Comment to the Editor

Response to Bistability in Apoptosis: Roles of Bax, Bcl-2, and Mitochondrial Permeability Transition Pores

Recently, a mathematical model of the mitochondrial apoptotic pathway was proposed. In that study, the robustness of different simplified signaling models with respect to parameter changes was also investigated. It was found that bistability achieved via cooperative ultrasensitivity is ''much more robust'' than other mechanisms such as inhibitor ultrasensitivity. We reinvestigate this interesting finding to reveal that it does not hold in such generality. Our results indicate that mechanisms other than cooperative ultrasensitivity, such as inhibitor ultrasensitivity, can confer a similar robust bistable performance. Thereby, these findings are not restricted to apoptosis signaling, but relevant to bistable signaling in general. In addition, example calculations indicate the potential practical relevance of inhibitor ultrasensitivity for generating robustness in apoptosis signaling.

INTRODUCTION

The recent publication by Bagci et al. (1) contains interesting results regarding the mitochondrial apoptotic pathway. While focusing on this pathway, the authors also investigate simplified general reaction mechanisms that generate bistable behavior. They state that a cooperative reaction mechanism is superior to other mechanisms, such as inhibitor binding, based on their finding that it is ''much more robust'' (1). The authors present this result in a general context, as robustness of bistable behavior is not only relevant to apoptosis, but to (bistable) signal transduction in general.

Robustness is an emerging concept in biology (2). Many biological processes have to function reproducibly irrespectively of perturbations like fluctuations in environmental and internal conditions. This is especially important when considering processes deciding on the cell fate, as is the case in apoptosis signaling. Apoptosis is a form of programmed cell death, and the outcome of the apoptotic reaction network is the decision on the survival or death of cells (3). Apoptosis can be envisioned and modeled as a bistable system (1,4,5). The normal nonapoptotic cellular state can be interpreted as the ''life steady state,'' in which no significant amount of caspases (which are at the heart of the apoptotic program) are activated, and there is the ''death steady state,'' in which the almost complete activation of caspases leads to apoptosis. Generally, bistability requires positive feedback and some ultrasensitive reaction mechanism as necessary but not sufficient ingredients (6–8). The three best-studied examples for generating ultrasensitivity are a cooperative reaction mechanism, inhibitors, or saturation effects (also called zero-order ultrasensitivity) (6).

We recently described simple models resembling core processes of apoptosis signaling and compared the above three mechanisms for generating ultrasensitivity and, together with positive feedback, bistability (9). The models are in fact similar to those described by Bagci et al. (1). Here we compare the robustness of these models using two different measures, one proposed by Ma and Iglesias (10) and one by our group (11).

Robustness measure according to Ma and Iglesias

Ma and Iglesias (10) consider single parameter variations for which they proposed a robustness measure that measures the minimal distance from a reference point in the parameter space to a bifurcation point.

Table 1 provides the robustness measures according to Ma and Iglesias (10) for the three models investigated. It can be seen that, for the parameters k_1, k_2 (mutual protease activation rate constants), and k_d (degradation rate constant), shared by the three models, the measures are all rather similar, not revealing any mechanism to be especially robust. Interestingly, the measure is especially small for the cooperative setup when considering the parameters unique to each model. For example, the cooperative model with reference parameter values is not bistable for either $n = 2$ or $n = 3$ (with n indicating the degree of cooperativity), but only in-between. Obviously, this measure is strongly dependent on the reference parameter set, which is not always easy to obtain. For example, the robustness measure for the parameter k_1 of the inhibitor model is far off the maximal value of 0.686, which can be achieved by varying k_1. Thus, the reference parameter choices were not obviously biased and this measure already provides a first indication that none of the three mechanisms appears to be especially robust compared to another.

Robustness measure using a Monte Carlo approach

We proposed a Monte Carlo-based approach to evaluate the robustness of bistable systems (11). Basically, in this approach, random parameter sets are drawn from predefined ranges and the relative frequency of occurrence of bistability provides an estimate of the volume in the parameter space allowing a bistable behavior and is used as a robustness measure.

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TABLE 1 Robustness with respect to parameter variations according to measure introduced by Ma and Iglesias (10)

Parameter	Cooperative	Inhibitor	Zero-order
k_1	0.214	0.262	0.302
k_2	0.282	0.187	0.171
k_d	0.131	0.119	0.302
n	0.155	—	—
k_f	—	0.879	—
K_b	—	0.967	—
K_M	—	—	0.889

The measure R is defined as $R(k) = 1 - \max\left\{\frac{k_{b_1}}{k}, \frac{k}{k_{b_2}}\right\}$, where k_{b_1} and k_{b_2} correspond to the closest bifurcation points to each side of a reference parameter k, i.e., $0 < k_{b_1} \le k \le k_{b_2} < \infty$. R is maximal at the geometric mean $k = \sqrt{k_{b_1} \times k_{b_2}}$, i.e., $R \in [0, 1 - \sqrt{k_{b_1}/k_{b_2}}]$. The formula reduces to $R(k) = 1 - k_{b_1}/k$ if $k_{b_2} \to \infty$. A robustness measure of 0 indicates that the parameter value is directly at a bifurcation point.

As can also be proven analytically, Table 2 shows that for $n = 1$ the system is not able to display a bistable behavior because ultrasensitivity is a necessary structural requirement. The cooperative models for $n > 1$ all allow a bistable performance in a rather large volume of the parameter space. Interestingly, for larger values of n, corresponding to increased cooperativity, the models do not become more robust. This can be interpreted using the observation that, as the Y_r nullcline becomes steeper with increasing n, it is also shifted to the right, counteracting an increase of the robustness (compare (9)). Unlike the cooperative model for $n > 1$, the inhibitor and zero-order model strongly depend on the values of their unique constants, i.e., only strong inhibitors or enzymes close to saturation allow a robust bistable performance similar to the cooperative model. Also, the cooperative model achieves slightly larger maximum values due to the additional transcritical bifurcation present in the two other models limiting their bistable parameter range (data not shown).

A simple calculation for apoptosis

The potential practical relevance of the theoretical finding can be shown by simple calculations for the apoptosis example. IAPs are known to be rather strong inhibitors of caspases with a reported $K_i = k_b \times Y_t/k_f = 7 \times 10^{-4}$ μM for XIAP binding activated caspase 3 (12,13). A total amount of protein $X_t = Y_t = 3 \times I_t = 1$ μM is within the range of reported values (14–16). Then, a k_b/k_f ratio of 10^{-3} or even smaller is a reasonable estimation for the considered reaction in the normalized setup analyzed above. Thus, the reference binding values and the strong inhibitors needed to achieve comparable robustness measures in the Monte Carlo test are not only theoretical hypotheses, but can already be found in apoptosis signaling.

TABLE 2 Robustness with respect to parameter variations evaluated by Monte Carlo approach (11)

	Cooperative	Inhibitor		Zero-order	
$n = 1$	0.00	$k_f = 0.1$, $k_b = 10^{-2}$	0.00	$K_M = 10^{-1}$	0.02
$n = 2$	0.41	$k_f = 1$, $k_b = 10^{-3}$	0.09	$K_M = 10^{-2}$	0.07
$n = 3$	0.37	$k_f = 1$, $k_b = 10^{-4}$	0.13	$K_M = 10^{-3}$	0.16
$n = 4$	0.35	$k_f = 10$, $k_b = 10^{-3}$	0.18	$K_M = 10^{-4}$	0.21
$n = 5$	0.34	$k_f = 10^2$, $k_b = 10^{-5}$	0.32	$K_M = 10^{-5}$	0.29
$n = 10$	0.29	$k_f = 10^6$, $k_b = 0$	0.37	$K_M = 10^{-10}$	0.36

We generate random parameter sets for k_1, k_2, and k_d (the parameters common to all three models when assuming k_m to correspond to k_d), so that each parameter is uniformly distributed in logarithmic space in the interval from 10^{-5} to 1. We evaluate 10,000 parameter sets for each model. This provides accurate values whose asymmetric binomial 95% confidence intervals are ~2% around the values provided.

DISCUSSION

We evaluate the robustness of bistable behavior to parameter variations for different reaction systems where ultrasensitivity, a necessary ingredient for bistability, is generated via cooperativity, or saturation (zero-order), or inhibitors. Using two previously described measures for robustness in bistable systems (10,11), we find that theoretically all three model structures allow a robust bistable performance. In all cases, the bistable property and its robustness is dependent on the right combination of parameters.

Comparing different models and different methods, we find that all methods tend to give biased results. The method introduced by Ma and Iglesias (10) is strongly dependent on a reference parameter set. However, experimental data rarely allow an exact choice of parameters. The exact results of the Monte Carlo approach are also affected by the parameter ranges assumed. Allowing for large parameter ranges or varying these ranges can attenuate this effect (11). The investigated cases reveal another problem common to both methods employed. All models show a saddle-node bifurcation limiting the bistable parameter range to one side. But only the parameter range of the inhibitor and zero-order model is also limited to the other side by a transcritical bifurcation. For the cooperative model, the unstable steady state asymptotically approaches the life steady state. Therefore, many of the parameter sets evaluated as mathematically bistable in the Monte Carlo approach can hardly be considered ''biologically bistable'', since the threshold is smaller than one molecule of activated caspases within a cell. While this explains why the maximal robustness measure of the cooperative model is slightly larger than those of the two other models, it also indicates the need for improved methods. For example, one could extend the Monte Carlo approach to pose additional requirements on the location of the steady states. Other approaches, not directly evaluating the property of bistability, are measuring the degree of ultrasensitivity (17), using overall coefficients developed in the framework of metabolic control analysis (18) or other global measures of robustness (2,19). Thus, the described analysis cannot be considered complete and cannot finally answer the questions of which reaction mechanism confers a better robustness. Also, both the models described here and those investigated by Bagci et al. (1) neglect residual activities of pro-caspases (zymogenicity) (20). Especially the bistability in the cooperative model is very susceptible when considering this kind of perturbation (9).

Summarizing, our results provide clear indications that none of the mechanisms evaluated here appears to be clearly superior regarding the robustness of bistable behavior with respect to parameter changes. Both measures described provide comparable results. We find that only the combination of different methods and a critical evaluation of the results enables conclusive insights. Simple calculations highlight the potential importance of (caspase) inhibitors in generating bistable behavior (during apoptosis). For apoptosis, the importance of inhibitors predicted through mathematical modeling was recently also confirmed experimentally on the single cell level (21). Additionally, these inhibitors can generate an implicit positive feedback, further enhancing the bistable behavior (5). Nevertheless, especially in the mitochondrial pathway of apoptosis, there are several potential cooperative steps in addition to inhibitors (21). In vivo, most likely a combination of different mechanisms will secure a tight switch (22).

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