Cellular responses to dynamic patterns of cytokine stimulation

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***Short Abstract* — Activation of NF-kB through exposure to inflammatory cytokines mediates signals for migration, differentiation, cell-cycle progression, and apoptosis. Time-varying properties in the concentration of cytokine, such as the duration of stimulus, influence the type of response elicited in a cell. To study the extent to which temporal properties of cytokine stimulation mediate cell fate decisions, we have developed a microfluidic flow system for precise control of cytokine concentration as a user-defined time varying function. By imaging cells that express reporters for NF-kB activation and exposing them to different patterns of stimulation in the microfluidic system, we aim to understand how cells decode time-varying cues from their environment to make cell fate decision.**

***Keywords* — NF-kB signaling pathway, TNF, microfluidics, environmental cues, temporal signaling patterns**

I. Introduction

Responding appropriately to molecular signals from the extracellular milieu, such as cytokines or growth factors, is essential for cellular adaptation and viability. The NF-kB signaling pathway, for instance, upon activation with tumor necrosis factor (TNF) activates transcription for a myriad of genes [1] responsible for anti-apoptotic and pro-inflammatory responses [2]. Additionally, the same pathway through its non-canonical branch activates genes inducing the opposite i.e. pro-apoptotic and anti-inflammatory response [3]. Thus, cells must decode and extract information from external signals to produce the corresponding response. It is apparent that time-varying properties of molecular signals play an important role in this information transfer. For example, short duration exposure to high concentrations of TNF can be more effective at killing than a TNF-pulse of longer duration [4], and the subsequent dynamics of NF-kB localization within the cell encodes multiple levels of responses that reflect the strength of stimulation[4]. Although these studies have begun to scratch the surface [5], cells are using molecular switches and dials that we do not currently understand to transmit information about TNF in their environment. Here, we develop a framework to more broadly define the capabilities of single cells when exposed to arbitrary user-defined patterns of TNF stimulation and use it to uncover molecular circuits that encode this information.

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II. Methods

To explore the role of temporal dynamics of external signals in cellular response, precise control of extracellular environment is needed. We have developed a microfluidic system for analog control over the cellular environment, exposing cells to user-defined temporal patterns of stimulation. The system consists of a hydrostatic pump connected to a microfluidic cell-culture chip. The hydrostatic pressure and hence the flow rates of cytokine and media are controlled by manipulating the heights of the corresponding reservoirs through the Arduino microprocessor. A passive mixer in the chip then dilutes the cytokine concentration as desired before the cell-culture chamber. We have developed a framework to control the device by a Hagen-Poiseuille equation based mathematical model and CFD simulations in Ansys-Fluent software. Given a user-defined input of concentration, time and position in the chip, our system generates the corresponding positions of heights to generate the desired concentration profile. We use live-cell imaging to track single-cells expressing a GFP reporter of NF-kB and measure the corresponding nuclear localization as a proxy for response to TNF stimulation. The system can hypothetically achieve an arbitrary-fold dynamic range in concentration, depending on parameters of the cell-culture chip, and we are working on adding extra functionalities to our system that permit simultaneous and independent control of multiple cytokines in the same chip.

III. Progress

We are testing a preliminary version of our system by monitoring the NF-kB response in cells exposed to linear and exponential ramps of TNF stimulation. Our early results show that ramp stimulations produce NF-kB responses that are distinct from those seen after a pulse or step increase in TNF concentration. These results may hint at the molecular architecture of circuits that transmit information about extracellular TNF into the NF-kB system. We envision that our microfluidic system may be adopted with remarkably low cost by labs without specialized expertise to enable studies of cellular systems in dynamic microenvironments.

References

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