

# A Computational Model of Apoptosis and Inflammation Signaling in Microglia

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## Introduction

Stress, such as that caused by stroke, initiates a number of responses at the cellular level in the brain which leave some cells dead, while others survive. Over-expressing the inducible heat shock protein 70 (Hsp70/Hsp72) has been shown to protect against cell death in models of cerebral ischemia [1]. However, the most important mechanisms of protection by Hsp72 in the setting of ischemia are still unclear, with multiple effects of Hsp72 on cell death pathways, inflammation and protein homeostasis demonstrated. In particular, how information from multiple pathways is integrated into the final decision on cell survival or death remains poorly understood. Microglia, immune cells of the CNS and first responders to a wide range of stresses within the brain, are known to play a role in the outcome from cerebral ischemia. Here we try to understand the signaling behavior which plays a key role in response to brain injury, focusing on microglial response.

Mathematical modeling has been used effectively to elucidate signaling properties involved in apoptosis [2], inflammation [3], and heat shock responses [4, 5]. However existing models focus on cell types characteristically different from parenchymal brain cells. We therefore develop a model that captures the signaling pathways of microglia. We focus on several pathways relevant to cell death decisions: inflammation and apoptosis by the intrinsic and extrinsic pathways.

## Model Development

We formulate a chemical kinetic model of nonlinear ODEs to describe the microglia signaling. Response to extracellular cytokines and intracellular stresses can influence whether caspases become activated, a key component in apoptosis. See Figure 1 for summary. Each reaction is modeled using mass action kinetics, which makes no *a priori* assumptions about time scales [6]. Initial values of chemical parameters are taken from the literature or estimated.

The intrinsic stress input is simulated as activation of BH3-only proteins. Intrinsic apoptosis signaling is transduced through the release of species from the mitochondria leading to Caspase-9 and Caspase-3 activation. The cytokines FasL and TNF $\alpha$  are external inputs to the model. Each can activate Caspase-8 and initiate Caspase-3 activation. TNF $\alpha$  stimulation also triggers NF $\kappa$ B activation. This inflammatory transcription factor controls the synthesis of proteins including those from the IAP and Bcl-2 anti-apoptotic families. Hsp72 is included under the control of a constitutive promoter. Hsp72 interactions considered are inhibition of species release from the mitochondria, interaction with Apaf-1 to prevent

Caspase-9 activation, and inhibition of IKK phosphorylation [7, 8].

Preliminary simulations have demonstrated qualitatively Caspase-3 activation in response to apoptotic inputs and  $\text{NF}\kappa\text{B}$  activation in response to  $\text{TNF}\alpha$ , consistent with previous models [2, 9], while overexpressing Hsp72 has decreased Caspase-3 activation in all circumstances.

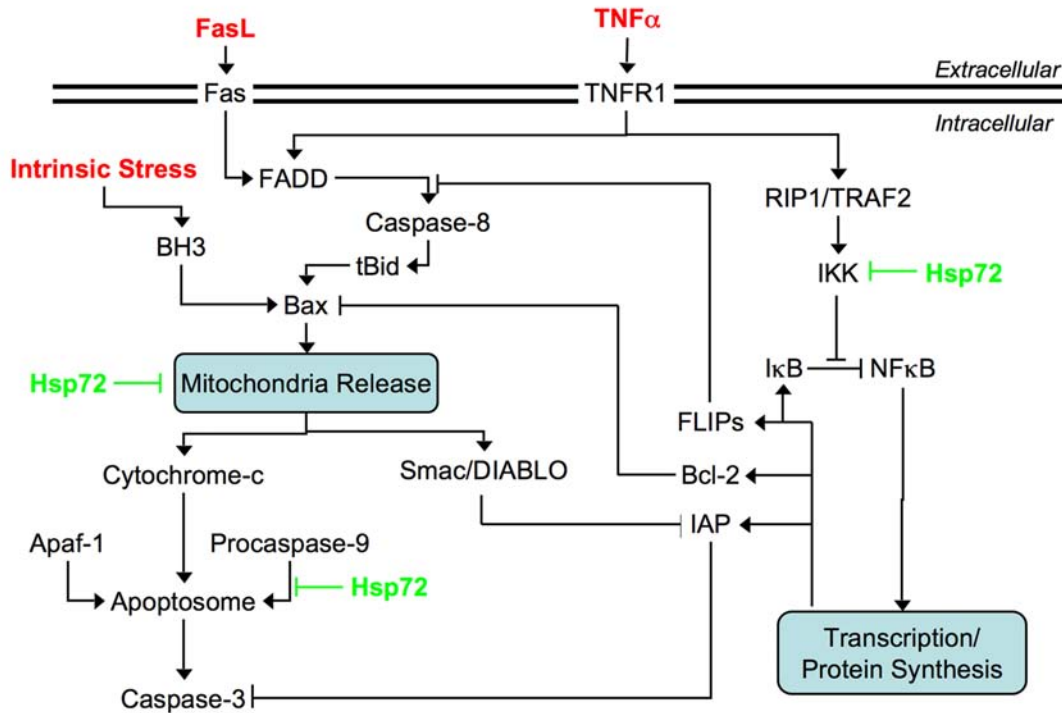


Figure 1: *Pathways involved in microglial apoptosis and inflammation signaling.* Model inputs (red) are Fas ligand (FasL),  $\text{TNF}\alpha$ , and intrinsic stress. Intrinsic stress induces the release of Cytochrome-c from the mitochondria and caspase activation, which executes apoptosis. Cytokine inputs FasL and  $\text{TNF}\alpha$  can lead to apoptosis via the caspase dependent extrinsic pathway.  $\text{TNF}\alpha$  signaling also activates the transcription factor  $\text{NF}\kappa\text{B}$  and leads to the synthesis of anti-apoptotic and inflammatory proteins as shown. Hsp72 (green) regulates signaling at several different points, including cytochrome-c and Smac/DIABLO release, IKK phosphorylation and apoptosome formation.

## Future Work

We are currently gathering *in vitro* experimental data for  $\text{NF}\kappa\text{B}$  and Caspase-3 activation in response to  $\text{TNF}\alpha$  and FasL treatment in microglia cell cultures. The data will be used to verify preliminary simulations, fit model parameters and design future experiments. A sensitivity analysis is also being performed to provide further insight into the model. Alternative models considering different levels of complexity will be explored.

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