

Cytokine Communication Networks in the Immune System

The immune system functions by a coordinated response of many cell types that exchange information via a complex communication network (Figure 1A). Important mediators of that intercellular communication are cytokines – small proteins that are secreted and can be detected by cells of the immune system, as well as by cells of other tissues. Thus, cytokines have crucial functions in the development, differentiation and regulation of immune cells.

module consists of autocrine feedback, where cells express a receptor for a cytokine which they secrete (Fig. 1B). Such feedback exists in the process of T cell activation, where the cytokine IL-2 is secreted upon activation and induces upregulation of the expression level of its own receptor. Other examples for such positive autocrine feedback include IFN- γ in Th1 cells, IL-4 in Th2 cells, and IL-21 in Th17 cells. We aim to better understand the functions of autocrine feedback and its importance

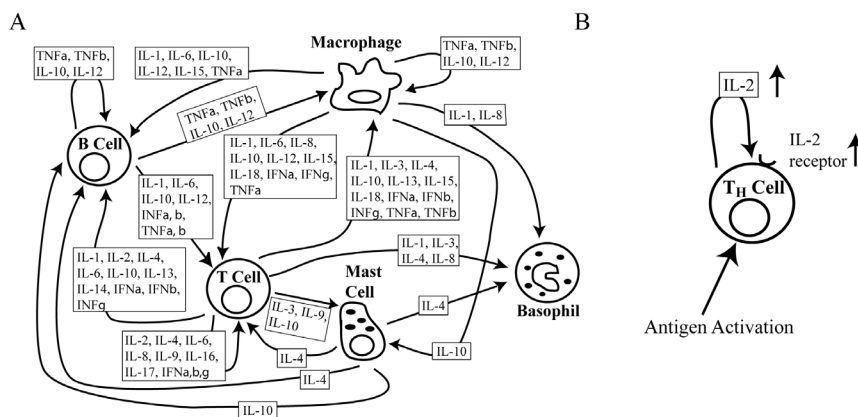


Fig. 1 The cytokine communication network. A) Immune cells communicate via a complex network of cytokines. The diagram is partial, and serves as an illustration (adopted from Biocarta). B) A network module: Autoregulation. Upon activation, T-helper cells secrete IL-2 and up-regulate expression of IL-2 receptor. Further cell proliferation depends on sufficient IL-2 signaling.

The motivation for our research is to obtain a better quantitative understanding of this intercellular communication network. We use a modular approach, characterizing the function of simple network modules that will provide the basic building blocks of the network. Studies at the level of single cells can avoid ambiguities that arise due to heterogeneous responses in cell populations, and provide quantitative data under well-defined conditions, which is necessary for mathematical modeling. We aim to combine quantitative experimental studies and mathematical modeling to gain new insights on the function of these networks.

Model Systems

Currently, we focus on network modules involved in T-cell activation and differentiation. The simplest

in the process of T-cell activation. Some questions of interest include: How does the feedback influence threshold for antigen specificity? Can one cell support its own proliferation, or is a quorum response required? Does feedback affect heterogeneity in a clonal population of cells? Applying mouse genetics, drugs and altered environment settings will serve to characterize the feedback loop and isolate its functions.

A second module of interest is combinatorial signaling: cells can express several cytokine receptors simultaneously, enabling response to combinations of cytokines. Differentiation of CD4⁺ T-cells into Th1/Th2 subsets serves as a paradigm for cell differentiation driven by combinations of external signals. We are quantitatively mapping the response of activated Th cells to various cytokine combinations and time-profiles, and

measure secreted cytokine profiles of cells during the differentiation process. These experiments at the single-cell level are expected to yield detailed information on how input parameters influence regulation and decision-making in this model differentiation process. Specific questions include: Is the response gradual or switch-like (all-or-none)? Is the differentiation process deterministic or random (Instruction vs. selection)? Modeling of this system will include effects of stochasticity in gene expression.

Another area of interest is the study of intercellular interactions in tumor microenvironments and their role in tumor development. In collaboration with Prof. Yinon Ben-Neriah and Dr. Eli Pikarsky from the Hebrew University, we study a mouse model for inflammation induced cancer. Here, the interactions between transformed hepatocytes and tumor-associated macrophages will be investigated at the single-cell level, inside simple microenvironments realized in microfluidics devices.

Methodology

We are developing and applying several techniques in our studies: A) well-defined cellular microenvironments realized inside microfluidics devices; B) ELISA based immunoassay and sensitive fluorescence microscopy for studying responses of single living cells in those devices; C) flow cytometry for high throughput, multi-parameter analysis of secreted cytokines and distributions of cellular parameters; and D) systems biology based mathematical modeling of network function based on experimental observations. The use of

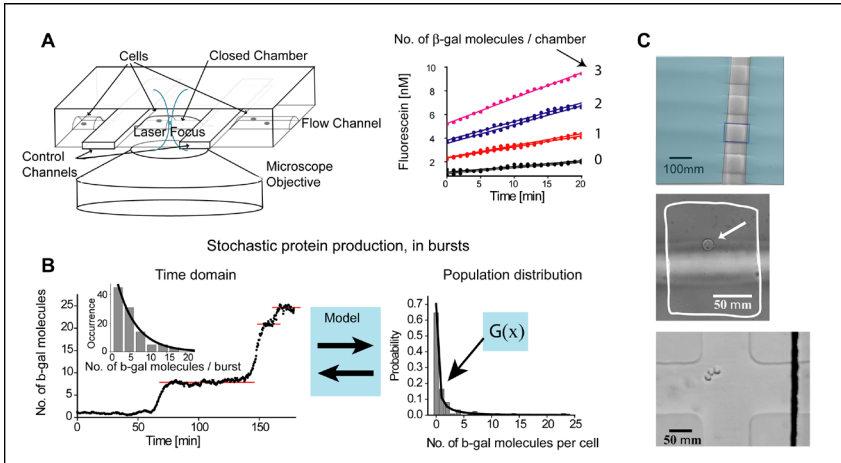


Fig. 2 Detection of protein expression with single-molecule sensitivity A) A two-layers microfluidics device for measuring protein expression in individual cells, with single-molecule sensitivity [Cai, L., et. al., 2006]. Cells containing the reporter β -galactosidase are trapped inside a closed miniature chamber. Right: The increase rate of fluorescent signal is proportional to the number of reporter molecules in the chamber. B) Quantitative measurement of protein production in living *E. coli* cells. Left: Proteins are produced in bursts of random time and amplitude. Inset: the number of protein molecules per burst follows an exponential distribution. Right: The same assay is used to count reporter molecules in a population of cells. The time domain behavior and the population distribution are related with an analytical model [Friedman, N., et. al., 2006] (solid line). C) Microfluidic devices used for cellular assays. Top: an image of the device shown in A, with closed chambers. Middle: An image of a mouse embryonic stem-cell inside one of the chambers of that device. Bottom: Primary activated mouse T-cells inside a microfluidics device.

advanced fluorescent microscopy and microfluidics systems enables studies of single living cells in well defined environments and under controlled interactions. These techniques extend a method we have developed for sensitive detection of gene expression in individual cells.

That method combines trapping of individual cells inside microfluidic chambers and enzymatic amplification to achieve sensitive fluorescence detection. Using this novel method we could study real-time protein expression events in living *E. coli* cells with single-molecule resolution [Cai, L., et. al., 2006]. This allowed us to observe for the first time individual protein production events, which occur in random bursts, and characterize their statistical properties (Figure 2). The method was also used to detect the steady-state population distribution of a protein expressed at a very low level, by counting the number of reporter molecules in individual cells. This was demonstrated in bacteria, yeast and mammalian cells. Our assay opens up possibilities for studying the process of protein expression, its control and dynamics, in single cells of diverse cell types in the unexplored regime of low expression levels. Possible examples include transcription factors involved in gene regulation, or the leakiness of silenced promoters in differentiated cells.

Mathematical modeling

We are applying mathematical modeling and numerical simulations to study simple modules of the cytokine communication network. In accord with experimental work, we are building models of the effects of autocrine feedback, and also of combinatorial cytokine signaling affecting differentiation of T-helper cells. The models will include effects of stochasticity in gene expression, which contribute to heterogeneity in cell populations. Recently, we developed an analytical theory that relates the dynamic random process of protein production in bursts, with variation in protein levels across a population of genetically identical cells [Friedman, N.,

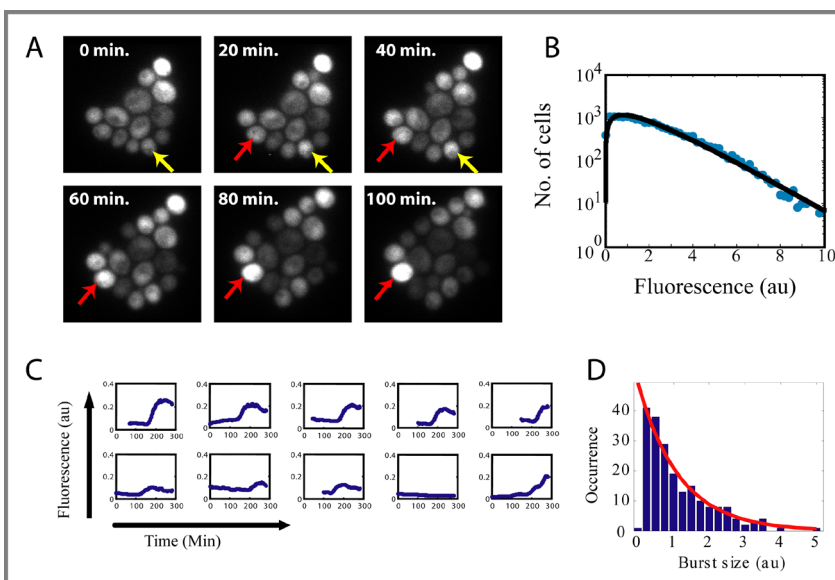


Fig. 3 Random bursts of gene expression in yeast cells. A) Six frames from a movie of yeast cells growing under normal (no stress) conditions. These cells express a fusion of the HSP12 protein to a yellow-fluorescent protein (YFP). Arrows indicate cells that produce the fusion protein in a burst. B) Distribution of fluorescence levels in these cells, measured by flow cytometry, showing a large variability in protein levels in the cell population. Black line is a fit with the analytical model (gamma distribution). C) Traces of YFP fluorescence level as a function of time for 10 cells from the experiment shown in A. Cells produce the protein in bursts with random timing and amplitude. D) Burst size follows an exponential distribution.

et. al., 2006]. This model was extended to analytically describe the shape of the distribution for the cases of negative and positive autoregulation, and the joint distribution of a repressor and a repressed protein (extrinsic noise). Additionally, in collaboration with Prof. Naama Barkai (Weizmann Institute), we applied our model to study how cells control the level of noise. Our results suggest that noise in gene expression contains mechanistic information about the underlying regulation processes, which is not available from the mean response (Figure 3).

Selected publications

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