A computational model of Bcl-2 regulated apoptosis: bistability revisited

Benjamin J Lansdell[1,2,3], Ruth Kluck[3], Colin Hockings[3], Erinna Lee[5], Douglas Fairlie[5], Federico Frascoli[2], Kerry A Landman[2], Terence Speed[4].

1 Department of Applied Mathematics, University of Washington, Seattle, USA; **2** Department of Mathematics and Statistics, University of Melbourne, Melbourne, Australia; **3** Molecular Genetics of Cancer division, **4** Bioinformatics division, **5** Structual Biology division, Walter and Eliza Hall Institute, Melbourne, Australia

Introduction

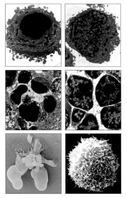
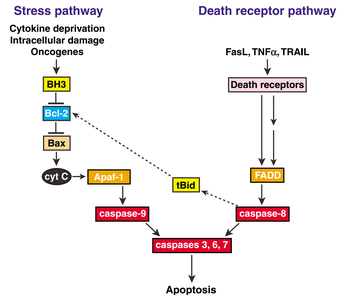


Figure: Apoptotic cells (left), healthy cells (right) [1]

- The Bcl-2 family or proteins are key regulators of the intrinsic apoptosis pathway
- Determining the mechanism that two of these proteins (Bak and Bax) use to control mitochondrial outer membrane permeabilisation (MOMP) and subsequent cytochrome c release is clinically important
- Bcl-2 family proteins have three roles: anti-apoptotic, pro-apoptotic effector and pro-apoptotis activator.

Aims

- Binding kinetics and large number of members confounds understanding: **develop mass-action kinetic model of a reduced mitochondrial assay. What interactions and mechanisms regulate cytochrome c release?**



Methods

- Extract mouse liver mitochondria containing endogenous membrane-integrated Bak, and minimal other Bcl-2 proteins
- Mitochondria co-incubated with pro-survival Mcl-1 and various levels of a Bim variant over 3 h; protein interactions quantified by co-immunoprecipitation, western blot and densitometry.

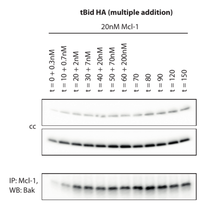


Figure: Cytochrome c release over 3hr incubation

- Where available, binding affinities determined through BIAcore measurements, other kinetic parameters obtained through a non-linear least squares fit between simulated and measured protein concentrations.

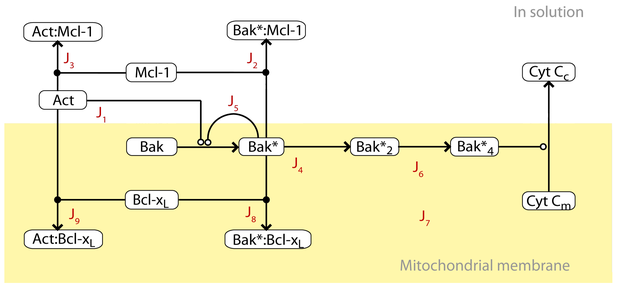


Figure: Interactions modelled

Direct and auto- activation of Bak

- **Is direct activation of Bak necessary?**
- Consistent with previous modelling [2] and experimental studies [3], a model which includes direct activation of Bak by Bim is shown to be more consistent with available kinetic binding data and MLM experiments, compared with a model which does not include direct activation.

Model	R^2
w. direct	0.86
w.out direct	0.82
direct+auto	0.87

Table: Goodness-of-fit of model to timing data

- In a model including Bak direct and auto-activation, more than 90% of the Bak is activated through interaction with Bim or Bid, suggesting a minimal role for auto-activation

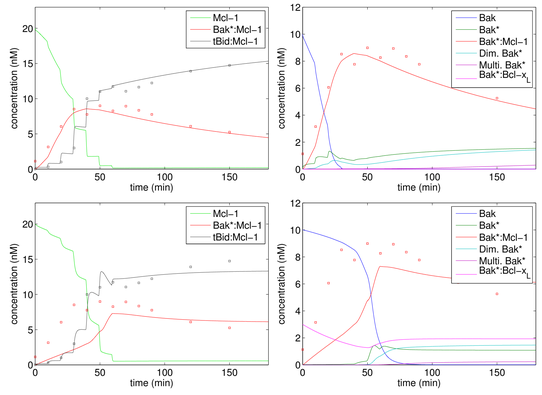


Figure: Simulated (curves) vs measured concentration (points) using fitted kinetic parameters. (Top) Simulation of model including direct activation, (bottom) simulation of model not including direct activation.

Bistable mechanisms

- **Previous studies propose bistability (apoptotic and non-apoptotic states both stable) is a key regulatory mechanism of apoptosis. Is it observed in the MLM assay?**
- Include protein production and degradation, gradually increasing the production rate of BH3-only stimulation
- Model does not produce an 'all-or-none', bistable response
- Predicts continuous transition between non-apoptotic and apoptotic states as a function of BH3-only stimulation.

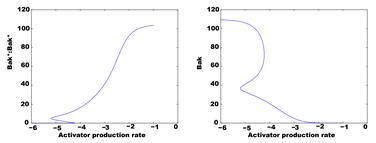


Figure: Example of bistable response. Steady state concentrations as a function of exponent of activator production rate. Parameters in which more than one steady state are present are bistable regions

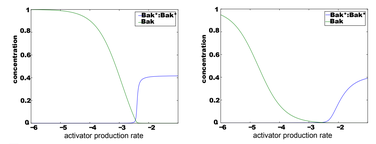


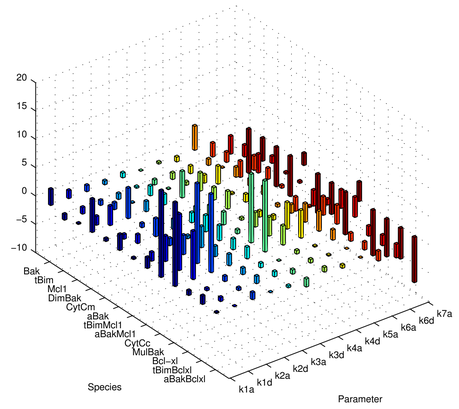
Figure: Response of model with experimentally constrained kinetics

- These results suggest that hystersis effects, if relevant in regulating intrinsic apoptosis, must contain more interactions than just those within the Bcl-2 family.

Robustness analysis

Parameter estimates

- **How sensitive are simulations to variation in binding rates?**
- Measure sensitivity according to [5]
- Perform for 2000 parameter sets chosen from log-uniform distribution $\Rightarrow$ gives measure of 'global' sensivity of parts of model
- Highlights interactions which most affect cytochrome c release



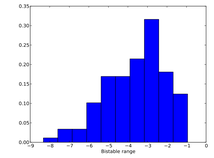


Figure: Exponent of bifurcation point (onset of bistability) from sampled parameters

Bistability

- **How robust is presence of bistability to parameter variations?**
- For 1000 randomly sampled parameters from log-uniform distribution, 20% contain bistability when BH3-only production rate varied
- For 1000 sampled parameters without direct activation, only 1% contain bistability
- Suggests bistability more robust in the presence of direct activation – as in [2]

Discussion

- This work shows how direct activation of Bak by BH3-only stimulus is necessary for the regulation of MOMP
- The understanding of dynamical mechanisms such as bistability within the Bcl-2 family is important to help the design of targeted anti-cancer drug therapies.
- Here we have shown such regulatory mechanisms, if they exist, must be found in other, more comprehensive, pathways related to intrinsic apoptosis.
- The model is novel in its being experimentally constrained by available binding rate data and knowledge of the Bcl-2 family.

References

1. D. Green, "Means to an End: Apoptosis and Other Cell Death Mechanisms." New York: Cold Spring Habor Labratory Press, 2011.
2. Chen, Chun; Cui, Jun; Lu, Haizhu; Wang, Rui; Zhang, Shuai; Shen, Pingping Modeling of the Role of a Bax-Activation Switch in the Mitochondrial Apoptosis Decision, 2007, Biophysical Journal, 92, 12, 4304-4315.
3. Czabotar, Peter E; Westphal, Dana; Dewson, Grant; Ma, Stephen; Hockings, Colin; Fairlie, W Douglas; Lee, Erinna F; Yao, Shenggen; Robin, Adeline Y; Smith, Brian J; Huang, David C S; Kluck, Ruth M; Adams, Jerry M; Colman, Peter M. Bax Crystal Structures Reveal How BH3 Domains Activate Bax and Nucleate Its Oligomerization to Induce Apoptosis. 2013. Cell, 152, 3, p519-531.
4. M. Bentele, I. Lavrik, M. Ulrich, S. Ster, D. Heermann, H. Kalthos, P. Krammer, and R. Eils, "Mathematical modeling reveals threshold mechanism in CD95-induced apoptosis," The Journal of Cell Biology, vol. 166, no. 6, pp. 839-851, 2004.

CONTACT: Benjamin Lansdell <lansdell@uw.edu>