Monte Carlo Simulation of Cell Death Signaling Predicts Large Cell-to-Cell Stochastic Fluctuations through the Type 2 Pathway of Apoptosis

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ABSTRACT Apoptosis, or genetically programmed cell death, is a crucial cellular process that maintains the balance between life and death in cells. The precise molecular mechanism of apoptosis signaling and the manner in which type 1 and type 2 pathways of the apoptosis signaling network are differentially activated under distinct apoptotic stimuli is poorly understood. Based on Monte Carlo stochastic simulations, we show that the type 1 pathway becomes activated under strong apoptotic stimuli, whereas the type 2 mitochondrial pathway dominates apoptotic signaling in response to a weak death signal. Our results also show signaling in the type 2 pathway is stochastic; the population average over many cells does not capture the cell-to-cell fluctuations in the time course (~1–10 h) of downstream caspase-3 activation. On the contrary, the probability distribution of caspase-3 activation for the mitochondrial pathway shows a distinct bimodal behavior that can be used to characterize the stochastic signaling in type 2 apoptosis and other similar complex signaling processes. Interestingly, such stochastic fluctuations in apoptosis signaling occur even in the presence of large numbers of signaling molecules.

INTRODUCTION

Programmed cell death, apoptosis, is one of the most important cellular processes that is critical to a wide variety of phenomena ranging from the normal development of multicellular organisms to maintaining homeostasis in an immune response (1,2). Two major pathways (type 1 and type 2) that mediate apoptotic cell signaling and their associated signaling molecules have been identified (1,2). However, differential activation of downstream signaling molecules in type 1 and type 2 pathways under differential cellular conditions were previously not well understood (2–4). In a series of experiments, Goldkorn and colleagues have shown that the timescale of cell death signaling can be slow (~10 h) for apoptosis under the action of an oxidative stress (see the study by Goldkorn et al. (4) and its reference list). The signaling mechanism that causes such slow apoptotic cell death, however, was not obvious from biologic experiments. In this article, we present a kinetic Monte Carlo model of cell death signaling to demonstrate how two apoptotic pathways are differentially activated under distinct apoptotic stimuli. Our results show that cells can use stochastic signaling through the mitochondrial type 2 pathway under weak apoptotic stimuli resulting in a slow activation of apoptosis.

Based on our simulations, we conclude that cell-to-cell variation (stochasticity) in apoptotic signaling can be significant under weak apoptotic stimuli. This stochasticity, however, cannot be captured by population average (average over many cells) behavior, as observed in previous attempts to mathematically model apoptosis signaling (5–8). Such stochastic effects were thought to be important only when a low copy number of a specific molecular species (~1–100) in a cell is involved (9,10). In contrast, our results show that cell-to-cell fluctuations in the type 2 pathway of apoptosis signaling occur even in the presence of a large number of molecules (~10,000–100,000 in mammalian cells). We attribute such stochastic effects to the structure of the type 2 apoptosis signaling network and to the low probability that an apoptosome signaling complex will form.

METHODS

Our stochastic simulation belongs to the kinetic Monte Carlo class of models, because we sampled individual molecules randomly and chose for either diffusion or reaction move. Instead of using a free energy-based Metropolis scheme (11,12), we used probabilistic rate constants for reaction and diffusion of signaling molecules (13). We simulated a single cell with a cubic lattice in which all the molecules were placed at different nodes of the lattice. Membrane-bound molecules like the death-inducing signaling complex were confined to six surfaces of the cubic box and allowed to diffuse only in two dimensions. Intracellular signaling molecules could diffuse inside the cubic box if they were in the free state, but they lost mobility when they formed multimolecular complexes. At each Monte Carlo move, a randomly chosen molecule was sampled to undergo either a diffusion or a reaction move, which are defined as follows. 1), Diffusion move: A molecule was allowed to diffuse to one of the neighboring nodes, four for membrane-bound molecules and six for cytosolic molecules, only if the neighboring site was not already occupied by any other molecule. 2), Reaction move: A molecule could undergo the following types of reactions: it could bind with a suitable molecule with which it can form a complex, it could dissociate into constituent free molecules, and it could undergo proteolysis activation reaction. We
typical simulation was run for $T$. We chose a time step of $10^{-4}$ s, and a typical simulation was run for $T = 10^{-3} - 10^{0}$ steps, that is, 1000–10,000 s. The probabilistic parameters used in our model were mapped to the macroscopic experimentally measurable constants in such a manner that a detailed balance condition was satisfied at each point in space. One novel aspect of our stochastic approach is that the timescale of one Monte Carlo step was set in such a manner that the diffusion constant of any single (low-density) molecule would match the experimentally measured diffusion constant of that molecule. However, the emerging timescale of cell death signaling in our simulations, which arise from the complex interplay of a large number of signaling species, cannot be predicted from the timescale of diffusion of a single molecular species. Each run of our simulation corresponded to apoptosis signaling observed at the single cell level.

Our stochastic computational model considers apoptotic signaling through two distinct pathways: 1), direct activation of caspase-3 by caspase-8 (type 1); and 2), activation of caspase-3 by mitochondrial cytochrome c through two distinct pathways: 1), direct activation of caspase-3 by caspase-8 and 2), activation of caspase-3 by mitochondrial cytochrome c release and apoptosome formation (type 2) (Fig. 1). Intracellular apoptosis signaling in our model was triggered by the activation of caspase-8 molecules at the cell surface that, in turn, diffused in the cytosol and activated both pathways of apoptosis signaling (Fig. 1). In the type 1 pathway, caspase-8 molecules directly catalyzed the cleavage reaction of procaspase-3 to generate caspase-3. In the type 2 pathway, caspase-8 bound with BID and catalyzed its truncation to form tBID that, in turn, bound to BAX to generate BAX2 complex molecules. BCL2, an antiapoptotic molecule, can inhibit both tBID and BAX, thus creating a local loop structure in the type 2 signaling cascade. The BAX2 complex leads to cytochrome c release from mitochondria and ultimately to the activation of caspase-3 (Fig. 1). Hence, caspase-3 activation at the end of both the type 1 and the type 2 pathways created a global loop structure in apoptosis signaling. Details of the signaling reactions and simulation schemes are provided in the Supplementary Material (Data S1).

To investigate cell death under varying strengths of apoptotic stimuli, we changed the concentrations of procaspase-8 molecules and explored its effect on the downstream signaling for the two different pathways. The results did not change if we activated the downstream signaling directly by caspase-8 and studied the effects of varying concentrations of caspase-8. Caspase-3 cleavage is irreversible and leads to apoptosis. Hence, caspase-3 activation was considered the readout for apoptosis signaling that decided the final cell fate. We followed the dynamics of signaling reactions by measuring the number of signaling molecules at regular time intervals. Each run of our simulation corresponded to apoptosis signaling observed at the single cell level.

RESULTS

By setting the appropriate kinetic constants to zero, we were also able to activate only one of the two pathways.

Type 1 pathway only

For the type 1 pathway, caspase-3 activation was fast (seconds to minutes) for all the cells in our simulations (Fig. 2, A). The population average over many cells (the average over many runs in our simulations) in this type 1 activation can capture the essential dynamics of direct caspase-8/caspase-3 activation (Fig. 2, A). We used a probability distribution-based approach (histogram plots of many single-cell observations) to characterize cell-to-cell stochastic fluctuations in apoptotic signaling. For the type 1 signaling, probability distribution of activated caspase-3 for various time points showed a gradual increase (Fig. 3, A).

Type 2 pathway only

In stark contrast to the type 1 pathway, the caspase-3 activation in individual cell simulations for the type 2 pathway varied by an order of magnitude from minutes to hours (Fig. 2, B). Such a scheme to obtain the timescale of complex biologic processes from a stochastic simulation is notably absent in the literature (14). Our simulations clearly show how very different timescales (approximately minutes to hours) can emerge from a stochastic simulation that could be matched against single-cell biologic experiments. Clearly, few cells with very slow activation of caspase-3 can shift the average time for peak caspase-3 activation to ~10 h. Such slow caspase-3 activation and subsequent apoptosis have been observed in experiments where apoptotic signaling was triggered by stress conditions such as an oxidative agent (4), and must be dominated by the type 2 pathway of apoptosis.

Another striking feature of caspase-3 activation that we observed for the type 2 pathway was that the completion of caspase-3 activation, once initiated, was relatively quick (approximately seconds to minutes) compared with the timescale over which cell-to-cell variations occur (approximately minutes to hours). The average over many runs of our simulations did not capture the cell-to-cell fluctuations observed in individual stochastic simulations (Fig. 2, B). The variance/average ratio (also called the Fano factor) for the number of activated caspase-3 molecules remained large (>1) for a long time (approximately hours). Interestingly, such large cell-to-cell stochastic fluctuations in type 2 apoptosis signaling occur even in the presence of a large number of molecules (9, 10). Stochastic fluctuation in the time course of caspase-3 activation initiation, coupled with the rapid activation of caspase-3 once it is initiated, led to a characteristic bimodal behavior for the probability distribution of caspase-3 activation (Fig. 3, B). One characteristic feature of this bimodal probability distribution is that it is not sym-
metric under time reversal \((t \rightarrow -t)\), which is a signature of a truly nonequilibrium process.

To investigate the cause of fluctuations in caspase-3 activation for the type 2 pathway, we considered the activation of downstream signaling molecules in that pathway. Our simulations showed small fluctuations in Bax activation, and subsequent cytochrome \(c\) release from the mitochondria led to large (an order-of-magnitude increase) fluctuations in apoptosome formation. Such cell-to-cell stochastic fluctuation in the time course of apoptosome formation mainly drives the fluctuations in caspase-9 and therefore caspase-3 activation, because rapid activation of those molecules was observed immediately after the formation of apoptosomes. Interestingly, only 1–5 molecules of apoptosome were sufficient to activate a large number of downstream caspase molecules, caspase-9 and caspase-3.

**Combined type 1 and type 2 pathways**

When both type 1 and type 2 pathways were combined, the presence of a large number of procaspase-8 (\(\sim 100\) nM or more) made the type 1 pathway dominant by rapid activation of caspase-3. As the concentration of procaspase-8 was lowered, however, the type 2 pathway dominated, because few caspase-8 molecules preferentially bind to Bid compared with caspase-3 molecules. Under weak stimulus, therefore, large cell-to-cell stochastic fluctuations through the type 2 pathway dominated the signaling behavior (results similar to those shown in Figs. 2, B, and 3, B). The addition of feedback loops, such as the proposed positive feedback loop between activated caspase-3 and pro-caspase-8, did not change our results, because a clear separation of timescales exists between the type 1 and type 2 pathways. Thus, the number of caspase-8 molecules or, equivalently, the strength of the apoptotic stimulus can be used as a control mechanism to selectively activate the type 1 or the type 2 pathway.

To check the robustness of our results, we carried out simulations for various sets of parameter values. For example, the activity of pro- and antiapoptotic B cell lymphoma-2 (Bcl-2) family proteins, other than the Bcl-2 protein itself, in the type 2 pathway was effectively considered by varying the concentration of Bcl-2 molecules. We also varied the rate constants of some specific reactions, such as that which governs caspase-3 activation, to mimic the effect of blocking such activation by inhibitory signaling molecules. However, such alterations in the signaling cascade did not change the stochastic signaling behavior through the type 2 pathway. Most importantly, a simple model of cell signaling has been developed in which a three-step (fast-slow-fast) pathway can capture the qualitative features of stochastic apoptotic sig-
naling (S. Raychaudhuri, unpublished), as observed in our full Monte Carlo simulation of cell death signaling. Therefore, the stochastic signaling behavior in the apoptosis signaling network is robust against changes in the details of the reaction kinetics. Recent experimental results showed rapid cytochrome c release from mitochondria in a cell type-independent manner (16). This same study (16) also found no evidence of caspase-dependent positive amplification of cytochrome c release, which is consistent with the findings of our computational studies.

DISCUSSION

Major signaling molecules of the apoptotic pathway and the network structure of the signaling reactions have been identified through biologic experiments (1–5). Mathematical modeling of apoptosis signaling was used to consider a set of kinetic rate equations of signaling reactions, and nonlinear effects of specific signaling molecules were analyzed (5,6). All-or-none type bistable behavior in apoptosis cell signaling experiments (17) was explained by fluctuations in environmental conditions (7) and by the low probability of apoptosisosome formation (8). In contrast to these earlier studies, our stochastic simulations of apoptosis clearly show that apoptotic cell signaling can vary even under identical cellular and environmental conditions and that a probability distribution-based approach can characterize such stochastic effects in cellular signaling. Specifically, our results show that, for a weak apoptotic stimulus, cells use the mitochondrial type 2 pathway and that cell-to-cell fluctuations in the caspase-3 activation can vary 1–10 h even under identical cellular and environmental conditions. Such large fluctuations in the time course of caspase-3 activation can explain the slow average apoptosis observed in cell death under oxidative stress (4). In a fluctuating environment, stochasticity in the time course of caspase-3 activation may be an adaptive mechanism for allowing a competing survival signal to win over a weak death stimulus.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

REFERENCES


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