Chromatin dynamics in IL-4 stochastic expression

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***Short Abstract* — Expression of Interleukin-4 (IL-4) by T-helper lymphocytes exhibits highly stochastic behavior at the single allele level. To elucidate the underlying mechanism, we combined mathematical modeling with experimental measurements of IL-4 biochemical rates. We attributed the probabilistic expression pattern to a chromatin opening step preceding transcription. We experimentally verified the central prediction of the model that IL-4 expressing cells retain an open chromatin state and can be re-activated with increased probability for a limited time. In this system, the amount of IL-4 produced by the population is tuned over a wide range by changing the rate of a stochastic step at the chromatin level.**

***Keywords* — IL-4 stochastic expression, chromatin changes, Th2 maturation, FACS, RT-PCR, ELISA.**

I. Purpose

Type-2 T-helper (Th2) lymphocytes control the adaptive immune response against extracellular pathogens through secretion of Interleukin-4 (IL-4) [1]. Upon antigen-stimulation, a fraction of cells transiently express IL-4. The strength of the immune response is tuned through controlling this fraction. By insertion of a GFP-reporter in one *il-4* allele, previous studies have shown that the probability to express IL-4 is regulated at the chromatin level [2]. Moreover, single-cell measurements have shown that the expression level among IL-4 producing cells varies over two orders of magnitude.

In this study, we combined quantitative measurements with mathematical analysis, to elucidate the mechanisms underlying the stochastic behavior of IL-4. We used a three-step model of the IL-4 expression, accounting for chromatin remodeling, transcription and translation. We analyzed how each of these steps contributes to expression variability.

II. Results

A. Noise does not arise from transcription or translation

To test if the high variability of IL-4 expression arises from low abundance (<100) of mRNA/protein or from transcriptional bursts, we estimated their copy numbers[3]. We used quantitative PCR, intracellular staining combined with flow cytometry, and Enzyme-linked Immunosorbent Assay (ELISA). Activated cells contained >500 mRNA copies (half-life ~1h). This value implies a constant and frequent transcription initiation (~1/second), forbidding a regime of transcriptional bursts. The absolute number of released proteins per cell was in the order of 10^6 per hour. Because of such high mRNA and protein numbers, the contribution of transcriptional and translational random events to the IL-4 variability was assumed negligible.

B. Model predicts a slow chromatin opening step

To understand the mechanisms underlying probabilistic IL-4 expression, we searched for the minimal model that could account for the observed behavior. We proposed that a slow (~hours) chromatin opening step must precede transcription initiation. Bimodality of expression arises because the opening is restricted to a time window of a few hours defined by T cell receptor signaling. The high variability among IL-4 expressing cells is a consequence of their asynchronous gene opening. In contrast to activation, the deactivation of the gene is a rapid process due to loss of transcription factors (~minutes), followed by slow closing of the gene (~days).

C. Experimental verification of short-term-memory

This model predicts that cells expressing IL-4, transiently memorize this event. After transcription is terminated, the chromatin remains open for a limited time (~days). To test this prediction, we purified IL-4 expressing cells by fluorescence-assisted cell sorting. These cells exhibited increased probability to express IL-4 when stimulated again within a few days. The fact that this probability decreases progressively, is due to the slow coming back of the chromatin to its initial closed state.

III. Conclusion

Through combining quantitative measurements with mathematical analysis, we found that the tuning of a single rate (chromatin opening) allows regulation of the amount of IL-4 produced by a population of Th2 cells. Such a mechanism, stochastic at the single cell level, can be used in multi-cellular organisms to tightly control the overall response.

References

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