NF- B signaling, dynamic range and stochastic switching under TNF- and LPS stimulation

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***Short Abstract* — Using a high-throughput microfluidic cell-culture system we analyze the response characteristics of the NF-KB pathway under TNF-α and LPS stimulation, and use mathematical modeling to show how stochasticity can give rise to a rich repertoire of responses at the single cell level.**

***Keywords* — Systems Biology, Microfluidics, Signaling, NF-KB, TNF-α, LPS, cell culture, modeling**

I. Introduction

THE highly conserved transcription factor NF-κB is activated by hundreds of different external stimuli (i.e. signaling molecules, virus, bacteria, physical stress and UV radiation) which in turn facilitates specific responses by translocating to the nucleus and coordinating the activation of more than one hundred genes [1]. Here we investigate the NF-κB response to multiple doses of the cytokine TNF-α and bacterial product LPS using a high-throughput microfluidic cell-culture platform [2] and fluorescent time-lapse microscopy, identify the quantitative differences between these responses, and explain the biochemical sources of variation and specificity of response using mathematical modeling.

II. Experimental results and modeling

In a single experiment, we have simultaneously measured the p65-GFP activity in more than 3000 mammalian cells (NIH-3T3) under identical conditions, for 8 different doses of both ligands and with a temporal resolution of 6 minutes [3]. We have tracked and analyzed cells images using a nuclear marker and custom software, and extracted nuclear localization intensity data with excellent quantitative quality to obtain the following results:

A. NF-KB oscillations under TNF-α are not damped

There is recent debate about whether or not NF-KB nuclear localization oscillations under TNF-α stimulation were damped. Our extended time-lapse measurements (12 hrs) indicate that, at the single cell level, these oscillations are continuous.

B. Thresholds, dynamic range and response time

We measure activity under 8 doses of both ligands, covering a range of 4 orders of magnitude. The cells start responding to doses above a certain threshold, and the number of cells activating increase significantly with the dose. The response time, defined as the time to the first peak after stimulation, was found to decrease from hundreds of minutes near threshold to below 30 minutes for near saturating doses. The standard NF-KB model [4] was modified and was used to explain the reduction in the response time as the stimulant dose increase.

C. Cell-to-cell variability and stochastic switching

The singe cell response is highly stereotyped at the high doses, while it is chaotic at the intermediate and low doses. We observe stochastic switching at the near threshold doses. Using the modified model, these phenomena were shown to possibly arise from relatively small fluctuations in key protein levels at low doses.

D. Analog vs. digital signaling

There is a striking difference between the way cells respond to LPS and TNF: in the TNF case the response is graded with nuclear localization intensity proportional to the dose, resembling analog signaling. For LPS stimulation, however, all of the NF-KB protein localizes in the nucleus even at the lowest doses, indicative of digital signaling. When cells switch on they respond fully, showing how NF-KB signaling differs for cell-cell communication (TNF) vs. a bacterial infection (LPS).

III. Conclusion

Our results, in addition to their biological significance, underlie the importance of high-quality, single-cell data in understanding and modeling biological systems, and demonstrate the efficiency of microfluidic techniques in obtaining such data easily and reproducibly.

References

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