NF-κB and IRF3 crosstalk signaling in MEFs

Maciej Czerkies[1], Zbigniew Korwek[1], Wiktor Prus[1], Sławomir Błoński[1], Bing Tian[2], Marek Kimmel[3], Allan Brasier[2], and Tomasz Lipniacki[1]

***Short Abstract* — NF-κB, IRF3 and AP-1 are most potent transcription factors controlling innate immune responses to pathogens. Combining single cell and population techniques with mathematical modeling we analyzed crosstalk of these pathways in mouse embryonic fibroblasts in response to LPS and poly(I:C). We found that both stimuli activate RelA (NF-κB) and c-Jun (AP-1) transcription factors, but only poly(I:C) leads to a significant IRF3 activation and IFNβ secretion. Paracrine regulation by IFNβ leads to inhibition of translation of negative regulators IκBα and A20 and causes that responses to poly(I:C) are switch-like in contrast to transient or oscillatory responses to LPS.**

I. Methods

Experimental methods involved live confocal imaging of MEFs with fluorescently tagged RelA (GFP) and IRF3 (strawberry); immunostaining in WT and RelA-deficient MEFs towards RelA, IRF3, STAT1, IκBα and A20; RT-PCR measurements of about 20 of NF-κB, AP-1, IRF3 and IFN regulated genes; Western blot analysis of cytoplasmic and nuclear extracts towards RelA, c-Jun, IRF3, A20, IκBα, IKKα/β, TBK1 and their phosphoforms; ELISA towards cytokines TNFα, RANTES and INFβ. Modeling combines bifurcation analysis of the ODE approximation with stochastic simulations of the corresponding Markov process.

II. Results

We found that both LPS and poly(I:C) activate mediating kinases IKKα/β and TBK1; interestingly, only poly(I:C) stimulation leads to activation of IRF3 (activated by TBK1) and triggering of transcription of IFNβ, IRF7, and RIG-1 and other interferon regulated genes. LPS stimulation leads to transient or oscillatory (in some cells) responses of NF-κB, in contrast to switch-like responses (preceded by one or two pulses in a fraction of cells) to poly(I:C) stimulation, with fraction of switched on cells increasing with stimulation dose. As confirmed by the experiment in which cells are costimulated by LPS and IFNβ, the difference in NF-κB responses is caused by IFNβ para- and autocrine regulation that leads to activation of Eif2ak2 and suppression of NF-κB inhibitors synthesis. This breaks the negative regulation of NF-κB (and IRF3) leading to build up of nuclear NF-κB and IRF3, followed in most cells by apoptosis. The IRF3 activation, which initially proceeds via TLR3 pathway, is stabilized by positive feedback involving strongly upregulated IRF7 and RIG-1 (which is IRF7 responsive).

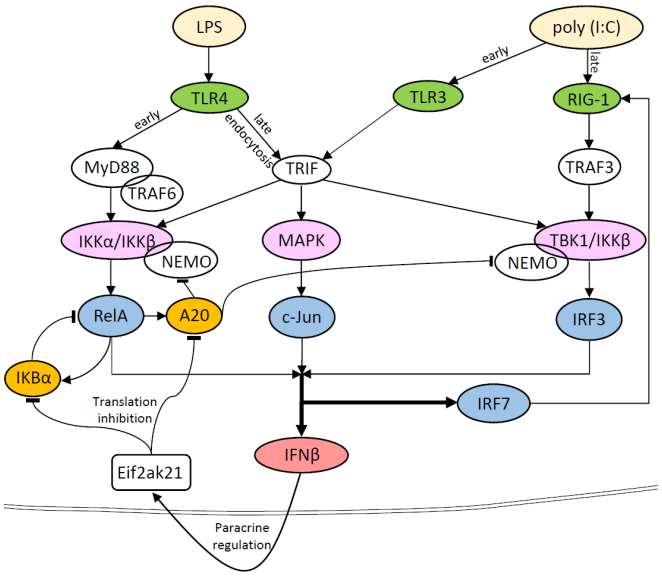

Fig.1. Simplified ideogram of the crosstalk of pathways.

III. Conclusion

We identified negative and positive feedbacks in NF-κB, AP-1 and IRF3 crosstalk, and confirmed by mathematical modeling the dynamically divergent responses to LPS (mimicking bacterial infection) and to poly(I:C) (mimicking viral infection). NF-κB is known for exhibiting oscillatory responses to TNFα, which are replaced by switch-like responses in A20-deficients cells. Here, we found that activation of the IRF3 pathway, or INFβ stimulation leads to the similar effect on NF-κB signaling, due to inhibition of translation. The switch-like behavior, frequently associated with cell fate decisions, is associated with bistability arising here due to the positive feedbacks in IRF3/IRF7 regulation. Observation of single cell dynamics shows that activation of IRF3 and NF-κB is followed by apoptosis.

Acknowledgements: This work was supported by Polish National Science Center grant

[1]Institute of Fundamental Technological Research, Warsaw, Poland. E-mail: tlipnia@ippt.pan.pl
[2]University of Texas Medical Branch (UTMB), Galveston, TX, USA E-mail: arbrasie@utmb.edu
[3]Department of Statistics, Rice University, Houston, TX, USA E-mail: kimmel@rice.edu