The Integration of Multiple Negative Feedback Regulators Directs Diverse NF-κB Signaling Dynamics

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Short Abstract — **The paradigm for transmission of information in mammalian cells is a stimulus-induced cascade of signaling events culminating in the expression or activation of downstream components. Signal transduction is seldom linear and the redundancies inherent in the network through combinatorial complexity complicate our understanding of the signaling process. The Nuclear Factor (NF) κB signaling pathway is central to a host of physiological responses and is controlled through a robust, non-linear network of interactions that responds to both homeostatic and stimulus-dependent controls. Through computational and biochemical studies, we have elucidated the interactions between multiple negative feedback regulators that enable stimulus and temporal-specific control of NF-κB responses.**

***Keywords* — Signal transduction, negative feedback**

I. Background

THE NF-κB family of transcription factors mediates diverse signaling responses to inter- and intracellular stresses. Most stresses that activate NF-κB do so via activation of the IκB kinase (IKK) that targets NF-κB inhibitor proteins, IκBs, to the ubiquitin-proteasome degradation pathway and thus allows free NF-κB to translocate to the nucleus. A great deal of combinatorial (multiple isoforms of IκB, NF-κB, etc) and temporal (diverse range of IKK dynamics) complexity complicates our understanding of how NF-κB activation is controlled [1].

A computational model was built and validated against experimental results to help explain how the network transduces signals [2]. The model uses ODEs to calculate the time-dependent changes in concentrations of proteins and mRNA in a network that contains IKK (the input to the model), NF-κB (the output of the model), and the IκBs. It includes reactions that describe IκB:NF-κB:IKK association and dissociation, synthesis and degradation of IκBs, and nuclear:cytoplasmic shuttling.

II. Results

The original model included only the predominant NF-κB negative feedback mediated by IκBα. Subsequent experimental studies revealed that another IκB isoform, IκBε, is also inducibly expressed upon NF-κB activation and provides negative feedback [3]. Its synthesis, however, is temporally delayed respective to that of IκBα. Inclusion of delayed IκBε synthesis within the model revealed that IκBε negative feedback is in anti-phase to IκBα and serves to dampen IκBα-induced NF-κB activity oscillations. IκBε can also provide homeostatic and dynamic compensation in IκBα-deficient cells.

A fourth IκB isoform, p100/IκBδ, was found to provide NF-κB regulation in response to developmental cues [3]. Incorporation of this new IκB into the model revealed that it mediates crosstalk between an initial inflammatory stimulus and a subsequent developmental cue. Recently, we reinvestigated the role of p100/IκBδ in response solely to inflammatory stimuli, in which its degradation is not induced [4]. We created a computational phenotyping tool to study NF-κB activation in wild-type vs IκBα- vs IκBδ-deficient systems and found that IκBδ provides regulation to long-lasting stimuli (i.e. pathogens) while IκBα is important for transient stimuli (i.e. cytokines and chemokines). Experiments confirmed these predictions.

An expanded model was constructed to include signaling upstream of IKK in response to TNF stimulation in order to delineate the functions of IκBα and A20 deubiquitinase [5]. Both proteins are strongly induced by NF-κB activation with similar temporal profiles, but function at different locations in the network—IκBα directly inhibits NF-κB while A20 deactivates the upstream TNF receptor activation complex. Naively, these negative feedbacks should be redundant, but we hypothesized that network topology would prevent such a simplification. Via simulations and experimental studies we found that IκBα and A20 are in fact non-overlapping and provide for different regulatory functions.

References

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