# Static versus Dynamic Control of TNF-induced Apoptosis by NF-κB and JNK

Paul Loriaux[1,2], Rebecca Delker[1], Shannon L. Werner[1] and Alexander Hoffmann[1]

***Short Abstract* — We present a mathematical model of the biochemical events governing the cell death decision in response to TNF stimulation. Specifically, we investigate a standing hypothesis that activation of the transcription factor NF-κB protects cells from TNF-induced apoptosis by moderating the activity of the pro-apoptotic stress kinase, JNK. Our findings suggest that instead, it is the basal activities of these proteins that predispose a cell towards one fate versus the other.**

***Keywords* — NF-κB, JNK, TNF, cFLIP, apoptosis, model.**

## I. Introduction

TUMOR necrosis factor alpha (TNF) is a pro-inflammatory cytokine that is released in response to trauma or infection. Binding of TNF to its cognate receptor (TNFR) results in direct activation of caspase-8 and the apoptotic machinery via the intracellular death domain of TNFR. Healthy cells, however, are highly resistant to TNF-induced apoptosis owing to concurrent activation of nuclear factor kappa B (NF-κB), a pro-inflammatory, anti-apoptotic transcription factor. Recent studies suggest that the anti-apoptotic function of NF-κB may be mediated through cFLIP, whose pseudo-caspase domain prevents activation of caspase-8 by ligand-bound TNFR [1].

Several mechanisms by which NF-κB regulates cFLIP have been proposed. Expression of cFLIP is known to be induced by active NF-κB. Additional regulation of cFLIP levels may be achieved by controlling the activity of JNK kinase [2]. JNK has recently been shown to induce the degradation of cFLIP by activating the E3 ubiquitin ligase, Itch [3]. The anti-apoptotic protein A20, also induced by NF-κB, functions upstream of JNK by inhibiting activation of the MAP3K responsible for activating JNK [4]. Similarly, accumulation of oxygen radicals (ROS) promote JNK activity by inhibiting the inactivating MAPK phosphatase [5]. Anti-ROS molecules FHC and Mn-SOD are induced by active NF-κB and have also been implicated in the regulation of JNK.

[1]Signaling Systems Laboratory, Department of Chemistry and Biochemistry, University of California San Diego

[2]Graduate Program in Bioinformatics, University of California San Diego. E-mail: ploriaux@ucsd.edu

## II. Results

To investigate the relative importance of these mechanisms under different physiological conditions, we have constructed a mathematical model of TNF-induced NF-κB activation and regulation of the JNK pro-apoptotic pathway. The model predicts, given a particular TNF stimulus and cellular state, whether mitochondrial outer-membrane permeabilization (MOMP) will occur, where MOMP is taken to be an irreversible switch-like event preceding cell death [6]. A novel modeling methodology allows us to explicitly define the cellular state prior to stimulation. Extensive simulation over a range of steady states in conjunction with experimental data from our lab suggests that it is the resting state and not the dynamic response of the cell that predisposes it to one cell fate over the other. These results are in good agreement with an earlier study demonstrating that the steady state abundances of upstream signaling proteins control the time at which MOMP is observed following stimulation with the TNF family member, TRAIL [7].

## References

[1] Irmler, M, et al. (1997) Inhibition of death receptor signals by cellular FLIP. *Nature* **388**, 123-125.

[2] Nakano, H (2004) Signaling crosstalk between NF-κB and JNK. *Trends Immunol* **25**, 402-5.

[3] Chang, L, et al. (2006) The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP (L) turnover. *Cell* **124**, 601-13.

[4] Lee, EG, et al. (2000) Failure to Regulate TNF-Induced NF-kB and Cell Death Responses in A20-Deficient Mice. *Science* **289**, 2350-4.

[5] Kamata, H, et al. (2005) Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. *Cell* **120**, 649-61.

[6] Albeck JG, et al. (2008) Modeling a snap-action, variable-delay switch controlling extrinsic cell death. *PLoS Biol* **6**, 2831-52.

[7] Spencer SL, et al. (2009) Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature* **459**, 428-32.