8}]^2}{K_1^2 + K_2 [\text{Casp8}] + [\text{Casp8}]^2} - d_{\text{Casp8}_2^*} \quad (3)$$

$$\begin{aligned} \frac{d[\text{Casp3}]}{dt} &= v_{\text{Casp3}} - v_2 [\text{Casp8}_2^*] [\text{Casp3}] \\ &\quad - v_3 \frac{[\text{Casp9}]_0 [\text{Casp8}_2^*]_{\tau} [\text{Casp3}]}{K_3 + [\text{Casp8}_2^*]_{\tau}} - d_{\text{Casp3}} \end{aligned} \quad (4)$$

$$\begin{aligned} \frac{d[\text{Casp3}^*]}{dt} &= v_2 [\text{Casp8}_2^*] [\text{Casp3}] \\ &\quad + v_3 \frac{[\text{Casp9}]_0 [\text{Casp8}_2^*]_{\tau} [\text{Casp3}]}{K_3 + [\text{Casp8}_2^*]_{\tau}} - d_{\text{Casp3}^*} \end{aligned} \quad (5)$$

In the above model, equation (1) is not essential for the apoptosis pathway, and is introduced only to reproduce the delay of caspase-8 activation in the experimental data (Fig. 3A). The coefficient 2 in (2) comes from the dimerization of active caspase-8.

The activation of caspase-8 is a complicated process requiring the involvement of DISC. These processes are composed of many various reactions including the recruitment of Fas-associated protein with death domain (FADD), the binding of FADD to FasC, the binding of FLIP or procaspase-8 to the FADD, and the generation of intermediate cleavage product Casp8₂: p41 [12]. Here, we simplified the process to only track the state of DISC, which is characterized by the number of procaspase-8 in the complex (Fig. 2). With this simplification and assuming the quasi-equilibrium of the intermediate complexes DISC_{*i*}, we obtain the flux of caspase-8 activity as given by

$$\frac{v_1 [\text{FasC}] [\text{Casp8}]^2}{K_1^2 + K_2 [\text{Casp8}] + [\text{Casp8}]^2}$$

in (2)-(3).

We assumed a first order reaction wherein caspase-8 and caspase-9 cleaved procaspase-3, in accordance with Okazaki et al. [12]. The active caspase-9 is normally induced by caspase-8 through the mitochondria. We omitted the detailed reactions in this pathway, and simply assumed that caspase-9 activity depended on caspase-8 activity by using a Michaelis-Menten type function. Here, a delay τ was introduced for the lag time needed to complete the intermediate reactions through mitochondria. In the present model, we took $\tau = 30\text{min}$ for type II cells, in accordance with Scaffidi et al. [14].

Molecule synthesis and degradation rates were assumed to be constants. Here, the degradation rates of the active form caspases were assumed to be two to three orders larger than those of the inactive forms.

2.3 Caspase-3 activity exhibited pulse increasing after introduction of apoptosis stimuli

To test the above model, we solved the equations numerically and compared the results with experimental data. In the simulation, most parameter values were taken from Okazaki et al. [12], with minor adjustment for some parameters due to the model modification. In the present study, we tuned the parameters to fit the experimental data for human Jurkat cells treated with 100ng/mol FasL (Fig. 3). In the simulation, the initial conditions were assumed to be at steady state prior the onset of FasL stimuli ($[\text{FasL}] = 0$). Thus, we have $[\text{Casp8}] = 33.33\text{nM}$, $[\text{Casp3}] = 200.00\text{nM}$, and $[\text{FasC}] = [\text{Casp8}_2^*] = [\text{Casp3}] = 0$ as initial conditions [8].

The simulation time courses of the concentration of procaspases and active caspases are shown in Figure 3. Figure 3(A)-(B) show the time-dependent reductions of procaspase-8 and -3. Figure 3(C) and (D) are time courses of active caspase-8 and -3; both showed pulse increasing. The activities increased rapidly at the beginning, reaching the maximum level after approximately 2 h, and then decreased to low levels. These results agreed qualitatively with previously reported experimental results [3, 13, 14]. The simulation reveals that the maximum level of active caspase-8 occurs at low saturation (about 2.8% of the maximum level) because the DISC formation is highly reduced in type II cells [14]. The caspase-3 activity reached the maximum value of about 40% saturation, approximately 30 min later than the time point when caspase-8 reached its maximal level. The caspase-3 activity was reduced to very low levels at the end of the simulation because of caspase-3 degradation and procaspase-3 reduction.

3 Conclusion

In this paper, we have proposed a simple mathematical model of Fas signaling-induced apoptosis. The model was developed based on the Fas-signaling pathway proposed by Hua et al. [8] and Okazaki et al. [12]. In the present model, we excluded most of the detailed reactions, and focused on the activation of caspase-8 and -3 in response to FasL stimuli. Rapid degradation of active caspases were highlighted in our model. The rapid degradation of caspase-3 has been reported in Tawa et al. [16], but was not considered in Hua et al. [8]. As active caspases are subject to rapid turn over, the caspase activations showed pulse increasing time course behavior following apoptosis stimuli exposure. This is in contrast to bistable steady states properties that have been suggested by others [1, 4, 10]. In addition, our results agreed qualitatively with experimental data [3, 13, 14].

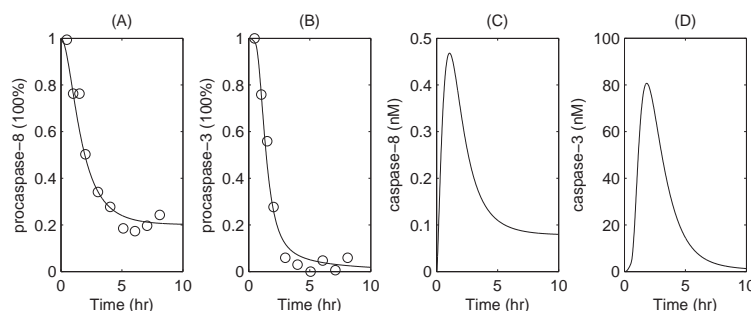


Figure 3: Model simulation results obtained for procaspase-8 (A), procaspase-3 (B), caspase-8 (C) and caspase-3(D). In (A) and (B), the experimental data (circles) were retrieved from Hua et al. [8, Fig. 3], which includes time courses for procaspase-8 and -3 in Jurkat cells in the presence of 100ng/ml FasL at room temperature. Parameters used are: $[FasL]_0 = 2.00nM$, $[Fas]_0 = 10.00nM$, $k_{on} = 9.09 \times 10^{-5}nM^{-1}s^{-1}$, $k_{off} = 1.00 \times 10^{-4}s^{-1}$, $v_1 = 2.65 \times 10^{-3}s^{-1}$, $K_1 = K_2 = 50nM$, $v_2 = 6.00 \times 10^{-5}nM^{-1}s^{-1}$, $v_3 = 2.60 \times 10^{-4}s^{-1}$, $[Casp9]_0 = 20.00nM$, $K_3 = 8nM$, $\tau = 30min$, $v_{Casp8} = 6.67 \times 10^{-4}nMs^{-1}$, $v_{Casp3} = 4.00 \times 10^{-5}nMs^{-1}$, $d_{Casp8} = 2.00 \times 10^{-5}s^{-1}$, $d_{Casp8_2^*} = 3.40 \times 10^{-3}s^{-1}$, $d_{Casp3} = 2.00 \times 10^{-7}s^{-1}$, $d_{Casp3^*} = 2.40 \times 10^{-4}s^{-1}$.

Biologically, a pulse increase of caspase-3 activity can be sufficient to trigger the irreversible downstream death program. These dynamic properties suggest the importance of timing for proper cell apoptosis, which has been previously reported in experiments with p53-induced apoptosis [6, 7].

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