

Immunology

The rise in computational systems biology approaches for understanding NF- κ B signaling dynamics

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The combination of mathematical modeling with imaging experiments helps to interpret how NF- κ B signaling is controlled.

Abstract

A study by Cheng *et al.* in this issue of *Science Signaling* highlights the distinct single-cell signaling characteristics conferred by pathways mediated by the adaptor proteins MyD88 and TRIF in the TLR4-dependent activation of the transcription factor nuclear factor- κ B (NF- κ B).

Nuclear factor- κ B (NF- κ B) is a transcription factor that is intimately involved in the inflammatory response. Although it was initially thought to act as a regulator of the immunoglobulin κ enhancer within B cells (1), it is now known that NF- κ B is ubiquitously expressed and tightly regulated in a cell type- and stimulus-specific manner (2).

Indeed, NF- κ B controls the transcriptional regulation of many genes, such as those induced in response to infections and cellular stress (3). Because of the wide-ranging gene products that are regulated through NF- κ B, it has been deemed a “master” regulator of inflammation (2). Activation of the transcription factor is controlled by two distinct signaling networks, which have been termed the canonical and noncanonical pathways. The canonical pathway is activated through Toll-like receptors (TLRs) and by the interleukin-1 receptor (IL-1R) superfamily. Signaling is mediated through interactions between the cytoplasmic Toll/IL-1R (TIR) homology domain and downstream adaptor proteins, including myeloid differentiation primary response gene 88 (MyD88) and TIR domain-containing adaptor gene inducing interferon- β (TRIF) (4).

A substantial body of knowledge has been generated through traditional “wet” laboratory experimentation since the discovery of NF- κ B almost 30 years ago. However, the complexity of the inflammatory process has been difficult to investigate through reductionist and linear analysis alone, because the dynamics of NF- κ B signaling are characterized by nonlinear kinetics as well as numerous feedback loops. To obtain detailed information about the mechanisms controlling the NF- κ B regulatory network, studies have increasingly used computational models, termed computational systems biology (5), to complement traditional wet-lab techniques. These studies use computational analysis to generate hypotheses about the mechanistic behavior of the signaling pathway. The *in silico*-derived hypotheses help further our understanding of the dynamics of the biological system by predicting how activation at the level of the receptor complex is propagated down the signal transduction pathway(s), and they improve our knowledge of the hierarchy and

significance of the individual regulatory steps.

A large number of computational models about NF- κ B signaling dynamics have been developed over the past 15 years, which we have recently reviewed (6). The first widely used model was developed in 2002 by Hoffmann *et al.* (7), and since then, the Hoffmann group has extensively used a computational systems biology approach to further our understanding of NF- κ B dynamics. Their latest study, by Cheng *et al.* (8) in this issue of *Science Signaling*, describes the distinct signaling characteristics conferred by the MyD88-controlled and TRIF-dependent pathways in the TLR4-stimulated activation of NF- κ B.

Cheng *et al.* performed a combination of in vitro and in silico experimentation to understand the specificity and redundancy between the MyD88-controlled and TRIF-dependent pathways in the activation of NF- κ B (Fig. 1). The authors first developed a mathematical model of TLR4 signaling that comprised three distinct functional modules: a TLR4 module for transduction of the extracellular signal into the activities of the downstream kinases IKK kinase (IKKK) and TANK-binding kinase 1 (TBK1) through the MyD88 and TRIF pathways; an interferon response factor (IRF) module, which transduces TBK1 activity to phosphorylated nuclear IRF3; and an existing NF- κ B module, which has been extensively tested and refined since its initial development (6, 7). Subsequent in vitro experimentation focused on the activation dynamics of IKK in response to the TLR4 ligand lipopolysaccharide (LPS) in bone marrow-derived macrophages derived from wild-type, *Myd88*^{-/-}, and *Trif*^{-/-} mice, together with analysis of NF- κ B dynamics [as measured by electrophoretic mobility shift assay (EMSA)] and the dynamics of IRF3 phosphorylation (as measured by Western blotting analysis).

Initial in silico experimentation with the mathematical model, calibrated to these in vitro dynamics, was unable to recapitulate the EMSA-determined NF- κ B activity profiles, and single-cell dynamics were obscured by a high degree of cell-to-cell variability. The authors resolved this discrepancy by recalibrating the model parameters with single-cell NF- κ B translocation dynamics gained through live-cell imaging of LPS-stimulated macrophages. This newly calibrated model was then used to explore the specific control of the MyD88- and TRIF-regulated pathways, including the role of endosome translocation, in encoding NF- κ B dynamics.

This combination of approaches enabled the authors to develop a predictive understanding of how TLR4-responsive NF- κ B dynamics in populations of single cells were encoded by MyD88 and TRIF. In particular, the authors observed (through flux analysis and single-cell imaging) that receptor endocytosis generated temporal separation between the activation of NF- κ B through both pathways; with the delayed activation through TRIF signaling ensuring that the initial first peak in NF- κ B activation is dependent on MyD88 signaling. Additionally, the authors observed molecular noise regarding NF- κ B activation by LPS and endosome maturation, which they believe was a result of extrinsic variation. After performing in silico experimentation to simulate this behavior, the authors conjectured that this extrinsic noise was the driving force for the variability of single-cell responses.

We believe that the work of Cheng *et al.* in this issue of *Science Signaling* exemplifies the benefits that a computational systems biology approach can bring to furthering our understanding of complex signaling networks, such as those controlling NF- κ B activity. We also believe that with the growing number of

computational models that look at varying aspects of NF- κ B signaling dynamics and that consider an increasing number of parameters (such as individual extracellular stimuli and gene transcriptional regulation), there is potential to merge these into a much larger system-wide model, which would enable us to simulate the signaling crosstalk that is inherent in complex biological systems.

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Fig. 1. TLR4 signaling through the MyD88-controlled and TRIF-dependent pathways. MyD88 is an essential adaptor protein for the canonical NF- κ B signaling pathway, which leads to the early-phase NF- κ B-dependent production of inflammatory cytokines. Conversely, the adaptor protein TRIF leads to the IRF3-induced and late-phase NF- κ B-dependent activation (9) of the production of IFN- β . Although both pathways may be activated through TLR4 at the plasma membrane, the TRIF-dependent pathway may also be activated through endosomal induction. Additionally, the TRIF-dependent pathway links to the canonical pathway for the early-phase NF- κ B-dependent production of inflammatory cytokines.

