GATES TO APOPTOSIS

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ABSTRACT

p53 is the key transcription factor controlling cellular responses to oncogenic stimulation and DNA damage. Its activity is tightly controlled by numerous feedback loops. In response to DNA damage, p53 promotes expression of proteins, which suppress cell cycle and activate DNA repair. If the damage is irreparable or the repair takes too long, the programmed cell death (apoptosis) is initiated.

In the current study we analyze the apoptotic module, a part of our larger p53 pathway model. In the model, the apoptosis is triggered due to the suppression of Akt activity and/or elevated level of p53 *killer*. p53 killer, *i.e.* p53 form phosphorylated at Ser-46 (in addition to Ser-15 and Ser-20), promotes synthesis of pro-apoptotic protein Bax. In healthy cells Bax is inactive due to binding to Bcl-2, another member of Bcl-2 family proteins. Suppression of Akt activity leads to the dissociation of Bax:Bcl-2 complexes and release of Bax. Therefore, two signals may lead to the accumulation of free Bax: one coming from elevated level of p53 killer, the other resulting from decreased level of active Akt. We demonstrated that, depending on parameters, the apoptosis can be controlled by the logic gate 'AND' as well as gate 'OR'. In the first case both signals are required simultaneously, while in the latter case any of the two signals suffices for the initiation of apoptosis.

INTRODUCTION

The tumor suppressor p53 plays a crucial role in the cellular response to genotoxic stress. After detection of the DNA damage, activated p53 serves as a potent transcription factor inducing the expression of numerous genes comprising a complex regulatory network. Multiple intertwined feedback loops, spanning diverse time scales, can suppress cell cycle and allow for the DNA repair in the interim. If the DNA damage is not repaired within a predefined timespan, specific feedback loops direct the cell toward apoptosis.

This late stage is driven by the Bcl-2 family proteins, including Bax, Bad and Bcl-2 proper, which are capable of creating heterodimers. Bcl-2 sequestrates free Bax, but it can be depleted through binding to Bad, which in turn in healthy cells is sequestered by other protein, 14-3-3. Released Bax promotes permeabilization of the outer mitochondrial membrane, leading to the efflux of cytochrome c and subsequent activation of caspase cascade responsible for irreversible cleavage of cellular proteins and cell death. Effective control of free Bax is therefore important for making appropriate decision about cell death or survival.

Building upon our previous results [1], we created a novel model of the p53 regulatory pathway. In this study we focus on its apoptotic module. The apoptosis is induced by either p53 killer

promoting the synthesis of Bax or in a p53-independent way, in which low activity of kinase Akt results in the disruption of Bcl-2:Bax complexes and release of free Bax. The latter mode can be enabled irrespectively of oncogenic stimulation by the withdrawal of growth factors. We analyze the dynamics of the apoptotic module with respect to input levels of p53 killer and active Akt. High level of the output signal – free Bax – indicates the initiation of apoptosis.

THE p53 MODEL

Ionizing radiation leads to DNA damage by creating double strand breaks, which are detected by ATM undergoing auto-phosphorylation. Active ATM activates p53 by phosphorylation at Ser-15 and Ser-20 [2]. Activated p53 serves as a transcription factor for genes coding for two groups of proteins having pro- or anti-apoptotic roles. In its basic active form named p53 *arrester*, it induces synthesis of its inhibitors Mdm2 and Wip1 [3] as well as proteins responsible for cell cycle arrest and DNA repair. The p53 killer form (phosphorylated additionally at Ser-46) activates expression of proteins mediating apoptosis (PTEN and Bax) [4].
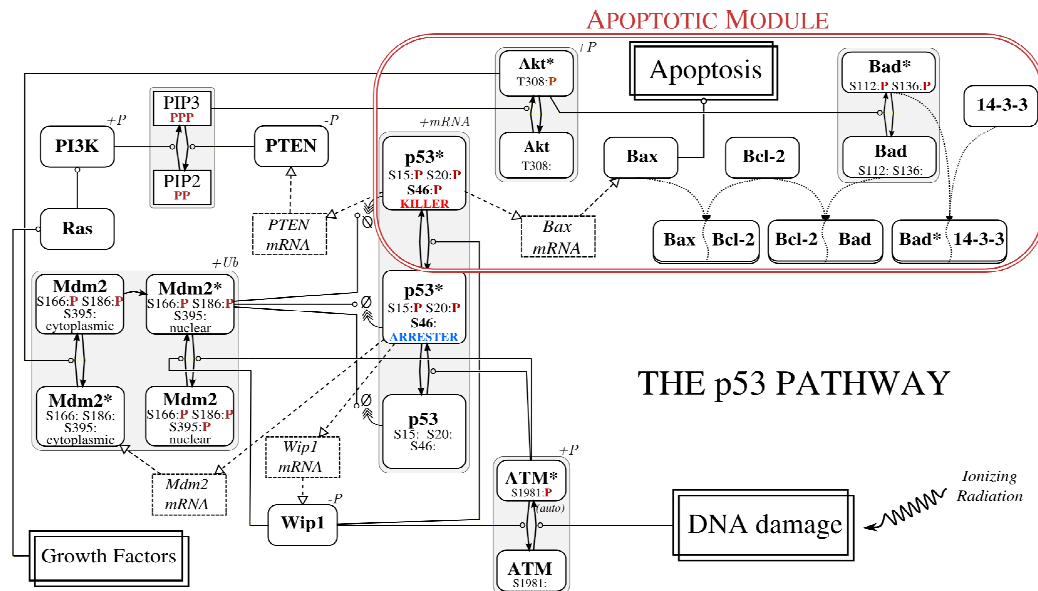

Figure 1. **Schematic representation of the model.** Triangle-headed dashed lines indicate transcription and translation, arrow-headed solid lines – protein transformation, circle-headed solid lines – activatory protein interactions.

The model (see Fig. 1) comprises four negative and two positive feedback loops.

Negative feedback loops:
- [p53 —o Mdm2 —⊣ p53] p53 arrester induces synthesis of Mdm2 which mediates p53 arrester degradation [5],
- [p53 —o Wip1 —⊣ ATM —o p53] p53 arrester induces synthesis of phosphatase Wip1, which switches off auto-activated kinase ATM, rendering it incapable of activating p53 to the arrester form,
- [p53 —o Wip1 —⊣ ATM —⊣ Mdm2 —⊣ p53] p53 arrester induces synthesis of Wip1, which switches off ATM so it cannot deactivate nuclear Mdm2, which mediates degradation of p53 arrester,
- [p53 —o Wip1 —o Mdm2 —⊣ p53] p53 arrester induces synthesis of Wip1, which activates nuclear Mdm2 directly, inducing degradation of p53 arrester.

Positive feedback loops:

- [p53 —o Wip1 —o p53] p53 arrester induces synthesis of Wip1, which increases level of p53 arrester by dephosphorylating p53 killer,
- [p53 —o PTEN —⊣ PIP3 —o Akt —o Mdm2 —⊣ p53] p53 killer induces synthesis of PTEN, which induces activity of p53 killer by protecting it from Mdm2-mediated degradation; this long loop involves PIP3 and Akt [6].

In the model, induced DNA damage triggers the repair process. The model reproduces oscillations of p53 level, which were observed experimentally and here are attributed mainly to the p53-Mdm2 loop and delayed degradation [5]. Restraints implemented by negative feedbacks and slow accumulation of PTEN grant irradiated cells time for the repair. In the case of small damage, the system returns to the initial state with low level of p53 arrester and low level of p53 killer (which corresponds to survival); however, if the damage is large, and is not repaired within a predefined time, p53 killer stabilizes on a high level and associated positive feedback becomes dominant; accumulating Bax forces the cell to commit apoptosis.

The analysis of the model shows that critical dose of IR initializing apoptosis is dependent on the expression level of anti-apoptotic phosphatase Wip1 and pro-apoptotic phosphatase PTEN. The experimental data shows that levels of Wip1 and PTEN expression strongly depend on the cell line. Interestingly, high level of Wip1 and low level of PTEN (as in breast cancer cell line MCF-7 [7]) give oscillations of p53 arrester and killer levels regardless of the extent of the IR dose. With the expression levels set accordingly, the model reflects the behavior of the cell line MCF-7. In normal cells with efficient mechanism of DNA damage repair and apoptosis, the critical IR dose is 2 Gy, *i.e.* higher dose leads to apoptosis, while for IR < 2 Gy the DNA is repaired and the cell returns to normal cell cycle.

RESULTS

The mathematical representation of the apoptotic module consists of 7 molecular species: mRNA of *Bax*, proteins: Bax, Bcl-2 (proper), Bad, 14-3-3, p53 killer and Akt, and 3 complexes Bax:Bcl-2, Bcl-2:Bad and Bad:14-3-3.

In healthy cells, level of p53 killer is low and level of active Akt is high. p53 killer serves as a transcription factor for Bax. Akt promotes cell survival by inactivation of pro-apototic Bax. This is accomplished by the following interactions: Normally, Bad is phosphorylated by Akt and bound with protein 14-3-3, while Bax remains bound to Bcl-2. Deactivation of Akt results in the dephosphorylation of Bad, which in dephosphorylated form binds preferentially to Bcl-2 (instead of to 14-3-3) displacing Bax from Bax:Bcl-2 complex, and thus leading to the release of apoptosis-inducing free Bax. The dynamic behavior of the module can be expressed with the following system of ordinary differential equations, where levels of phosphorylated Bad and Akt are denoted Bad* and Akt*:

$$\frac{dBax_{mRNA}(t)}{dt} = \underbrace{h_3}_{\substack{basal \\ transcription}} + \underbrace{p_2 \frac{p53_{killer}^2}{h_2^2 + p53_{killer}^2}}_{p53-induced\ transcription} - \underbrace{d_{13}Bax_{mRNA}}_{transcript\ degradation}$$

$$\frac{dBad(t)}{dt} = \underbrace{d_{19}Bad*}_{\substack{implicit \\ dephosphorylation}} + \underbrace{u_1\{Bad:Bcl2\}}_{dissocation} - \underbrace{p_7 Akt*Bad}_{phosphorylation} - \underbrace{b_1 Bad\ Bcl2}_{association}$$

$$\frac{dBad*(t)}{dt} = \underbrace{p_7 Akt*Bad}_{phosphorylation} + \underbrace{u_2\{Bad*:14-3-3\}}_{dissociation} + \underbrace{p_7 Akt*\{Bad:Bcl2\}}_{\substack{Akt*-induced\ dissociation \\ via\ Bad\ phosphorylation}} - \underbrace{d_{19}Bad*}_{\substack{implicit \\ dephosphorylation}} - \underbrace{b_2 Bad*\{14-3-3\}}_{association}$$

$$\frac{d\{Bad:Bcl2\}(t)}{dt} = \underbrace{b_1 Bad\ Bcl2}_{association} - \underbrace{u_1\{Bad:Bcl2\}}_{dissociation} - \underbrace{p_7 Akt*\{Bad:Bcl2\}}_{\substack{Akt*-induced\ dissociation \\ via\ Bad\ phosphorylation}}$$

$$\frac{dBax(t)}{dt} = \underbrace{t_5 Bax_{mRNA}}_{\text{translation}} + \underbrace{u_3\{Bcl2:Bax\}}_{\text{dissociation}} - \underbrace{b_3 Bcl2\,Bax}_{\text{association}} - \underbrace{d_3 Bax}_{\text{degradation}}$$

$$\frac{d\{Bcl2:Bax\}(t)}{dt} = \underbrace{b_3 Bcl2\,Bax}_{\text{association}} - \underbrace{u_3\{Bcl2:Bax\}}_{\text{dissociation}} - \underbrace{d_3\{Bcl2:Bax\}}_{\text{degradation of } Bax}$$

$$\frac{dBcl2(t)}{dt} = \underbrace{u_1\{Bad:Bcl2\}}_{\text{dissociation}} + \underbrace{u_3\{Bcl2:Bax\}}_{\text{dissociation}} + \underbrace{d_3\{Bcl2:Bax\}}_{\text{degradation of } Bax} + \underbrace{p_7 Akt^*\{Bad:Bcl2\}}_{\substack{\text{Akt*-induced dissociation} \\ \text{via } Bad \text{ phosphorylation}}} - \underbrace{b_1 Bad\,Bcl2}_{\text{association}} - \underbrace{b_3 Bcl2 Bax}_{\text{association}}$$

$$\frac{d\{14-3-3\}(t)}{dt} = \underbrace{u_2\{Bad^*:14-3-3\}}_{\text{dissociation}} - \underbrace{b_2 Bad^*\{14-3-3\}}_{\text{association}}$$

In a healthy cell we assume the level of p53 killer = 0, and that the whole pool of Akt ($2\cdot10^5$ molecules) is in the phosphorylated form.

The apoptotic module can combine levels of active Akt and p53 killer as input signals in a manner similar to logic gates OR and AND, depending on the total amount of Bad (1.5×10^5 molecules in the case of gate OR, 0.4×10^5 for gate AND) and maximal transcriptional activity of p53 (rate for gate OR is 3 times larger than rate for gate AND). To activate apoptosis, gate OR requires at least *one* sufficiently strong input signal: decreased level of active Akt or increased level of p53 killer. Single signal is insufficient for gate AND, which requires *both* signals simultaneously.

In the healthy cell steady state, for OR and AND gates, levels of complexes Bad*:14-3-3, Bax:Bcl-2, Bcl-2:Bad, are depicted in Fig. 2A and Fig. 3A, respectively. In both cases, Bad* is bound to 14-3-3, while Bad is bound to Bcl-2; all Bax is bound to Bcl-2. In order to observe processing of signals by the two logic gates, we assume that cells are stimulated with input signals with maximal amplitudes. This is we assume that all Akt is phosphorylated (no signal) or all Akt is unphosphorylated (positive signal). Similarly p53 killer = 0 corresponds to no signal, while p53 killer = ∞ (which corresponds to maximal transcriptional rate for Bax) corresponds to positive signal.

Lower than normal level of active Act induces dephosphorylation of Bad*, its dissociation from the complex with 14-3-3, and its dimerization with Bcl-2. In the case of the OR gate, dephosphorylation of all Akt is sufficient for the release of free Bax from the Bax:Bcl-2 complex (total amount of Bad is much larger than total amount of Bcl-2; Fig. 2B). In the case of the AND gate (Bad < Bcl-2 – Bax), and thus even the release of all Bad from complexes with 14-3-3 is insufficient for the release of free Bax (see Fig. 3B).

Higher than normal level of p53 killer leads to the increased level of Bax. In the case of gate OR, due to assumed high transcriptional p53 activity, level of Bax may overpass the level of Bad, which results in the appearance of free Bax (Fig. 2C). For gate AND the maximal transcriptional activity of p53 is lower, and level of Bax may not overpass level of Bcl-2, and thus all Bax remains complexed with Bcl-2.

For gate AND both signals are required for the release of free Bax (see Fig. 3D). This is accomplished due to the increase of Bax level, and simultaneous release of Bax from Bcl-2 due to its binding to Bad.
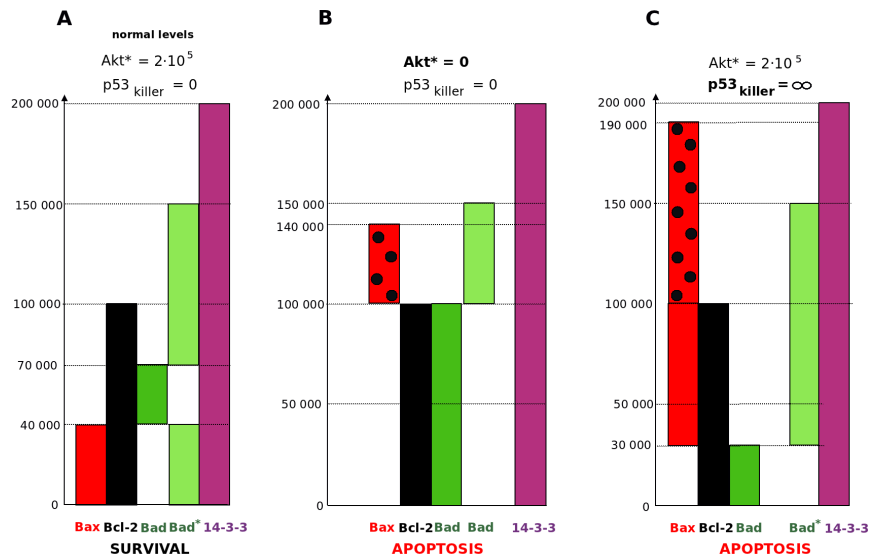
Figure 2. **Gate OR**. Panel A: initial levels of the complexes: Bad*:14-3-3, Bax:Bcl-2, Bcl-2:Bad. Panel B: single positive signal *absence of phosphorylated Akt* is sufficient to release Bax. Panel C: the other positive signal *max. transcriptional activity of p53 killer* is sufficient to release Bax. Columns sharing adjacent sides denote complexes. For example on Panel A, 40,000 molecules of Bcl-2 is complexed to Bax, 30,000 molecules of Bcl-2 is complexed to Bad, while 30,000 molecules of Bcl-2 remain free.
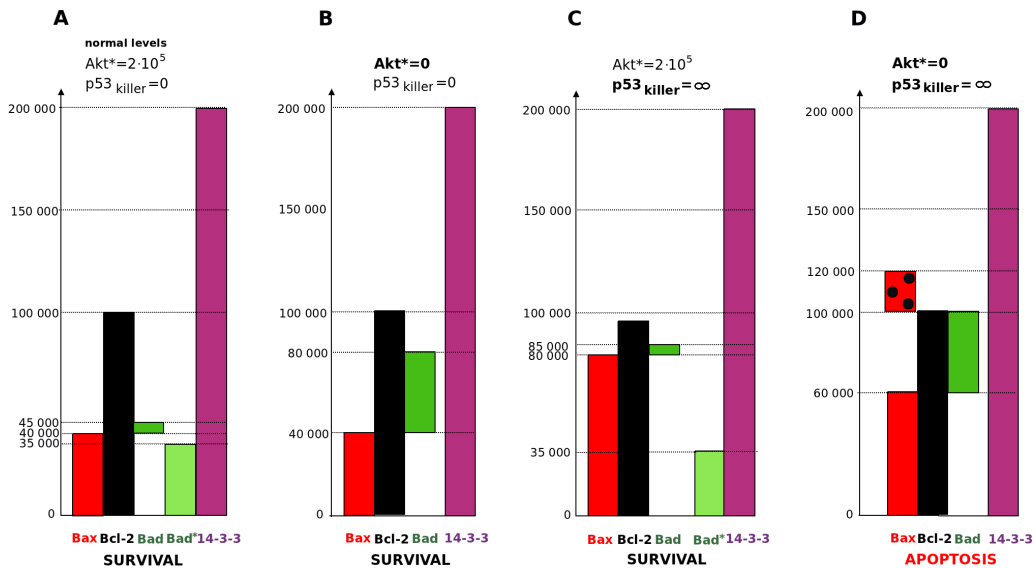

Figure 3. **Gate AND**. Panel A: initial levels of the complexes: Bad*:14-3-3, Bax:Bcl-2, Bcl-2:Bad. Panel B: single positive signal *absence of phosphorylated Akt* is insufficient to release Bax. Panel C: single signal *max. transcriptional activity of p53 killer* is insufficient to release Bax. Panel D: both signals result in the release of Bax. Columns sharing adjacent sides denote complexes.

CONCLUSIONS

Robust response to genotoxic stress enables cells to preserve their genome integrity and suppress oncogenic transformation. As an extension of our p53 pathway model, we introduced the apoptotic module, which is responsible for generating precise decisions on cell death or survival. Involved regulatory proteins span a complex network of interactions, which can serve as the common template of the decision unit determining cell fate. By analyzing the steady states of the module under various conditions defined by steady state expression level of regulatory protein Bad and maximal transcriptional rate for p53 regulated Bax, we found two modes of triggering the apoptosis. The module aggregates input signals in the form of level of p53 killer and active Akt, and can generate digital output in the manner analogous to either logic gate AND or gate OR.

Various cell lines are characterized by different expression level of regulatory proteins comprising the p53 pathway. This can be attributed to methylation of gene promoters, which render them less approachable to transcription factors. Additionally, diverse cell types can be sensitive to different external stimuli. We therefore hypothesize that our generic module, when specialized according to the particular cell lines, can dynamically reconfigure the network and utilize different logic gates. Consequently, the module operates effectively independently of the cellular specialization, and is capable of inducing apoptosis which can suppress tumor growth.

This findings could be useful for planning (targeted) cancer therapy. In case of some cell types, the apoptosis can result only from the simultaneous presence of both pro-apoptotic signals, which were considered in the model. Level of p53 killer should be elevated (e.g. through radiotherapy or chemotherapeutic inhibition of the activity of Wip1) as well as the pathway leading from growth factors should be inhibited (e.g. by cytotoxic inhibition of growth factor receptors).

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