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Robustness analysis identifies the plausible model of the Bcl-2 apoptotic switch

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Abstract **In this paper two competing models of the B-cell lymphoma 2 (Bcl-2) apoptotic switch were contrasted by mathematical modeling and robustness analysis. Since switch-like behaviors are required for models that attempt to explain the all-or-none decisions of apoptosis, ultrasensitivity was employed as a criterion for comparison. Our results successfully exhibit that the direct activation model operates more reliably to achieve a robust switch in cellular conditions. Moreover, by investigating the robustness of other important features of the Bcl-2 apoptotic switch (including low Bax basal activation, inhibitory role of anti-apoptotic proteins and insensitivity to small perturbations) the direct activation model was further supported. In all, we identified the direct activation model as a more plausible explanation for the Bcl-2 apoptotic switch.**

Keywords: Apoptosis; Bcl-2 apoptotic switch; Model; Robustness; Plausibility; Ultrasensitivity

1. Introduction

Apoptosis is an essential process for multi-cellular organisms to eliminate unwanted cells in order to achieve tissue remodeling and homeostasis. Robust decision matrixes are required for cells to conduct all-or-none, irreversible demise in response to diverse developmental cues or cellular stresses. Dysregulation of apoptosis contributes to cancer, developmental defects, autoimmune diseases and certain neurodegenerative disorders [1,2].

Mitochondria play a central role in apoptosis decisions by sensing upstream apoptotic signals and responding to them via mitochondrial outer membrane permeabilization (MOMP) [2]. MOMP, a rapid all-or-none process, is usually thought to be a 'point of no return' in the mitochondrial apoptosis pathway [3]. It releases multiple apoptogenic factors such as cytochrome *c* from the mitochondrial intermembrane space (IMS) to the cytosol and nucleus, where cellular demolitions are initiated. MOMP is mainly governed by intricate interactions between three subclasses of the B-cell lymphoma 2 (Bcl-2) protein family [4–10]. Two subclasses promote apoptosis: the subfamily of BH3-only proteins (including Bid, Bim, Puma, Bad, Noxa) sense and integrate cellular damage signals. Somehow BH3-only proteins activate the subfamily of multidomain proteins Bax and Bak, which form mitochondrial apoptosis channel (MAC) and permeabilize the mitochondrial outer membrane. This process is opposed by the anti-apoptotic subclass (Bcl-2, Bcl-xL, Bcl-w, A1 and Mcl-1) which share sequence conservation through BH1–4. It has been widely embraced that Bcl-2 protein family constitutes a crucial checkpoint in apoptosis [4,5].

Considering the complexity and irreversibility of MOMP, unrestrained activation of Bax and Bak is supposed to be the ultimate commitment to cell death [11]. However, exactly how Bax/Bak activation is regulated is still unclear. Two most elusive questions are (1) whether multidomain proteins Bax and Bak are activated directly by BH3-only proteins or not and (2) in which way do anti-apoptotic proteins exert their roles to inhibit Bak/Bak activation. Multiple models explaining these questions can be distilled into two distinct ideas, namely the direct activation model and the indirect activation model [8,9]. The direct activation model holds that select BH3-only proteins termed as 'activators', including Bim, Bid and, perhaps, Puma, can directly activate Bax and Bak. Anti-apoptotic proteins inhibit this process by sequestering activators. The remaining BH3-only proteins, termed 'sensitizers' or 'enablers', act only by displacing the activators from the anti-apoptotic proteins, sensitizing the activation of Bax and Bak. In the indirect activation model, on the other hand, anti-apoptotic proteins inhibit apoptosis by sequestering Bax and Bak. All BH3-only proteins activate Bax or Bak indirectly by binding and inactivating their specific anti-apoptotic relatives that constrain them. The relative killing potency of different BH3-only proteins is explained based on their relative affinities for anti-apoptotic partners. Fig. 1 schematically illustrated the structures of the two models. Recently, compelling new evidence was reported in support of both models [6,7]. However, proving which model is more telling by means of experimental approaches seems difficult thus far [8,9].

Robustness analysis provides an alternative strategy to exploit the plausibility between different models of the biological system. It has been widely embraced that robustness is a necessary property of biological organisms [12]. The robustness of certain features of regulatory networks such as adaptations, oscillations and so on, has been extensively studied [13–20]. Since comparable robustness is required for models that attempt to explain the system, robustness can be employed as a measure of the quality of a model. This notion has been

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Abbreviations: Bcl-2, B-cell lymphoma 2; MOMP, mitochondrial outer membrane permeabilization; IMS, intermembrane space; BH domain, Bcl-2 homology domain; MAC, mitochondrial apoptosis channel; ODEs, ordinary differential equations

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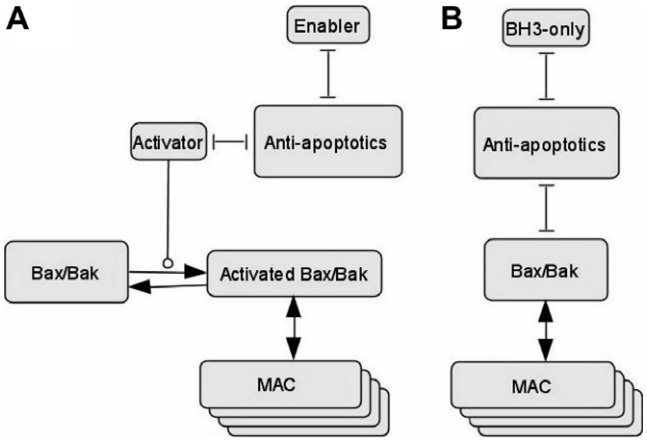

Fig. 1. Outline of the two models of the Bcl-2 apoptotic switch. (A) The direct activation model. (B) The indirect activation model. Rectangles represent molecular species. Arrows represent material flow. Black line ended with a cycle denotes catalyzed reaction and those terminated by a bar denote interaction and inhibition.

appreciated for a long time. Savageau suggested that parameter sensitivities can be used to evaluate the performance of biochemical systems [21]. Morohashi et al. analyzed two models of the biochemical oscillator, and first proposed that parametric robustness can be regarded as a criterion for determining plausibility between different models [22]. Eissing et al. compared two different models of apoptosis signaling by evaluating their robustness of bistability [23]. They also compared bistable performances given by three different mechanisms in signal transduction [24,25]. Besides, a variety of methods for quantifying parametric robustness have been developed recent years [26,27].

Here, we examined the parametric robustness of the two models of the Bcl-2 apoptotic switch by numerical simulations. We mainly focused on whether these two models have ultrasensitive responses to apoptotic stimulus. The sensitivities of the responses were quantified by estimating the relative amplification coefficient, which has been proved more representative than Hill coefficient [28]. By analyzing the ultrasensitivities of the two models, we suggested that the direct activation model operates more reliably to achieve switch-like behavior under cellular conditions with variations of the internal 'parameters'. In addition to ultrasensitivity, the robustness of three other salient features of the Bcl-2 apoptotic switch, including low Bax basal activation, inhibitory role of anti-apoptotic proteins and insensitivity to small perturbations, were investigated. The results further supported the direct activation model. Thus, the robustness analysis performed in this study provides a useful strategy to exploit the plausibility between alternative models for cellular behaviors and identified the direct activation model as a more plausible explanation for the Bcl-2 apoptotic switch.

2. Materials and methods

We constructed the two models of the Bcl-2 apoptotic switch preserving the core information of biological events. For example, all kinds of anti-apoptotic proteins are designated as 'Bcl2' despite different affinities they have and, 'Bax' is used to represent the multidomain

Table 1
Reactions and parameters of the two models

(A) Reactions[a]

Models	Reactions	k+	k−
Direct activation model	Ena + Bcl2 ⇌ EnaBcl2	k-BH3-Bcl2	kr-BH3Bcl2
	Act + Bcl2 ⇌ ActBcl2	k-BH3-Bcl2	kr-BH3Bcl2
	InBax ⇌ Bax	k-InBax[b]	k-Bax
	n Bax ⇌ MAC	k-o	kr-o
Indirect activation model	BH3 + Bcl2 ⇌ BH3Bcl2	k-BH3-Bcl2	kr BH3Bcl2
	Bax + Bcl2 ⇌ BaxBcl2	k-Bax-Bcl2	kr-BaxBcl2
	n Bax ⇌ MAC	k-o	kr-o

(B) Parameters[c]

Abbr.	Descriptions	Value[d]	Ref. and comment
$[Ena]_0$	Initial concentration of Ena	2	[29,30]
$[Act]_0$	Initial concentration of Act	1	[29,30]
$[BH3]_0$	Initial concentration of BH3	3	The sum of $[Ena]_0$ and $[Act]_0$
$[InBax]_0$	Initial concentration of InBax	60	[29–31]
$[Bcl2]_0$	Initial concentration of Bcl2	30	Estimated from $[InBax]_0$ and the ratio of Bax/Bcl2 [32–34]
k-BH3-Bcl2	Dimerization of BH3and Bcl2	0.0001	[35–37]
kr-BH3Bcl2	Dissociation of BH3-Bcl2dimer	0.001	[35]
k-InBax	Act-mediated Bax activation	0.001	The same as used in [38]
k-Bax	Bax inactivation	0.001	Estimated from k-InBax
k-Bax-Bcl2	Dimerization of Bax and Bcl2	0.0001	The same as k-BH-Bcl2
kr-BaxBcl2	Dissociation of Bax-Bcl2 dimer	0.001	The same as kr-BH3 Bcl2
k-o	Oligomerization of Bax	0.001	Estimated
kr-o	Dissociation of Bax oligomer	0.001	Estimated
n	Cooperative coefficient	4	[39]

[a]Abbreviations used: Ena (Enabler), Act (Activator), BH3 (All BH-3 only proteins), InBax (Inactive Bax/Bak), Bax (Activated Bax/Bak), MAC (Bax/Bak pore), Bcl2 (Anti-apoptotics), EnaBcl2 (Enabler–Bcl-2 dimer), ActBcl2 (Activator–Bcl-2 dimer), BaxBcl2 (Bax–Bcl-2 dimer).
[b]The forward reaction (Bax activation) is catalyzed by Act.
[c]Range: To reflect the degree of uncertainty we defined statistical distribution for each parameter. All the rate constants are assigned four magnitudes of variations (0.01–100) around the reference value. The initial concentrations of different species are assigned one magnitude of variations. The cooperative coefficient n is assigned a half magnitude of variations.
[d]Units: The total amounts of different species are expressed in units of nanomolar. The first and second rate constants are expressed in units of $second^{-1}$ and $nanomolar^{-1}$ $second^{-1}$.

proteins since Bak can be regard as a kind of MOM integrated Bax. We presented mathematical modeling of the two models with ordinary differential equations (ODEs). The law of mass action for biochemical reactions was used. The reactions and parameters of the two models of Bcl-2 apoptotic switch are described in Table 1. Details of the kinetic equations as well as descriptions for the robustness assessment are presented in Supplement. Differential equations of our models were solved mathematically using the ODE23s routine of Matlab 6.5 (The Mathworks Inc., Natick, MA). The evaluation of parametric robustness was also implemented in Matlab 6.5.

3. Results

3.1. Switch-like behaviors of the two models

We first examined if the two models with reference parameter sets have ultrasensitive responses to apoptotic stimulus. We suppose that the concentration of BH3 increases linearly with the apoptotic stimuli, while details such as expression, degradation and post-translational regulation are not considered for simplicity:

$$[\mathrm{BH3}] = [\mathrm{BH3}]_0 + F \times [\mathrm{BH3}]_0 \quad (1)$$

Here, $[\mathrm{BH3}]_0$ stands for the initial concentration of BH3; F stands for the increased folds of $[\mathrm{BH3}]_0$ with respect to the input stimuli ($F \in [0,20]$).The upper limit of F is set to 20 in our paper, which means that the concentration of BH3 can have a maximum 20-fold increase according to the apoptotic stimuli. For simplicity we regard F as the input stimuli in our analysis. Similarly, in the direct model we have

$$[\mathrm{Act}] = [\mathrm{Act}]_0 + F \times [\mathrm{Act}]_0 \quad (2)$$

$$[\mathrm{Ena}] = [\mathrm{Ena}]_0 + F \times [\mathrm{Ena}]_0 \quad (3)$$

Here, $[\mathrm{Act}]_0$ and $[\mathrm{Ena}]_0$ are the initial concentrations of Activator and Enabler, respectively. Both Activator and Enabler simultaneously changed to reflect the apoptotic stimuli in our modeling. For each model with a given stimulus, the steady-state activity of Bax is assessed as response. It is widely embraced that activated Bax is the major component of the cytochrome *c* release channel MAC and, in the apoptotic cells, the amount of oligomeric Bax increases significantly [40]. So in our simulations the percentage of oligomeric Bax to the total amount of Bax is calculated to indicate the activity of Bax. Fig. 2A shows the stimulus–response curves for the two models.

The ultrasensitivities of the two curves were quantified by two methods. We first estimated the Hill coefficients of the two response curves (Fig. 2B) using the definition given by Goldbeter and Koshland [41]. However, high Hill coefficients ($h > 1$) here do not sufficiently imply ultrasensitive responses since both models have significant basal activations (Fig. 2A) which could make the sensitivity of the responses overestimated. Neither can we tell which response is more ultrasensitive by comparing their Hill coefficients. To circumvent this problem, the relative amplification approach proposed by Legewie et al. was used [28]. Fig. 2C shows the relative amplification plots of the two models. The relative amplification coefficients are estimated for both two models. The values for the relative amplification coefficient of different models have direct biochemical meanings and are comparable to each other. Our results indicate that none of the two models is ultrasensitive, or to say 'more sensitive than the Michaelis–Menten equation'. Besides, the direct activation model (with n_R = 0.074) seems to be more sensitive than the indirect activation model (with n_R = 0.056).

However, there is still a long way to go before we can tell which model is more plausible. The reference parameter sets used here only give a rough estimation of the physiological conditions and led to a biased calculation. To solve this problem we have to examine the influence of parameter variations on the ultrasensitivities of the two models.

3.2. Parametric robustness of ultrasensitivity

Ultrasensitivity is a key property of the Bcl-2 apoptotic switch. Thus, comparing the parametric robustness of the ultrasensitivity can be used as a meaningful discrimination criterion between the two models.

3.2.1. Single parameter changes. In order to study the influence of individual parameter changes on the sigmoidality of the stimulus–response curve, we plot the relative amplification coefficient as a function of the single parameter variation (PV) for each model. Here, the parameter variation is defined as PV = $p_i/p_{i,\mathrm{ref}}$, where p_i is the value of the ith parameter of the model and $p_{i,\mathrm{ref}}$ is its corresponding value of the reference set. Fig. 3 shows the results for both the models of the Bcl-2 apoptotic switch.

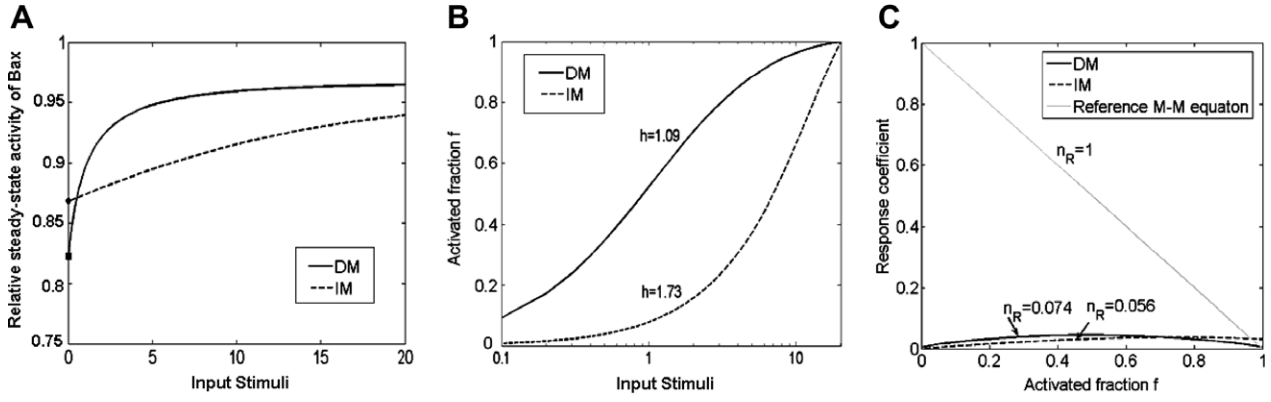

Fig. 2. The stimulus–response curves and the relative amplification plots for the two models. (A) The relative steady-state activity of Bax is plotted as a function of the input stimuli. (B) For each model the activated fraction f is calculated and plotted as a function of the input stimuli. (C) Relative amplification plot of the two models. Note that the Michaelis–Menten equation is used as the reference response. (IM: the indirect activation model. DM: the direct activation model).

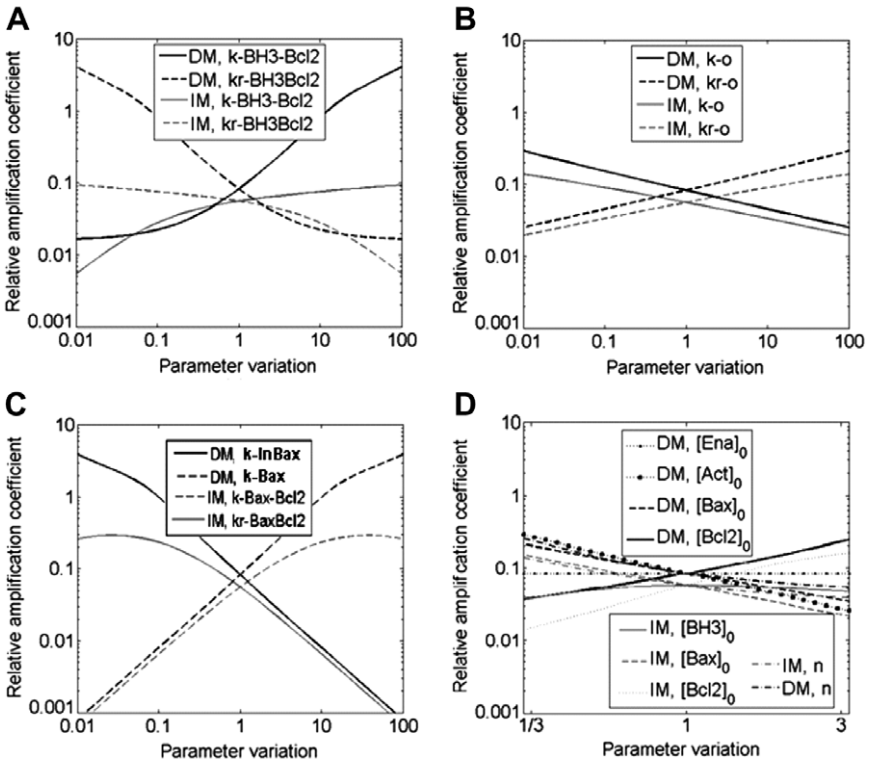

Fig. 3. Relative amplification coefficient of the stimulus–response curve in each model as a function of the parameter variation. For comparison purpose the pairs of parameters employed by both models are plotted in the same subplot, with (A) k-BH3-Bcl2, k-BH3Bcl2 and, (B) k-o, kr-o. The pairs of parameters specific to two models ([k-Bax, k-InBax] for the direct activation model and, [k-Bax-Bcl2, k-BaxBcl2] for the indirect activation model) are plotted in (C). (D) Refers to the changes in the initial concentrations of the Bcl-2 family proteins. Note that $[Act]_0$ and $[Ena]_0$ in the direct activation model are integrated into $[BH3]_0$ in the indirect activation model. The effect of the cooperative coefficient n is also shown in (D).

For both models, variations of most parameters (k-o, kr-o, $[Ena_0]$, $[Act_0]$, $[Bax_0]$, $[Bcl\text{-}2_0]$, n for the direct activation model, and k-o, kr-o, $[BH3_0]$, $[Bcl\text{-}2_0]$, n for the indirect activation model) have small influence on ultrasensitivity. This kind of single parameter insensitivity is usually regarded as robustness for steady-state systems. However, to prove that a system has robust 'dynamical' function, such as switch-like behavior, adaption or oscillation, the size of parameter window in which the model exhibits these behaviors should also be considered. Here, the size of parameter window in which the model exhibits ultrasensitivity (with $n_R > 1$), should be taken as a criterion for robustness quantification. In our results, with variation of certain parameters (k-BH3-Bcl2, kr-BH3Bcl2, k-Bax and k-InBax), the direct activation model can employ a parameter window of ultrasensitivity (Fig. 3A and C). However, the indirect activation model can never show ultrasensitivity even if individual parameter is varied in full physiological ranges. This difference suggests that the direct activation model outperforms the indirect activation model with respect to robustness in single parameter analyses.

3.2.2. Global parameter variations. Cell-to-cell variations and environmental differences may lead to multiparametric uncertainty, which means a group of parameters differ from their 'nominal' values at a time. It is possible that the effects of different parameter variations can compensate or, enforce each other. We here investigated how systematic parameter changes influence the ultrasensitivities of the two models.

To measure robustness we employed the frequency of occurrence of ultrasensitive response of the model with 3000 random parameter sets for each model. Similar to [13,19,20], we plot for each parameter set the calculated relative amplification coefficient as a function of the total parameter variation TPV in the scatter plot (Fig. 4). TPV is defined as the total order of magnitude of parameter variation [13,19,20]:

$$\mathrm{TPV} = \sum_{i=1}^{n_{\mathrm{para}}} \left| \log_{10} \frac{p_i}{p_{i,\mathrm{ref}}} \right|. \tag{4}$$

Here, n_{para} is the number of the parameters in each model and p_i and $p_{i,\mathrm{ref}}$ are parameters in the test set and reference para-

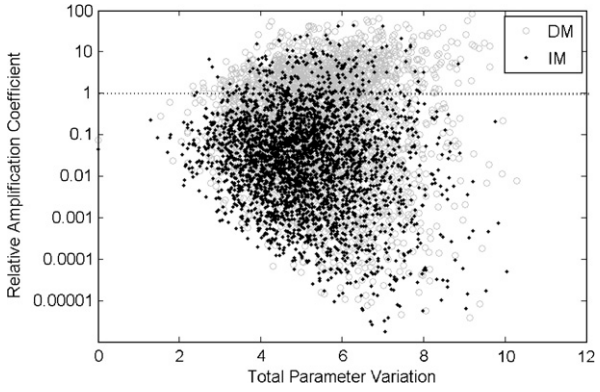

Fig. 4. Robustness of ultrasensitivity with respect to global parameter variations. Note that the grey dotted line in the figure. Parameter dots above this line ensure ultrasensitivity of the model. Shown is one representative result of the three independent simulations.

meter set, respectively. Thus, TPV can be envisioned as a measure for distances in kinetic parameter sets. It can be seen that the direct activation model has a much larger volume of the parameter space ensuring ultrasensitivity. We found 996 parameter sets that exhibited ultrasensitive responses (with $n_R > 1$) for the direct activation model (Fig. 4, grey open circles, upper the dotted line). However, for the indirect activation model, the number of parameter sets exhibiting ultrasensitive response (with $n_R > 1$) is only 217 (Fig. 4, black filled dots, upper the dotted line). Therefore, as has a larger volume of parameter space allowing the switch-like behavior, the direct activation model confers a better robustness of ultrasensitivity with respect to global parameter variations.

3.3. Robustness of other important features

Comparing the ultrasensitivity of Bax activation cannot be considered complete to discriminate between the two models since there are other important features of the Bcl-2 apoptotic switch, for example, the inhibitory role of anti-apoptotic proteins. It has been widely embraced that anti-apoptotics can inhibit apoptosis by suppressing Bax activation and oligomerization. Anti-apoptotics (Bcl-2, Bcl-xL, etc.) overexpressing cells were reported to be apoptosis-resistant, and with suppressed Bax oligomerization [42]. Besides, in non-apoptotic cells Bax should predominately exist as inactivated monomers [11]. Furthermore, cells can preserve low activity of Bax even with small perturbations of the apoptotic stimuli [43]. To circumvent such problems we extended the robustness analysis by posing additional requirements to the models.

Simulations under four different initial conditions were performed with 3000 random parameter sets for each model. Calculations were performed to address different features of the Bcl-2 apoptotic switch (Table 2). First, low basal activation of Bax was checked by the Range Test. We supposed that in non-apoptotic cells the basal activation level of Bax should below a threshold while with apoptotic stimuli the activity of Bax should exceed it. The number of parameter sets ensuring this property was calculated and its relative frequency was used as a measure of robustness (Fig. 5A and B, Range Test). Similarly, the robustness of other two properties can be estimated (Fig. 5A and B). In Inhibition Test we supposed that with apoptotic stimuli the activity of Bax should exceed the threshold while it should be reduced back below the threshold when anti-apoptotic proteins were added. Insensitivity Test means that the model should preserve low activity of Bax in non-apoptotic condition and, even with small perturbations of the apoptotic stimuli. For all these tests, semi-quantitative simulations were performed since a unified quantitative model is not yet practical.

Table 2
Three tests for evaluation of other features of the Bcl-2 apoptotic switch[a]

Tests	Normal	Stimuli	Stimuli + protection	Small perturbation
Range Test	$<T$	$>T$	–	–
Inhibition Test	–	$>T$	$<T$	–
Insensitivity Test	$<T$	–	–	$<T$
All	$<T$	$>T$	$<T$	$<T$

[a]We roughly use an increased 15-folds of BH-3 only proteins to mimic the stimuli, and an increased 15-folds of anti-apoptotics to mimic the protection experiments. 33.3% of the stimuli were used to test the model's insensitivity to small perturbations. A threshold of the relative activity of Bax (T) is defined here. We used $T = 0.5$ in our simulations, which means a half of Bax homo-oligomerized.

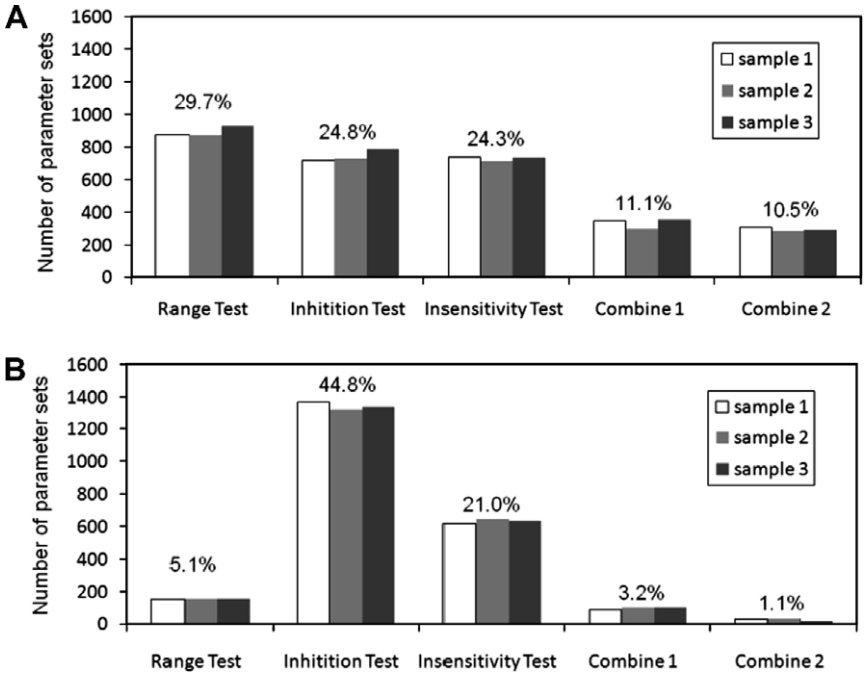

Fig. 5. The number of total parameter sets that ensure desired features of the Bcl-2 apoptotic switch for each model. (A) The direct activation model. (B) The indirect activation model. For each model the simulations are repeated using three independent samples, each consisting of 3000 latin sets of parameters. The mean of three samples in each test is given above the bars. Range Test: low Bax basal activation. Inhibition Test: inhibitory role of anti-apoptotic proteins. Insensitivity Test: insensitivity to small perturbations. Combine 1: proportion of parameters which can ensure all three features simultaneously. Combine 2: combination of these three tests with the ultrasensitivity analysis.

Fig. 5 shows the result of these tests. It can be seen that the direct activation model is more likely to achieve low basal activation and employ insensitivity to small perturbations. The indirect activation model is more likely to achieve the inhibitory role of anti-apoptotic proteins. If all three features are considered simultaneously, the direct activation model employs a larger parameter space than the indirect activation model (Fig. 5A and B, combine 1). Then we combined these tests with the results of the ultrasensitivity analysis. For the indirect activation model, we found only 33 out of the 3000 random parameter sets can ensure both these features and ultrasensitivity. However, 316 out of the 3000 parameter sets can meet all the requirements (Fig. 5A and B, combine 2) in the direct activation model. Thus, we concluded that the direct activation model confers a better robustness in contrast to the indirect activation model.

4. Discussion

Biological signaling networks are complex and difficult to untangle. Usually, wet lab experiments alone are not of much help. Contradictory results are reported by different groups based on different experimental systems. So it is common to see that multiple competing models for a regulation are postulated in literature. As a particular example discussed in this paper, two distinct models of the regulation of the Bcl-2 network, which constitutes a robust switch that regulates apoptosis, were supported by different groups and contradictory evidences [6–9]. Proving which model is more plausible based on intuitions is not possible because of the complexity of the system. An alternative strategy to exploit the plausibility between different models of the biological system is mathematical modeling. It is widely embraced that faithful models should not rely on fine tuned parameters to reflect biological systems. Thus, determining the robustness of mathematical models can help distinguish between more and less plausible models, as has been discussed previously [21–25].

Ultrasensitivity was employed as a first criterion for comparison. It was quantified by estimating the relative amplification coefficient, which has been proved more representative than Hill coefficient when basal activation is high. Here, the interactions of Bcl-2 family proteins are not typical enzymatic reactions and both models can have high basal activation. In the direct model, Bax can be activated directly by the initial part of Activator and thus make up the basal activation. In the indirect model, Bax can oligomerize spontaneously in non-apoptotic condition. Thus, in this paper the relative amplification approach proposed by Legewie et al. provides a better choice for us to quantify the ultrasensitivity.

Our results reveal that the direct model is more likely to be ultrasensitive than the indirect model with parameter variations. It is mainly due to the different regulating mechanisms of the two models. In the direct model, Bax are activated directly by Activator before they can oligomerize into pores. To have ultrasensitive responses, the ability of Activator to activate Bax should be low (Fig. 3C), or the binding affinity between Activator and Bcl2 should be high (Fig. 3A). While in the indirect model, Bax can oligomerize spontaneously. The reason why Bax do not form pores significantly in non-apoptotic condition is that Bcl2 can sequester most Bax. That requires a high binding affinity between Bcl2 and Bax. What is more, in order to disrupt the binding between Bax and Bcl2, the binding affinity between BH3 and Bcl2 should also be considerably high. We can find that the requirements for the direct model are more restricted than the direct model. Thus, its natural that the direct model is more likely to be ultrasensitive than the indirect model with respect to parameter variations.

We also performed robustness analysis of other important features of the Bcl-2 apoptotic switch in addition to ultrasensitivity. Single property analysis usually gives limited or even misleading results. For instance, in our simulations ultrasensitivity does not ensure low basal activation of Bax, which is required according to experimental observations [11]. So, several key features of the Bcl-2 regulation network were accounted in a unifying way, including ultrasensitivity, low basal activation, anti-apoptotic inhibition and insensitivity to fluctuations. Ultrasensitivity was quantitatively evaluated by estimating the relative amplification coefficient. Other properties were assessed by evaluating the Boolean output for robustness. Obviously, such a combined analysis of different properties can give more reasonable results.

Another important question is how to measure the robustness objectively. In tradition, robustness is evaluated by repeated simulations varying one or two parameters while leaving all other fixed. This requires an exact parameter point in the multidimensional parameter space. However, this particular 'point' is impossible – or at best – difficult to determine. Instead, statistically distributions of the parameters are more reasonable to reflect the inherent uncertainty of the biological systems. Moreover, since simultaneous parameter variations may lead to non-linear effects, it is more important to assess the robustness with respect to global parameter variations. To address this question we proposed a Monte Carlo based approach – sampling parameters from the distributions. The volume of expected features – such as ultrasensitivity – in the parameter space was estimated. Then the ratio of the volume gives comparable results between different models. Similar idea has been applied in [16–18,23,25].

It should be noted that the robustness examined in this paper is only parametric robustness. Other perturbations such as noise and time delays were not taken into consideration. Another limitation of this study is that the results of the Monte Carlo approach are still affected by the parameter ranges we defined. In our simulations, four magnitudes of variation for most parameters are allowed thus this effect can be attenuated. However, it should be expected that more numerically estimated quantities can be made used and therefore less biased results can be achieved. Finally, there are other hypotheses of the Bcl-2 regulatory network, including the binding of activated Bax to anti-apoptotic proteins [8] and Bax/Bak auto-activation [44]. Indeed, we have revealed in our former study that when considering the binding of activated Bax to anti-apoptotic proteins, the direct activation model can show bistability, forming a bio-switch [45]. This is quite interesting. However, since these hypotheses have not yet been well received, we mainly focused on the two widely accepted models in this paper.

Notwithstanding these limitations, the parametric robustness analysis performed in this paper successfully selected a more plausible model from the two competing models of the Bcl-2 apoptotic switch.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.09.063.

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