Single cell processing of inflammatory signals via the NF-κB network

Christopher Boddington, Antony Adamson, James Bagnall, David Spiller, Dean Jackson, Mike White, Pawel Paszek

Faculty of Life Sciences, University of Manchester, Manchester, UK

Abstract. Immune cells function in highly dynamic environments communicating through a set of cellular and molecular cytokine networks. Many inflammatory signals (e.g. Tumor Necrosis Factor alpha, TNFα) converge on just a few key transcription factors such as Nuclear Factor kappa B (NF-κB). Using stochastic differential equations and live-cell imaging we show that there exists a unique refractory period contained within each cell that controls responsiveness of the NF-κB to periodic TNFα stimulation. The refractory period is however heterogeneous in a population, but surprisingly fixed in an individual cell over time. Using sensitivity analyses we hypothesize that multiple transcriptional feedbacks independently regulate this refractory period, which may help prevent out-of-control inflammatory states, characteristic of many autoimmune diseases.

1 Background

The NF-κB family of transcription factors critically regulates innate immune responses and inflammation, and has a key role in cell division and apoptosis [1]. NF-κB must decode noisy extracellular cytokine signals and encode intercellular information leading to specific cell fate decisions. Using time-lapse confocal microscopy we study the single cell dynamics of the NF-κB system. We showed that in response to cytokine stimulation, NF-κB oscillates between the cytoplasm and the nucleus and the frequency of oscillations controls target gene expression [2, **?**]. Oscillations between individual cells appear asynchronous and we hypothesised that this is due to a dual IκB negative feedback that drives cellular heterogeneity and allows robust population responses [4]. By developing a more quantitative picture of NF-κB regulation, we can begin to understand how noisy inflammatory signals are encoded and regulated by single cells.

2 Results

Using single-cell time-lapse imaging data we have shown that pulsatile treatment with TNFα results in a synchronized response at the pulsing frequency. At high pulsing frequencies, the response is a digital response in activation, that is hetrogenious in a population. A cell exhibits a transient refractory period of

unresponsiveness following a cytokine pulse that is unique to an individual cell, and we have shown it is constrained between the IκB kinase and cytokine receptor in the signal transduction pathway.

We have developed a new cell model of the NF-κB system specifically considering regulation via extrinsic noise in proteins levels (e.g. receptors, kinases) and intrinsic noise in stochastic gene transciprtion. In particular, we focus on the A20 and IκBα feedbacks and their differential regulation over NF-κB responses. A dynamic local and global sensitivity analysis suggests both feedbacks regulated NF-κB oscillations following TNFα stimulation, whereas A20 mediates a cells refractory period and population hetrogeneity following pulsatile stimulation.

We also found that cell responses are based on pre-exisiting variation in cell sensitivities. Cells that responded to 2 short pulses of TNFα will respond deterministically following after a subsequent set of pulses. This suggests pre-exisiting variation in internal variables imprinted on the system network is key to cell responses.

3 Conclusion

Single-cell network imprinting of variable parameters generates hetrogeneity at the population level. Understanding this hetrogeneity using multi-scale mathematical modelling will allow a better understanding of inflammatory processes.

References

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