Komitet Mechaniki Polskiej Akademii Nauk

Politechnika Rzeszowska
im. Ignacego Łukasiewicza

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INNATE IMMUNE RESPONSES AT SINGLE CELL LEVEL

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Innate immunity forms the first line of defense, limiting spreading of infection before the adaptive immune response is activated. In the first phase of the innate immune response, cells detect pathogens or their fragments with their membrane and cytoplasmic receptors. This leads to activation of the regulatory systems of the transcriptional factors NF-κB, IRF3 andAP-1 families, Fig.1. These factors jointly regulate the activity of a several hundred genes responsible for inducing inflammation, antiviral protection, proliferation and apoptosis. In particular, they induce production and secretion of proinflammatory cytokines (among them IL-1, TNFα) as well as Interferons α and β. These cytokines are mediators of the second phase of the cellular innate immune response in cells that did not encounter the pathogen.

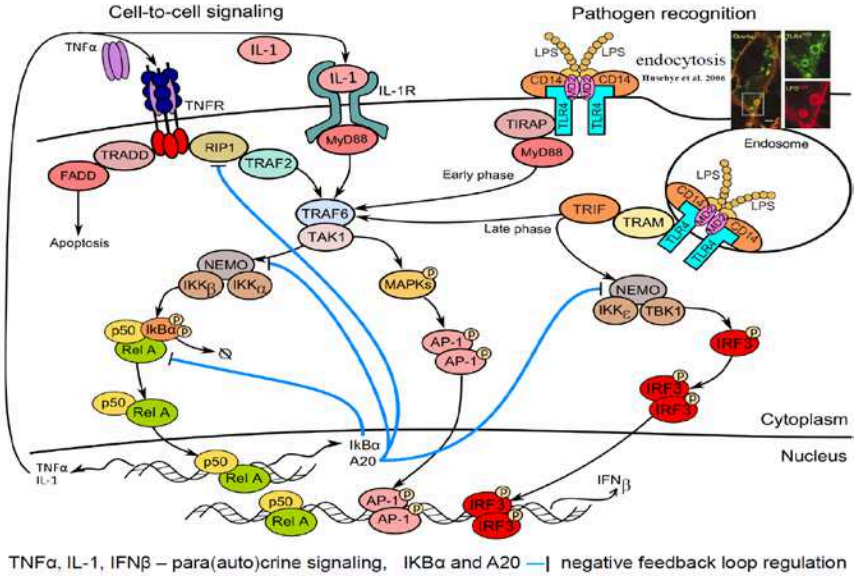

Fig. 1. Example of pathogen recognition: LPS induced signaling. LPS (Lipopolysaccharide – outer membrane of Gram-negative bacteria) is recognized by CD14 co-receptor, which transfer it to TLR4 leading to its activation, and binding of adaptor protein Myd88. As a result kinase TAK1 is activated and transmits signal to transcription factors p50-RelA (NF-κB) and AP-1 (early phase ~ 30 min). CD14 induced endocytosis of CD14-TLR4-LPS complexes leads to TRIF mediated activation of IRF3 and p50-RelA (late phase). Activation of transcription factors p50-RelA, IRF3 and AP1 leads to their nuclear translocation and synthesis of cytokines: TNFa, IL-1 and IFNβ that regulate via para(auto)crine signaling the second phase of innate immune responses. Transcriptional activity of p50-RelA and IRF3 is controlled by p50-RelA inducible proteins IkBα and A20 (negative feedbacks).

Single cell experiments (Nelson et al. 2004) as well as mathematical modeling (Lipniacki et al. 2006) demonstrated that individual cell responses are very heterogeneous and the population average is not a good representative of single cell behavior. Such experiments use fluorescently tagged transcription factors NF-κB, and recently (in our group) IRF3, and allow for a real time analysis their translocation between cytoplasm (where they are inactive) and nucleus (where they can induce expression of genes).

I will make a short overview on innate immune responses and then discuss the single cell mathematical modeling of NF-κB regulatory system (Fig. 2) under TNFα stimulation at various doses of the cytokine. As will be shown, the regulatory process is highly stochastic, and intrinsic and extrinsic components of noise are both responsible for the observed heterogeneity of the responses, Fig. 3. We propose an approach combining ordinary differential equation (ODE), which allow for bifurcation analysis, and Markov processes which accounts for stochasticity in the process. Based on the stochastic modeling we have theoretically predicted that cells can be activated by binding of one or few TNFα molecules (Lipniacki et al. 2007) and later demonstrated it experimentally using microfluidic system that allowed for precise dosage of TNFα (Tay et al. 2010). Interestingly, the fraction of activated cells decreases with the TNFα dose, but the expression of early NF-κB dependent genes in responding cells is independent to the dose.

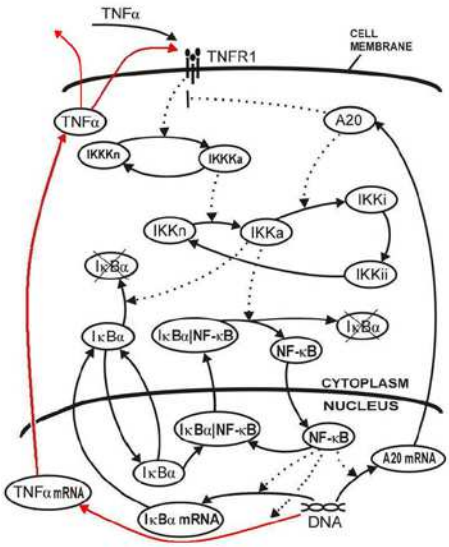

Fig. 2. Schematic of NF-κB regulatory module (based on Lipniacki et al. 2007). Solid lines denote transitions. Dotted lines denote influence, positive for arrowhead lines, negative for hammerhead lines. From TNFα receptors (TNFR1) signal is transmitted via IKKK and IKK kinases. Transcriptional activity of NF-κB is regulated by two negative feedback loops. The "inner" one is mediated by IkBα, which sequesters NF-κB in its inactive form in cytoplasm. Upon the signal active IKK (IKKa) phosphorylates IkBα leading to its proteolitycal degradation. Liberated NF-κB translocates to the nucleus and triggers transcription of inhibitors IkBα and A20. Synthesized IkBα enters the nucleus, binds NF-κB and transport it back to cytoplasm. If the signal persists, IkBα is again degraded, leading to NF-κB cytoplasmic-to-nuclear oscillations. Activity of kinase IKK is attenuated by A20, which mediates the outer negative feedback loop. In the A20 knockouted cells, IKKa remains at high level preventing accumulation of IkBα protein and in turn leading to persistent nuclear NF-κB occupancy. In some cell lines (including cancer), the positive autocrine regulation via TNFα, leads to the oscillations of NF-κB system, which can be triggered spontaneously in the absence of external stimuli.

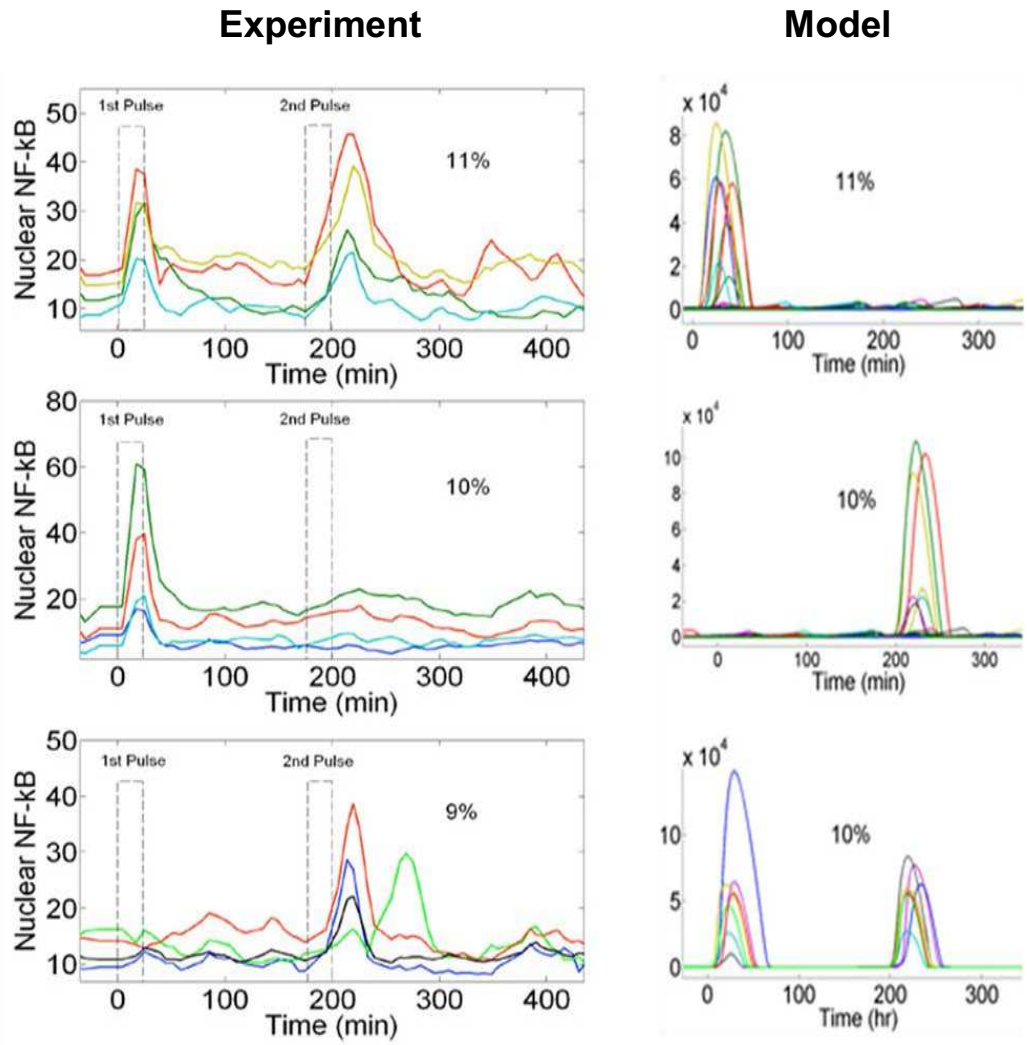

Fig. 3 Single cell responses to two weak TNFα pulses: experiment and model. Each color corresponds to single cell trajectory. Cells were stimulated by two 20 min long pulses of 0.1 ng/ml TNFα, separated by 180 min. The population of cells responding to first, second, or both pulses was about 10%. Such behavior results from coexistence of two type of noise: extrinsic (initial heterogeneity rendering some cells more sensitive than others), and intrinsic resulting from small amount of cytokine molecules per cell.

LITERATURA:

[1] T. Lipniacki, P. Paszek, A. R. Brasier, B. Luxon, M. Kimmel, Stochasticity in early immune response, Biophysical Journal 90 (2006) 725–742.

[2] D. E. Nelson et al. Oscillations in NF-kB signaling control the dynamics of gene expression, Science 306 (2004) 704–708.

[3] T. Lipniacki, K. Puszynski, P. Paszek, A. R. Brasier and M. Kimmel, Single TNFα trimers mediating NF-κB activation: Stochastic robustness of NF-κB signaling, BMC Bioinformatics 8 (2007) 376.

[4] S. Tay, J. J. Hughey, T. K. Lee, T. Lipniacki, S. R. Quake, M. W. Covert, Single-cell NF-κB dynamics reveal digital activation and analogue information processing, Nature 466 (2010) 267–272.