Stochastic regulation of NF-κB pathway

T. Lipniacki[1], P. Paszek[2], A. Brasier[3], B. Luxon[3], M. Kimmel[2]

Keywords: Stochastic regulation of transcription, molecular pathways, NF-kappaB

1 Model formulation.

Typically, in an animal cell there are tens or hundreds mRNA molecules of a given species and tens of thousand of corresponding protein molecules. Therefore processes such as mRNA translation, formation and degradation of protein complexes, and catalytic and spontaneous degradation, which involve a large number of molecules, may be modeled by ordinary differential equations (ODEs). In contrast, in a single cell, the regulation mRNA transcription may be discrete, governed by stochastic events of binding and dissociation of transcription factors. The stochasticity in regulation of transcription leads to large variability among cells. Since a given cell reacts to its own mRNA and protein levels, and not to the average levels in the population, the information about this variability is very important. In this work we apply our model on the NF-κB regulatory module [1], Fig. 1, to the single cell by modeling the transcriptional part of the regulatory network using a stochastic switch. The mathematical representation of the model consists of 14 ODEs accounting for: formation of complexes and their degradation, transport between nucleus and cytoplasm, and transcription and translation, together with 4 equations accounting for binding and dissociation probabilities of NF-κB molecules to regulatory sites in A20 and IκBα promoters. The simulation time is split into small time intervals Δt. Within Δt's, the ODEs are solved using the fourth order MATLAB solver. At the end of each interval, the

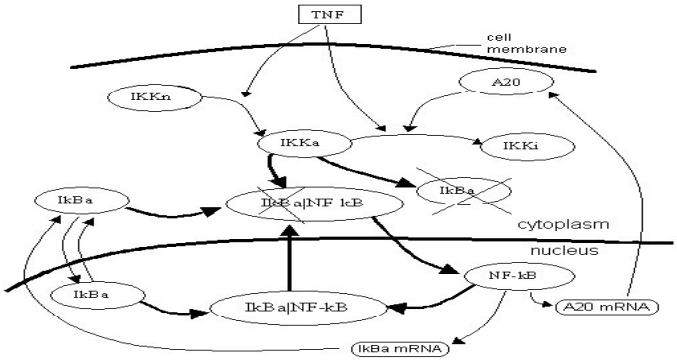

Figure 1: The model involves two-compartment kinetics of the activators IKK and NF-κB, the inhibitors A20 and IκBα, and their complexes. In resting cells, the unphosphorylated IκBα binds to NF-κB and sequesters it in an inactive form in the cytoplasm. In response to extracellular signals such as TNF, IKK is transformed from its neutral form (IKKn) into its active form (IKKa), capable of phosphorylating IκBα, leading to IκBα degradation. Degradation of IκBα releases NF-κB, which enters the nucleus and triggers transcription of the two inhibitors and numerous other genes. The newly synthesized IκBα leads NF-κB out of the nucleus and sequesters it in the cytoplasm, while A20 inhibits IKK converting IKKa into the inactive form IKKi, a form different from IKKn but also not capable of phosphorylating IκBα. Bold arrows stand for very fast kinetics.

[1] IPPT PAN, Warsaw, Poland and Department of Statistics, Rice University, E-mail: tomek@rice.edu
[2] Department of Statistics, Rice University, Houston, TX, USA
[3] University of Texas Medical Branch, Galveston, TX, USA

binding and dissociation probabilities are calculated, and the status of the two promoters, which may be ON or OFF, is evaluated and kept constant during the next time interval.

2 Results.

The stochasticity of the model implies that simulations, which mimic the behavior of single cells, performed with the same model parameters and the same initial conditions, are different. The averaged outcome resembles that of the deterministic model [1] and fits well the experimental data obtained for a population of cells, data not shown. Here we focus on variability in cell kinetics.

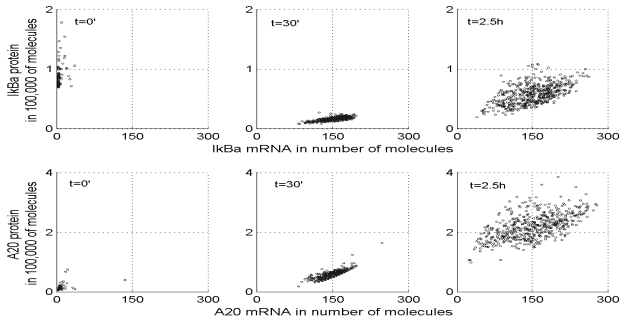

Figure 2: The scatter plots show the abundance of protein versus its mRNA at three time points, for inhibitors IκBα and A20. Prior to TNF signal there is relatively little of IκBα mRNA molecules, while the IκBα protein (which is mostly complexed with NF-κB) is abundant. Then at 30min most of the IκBα protein is degraded, but the number of mRNA molecules is large due to NF-κB induced transcription. For A20, initially there is little of both protein and mRNA, then the growing amount of transcript is followed by the growing amount of protein. The broadening of the distribution in time is caused by the desynchronization of cells due to stochasticity.

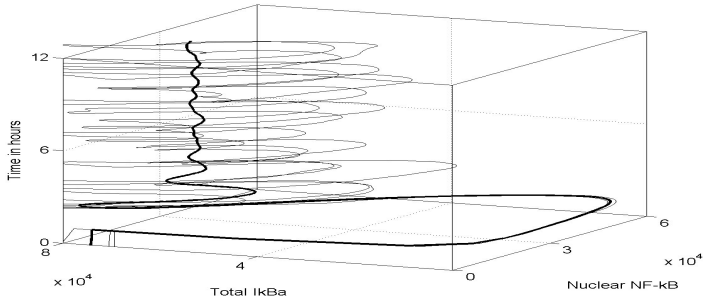

Figure 3: The single cells trajectories (thin lines) keep oscillating despite the equilibrium distribution is reached, and the average trajectory (bold line), a construct resulting from averaging over population, stabilizes.

Patterns of variability indicate that the averaging is accomplished by cancellation of phases of oscillations in individual cells. Fig. 3 shows that none of cells behaves like an average. This outcome suggests experimental testing by following individual cells, which currently is underway.

References

[1] Lipniacki, T., Paszek, P., Brasier, A., Luxon, B. and Kimmel, M. (2004) Mathematical model of NF-kappaB regulatory module. *J. Theor. Biol.,* in press.