Intra Microfluidic Calcium Cycling and Balance in T Cells

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***Short Abstract* — Calcium fluctuations are an integral component of T-cell activation and the broader immune response. Measurement of the intracellular levels of Ca in T cells is a nontrivial process due to the complexities of Ca regulation. By careful selection of chemical treatments, the nature of Ca sequestration by the cell after initial release can be elucidated. Once the threshold of Calcium released in the cytosol is reached, Calcium release-activated calcium (CRAC) channels, integral membrane proteins that further allow Ca influx, open initiating the activation cascade.**

***Keywords* — Calcium, T-cells, Modeling, SERCA pump, CRAC channel, microfluidics**

I. Purpose And Methods

Cytosolic free calcium is an important intracellular messenger. Previously in T-cells it has been shown that calcium cycling at different frequencies can alter transcription in specific genes[1]. Calcium signaling contributes to the regulation of numerous cellular processes in various cell types including liver cell, epithelial cells, leukocytes, muscle cells and neurons. Normal calcium levels are maintained by the Sarco/Endoplasmic Reticulum Ca^{2+}(SERCA)-ATPase pump that maintains a 10000 fold gradient across the SR membrane[3]. Understanding of intracellular regulation of calcium and the interplay between regulatory mechanisms, store depletion and sequestration is of great importance.[2]

Microfluidic systems provide many advantages for cellular studies. Calcium signaling in single cells can exhibit unexpected heterogeneity that is not discernable in studies of bulk cell populations or in studies using high-throughput flow cytometry systems. Miniaturization allows for precise control of the cellular microenvironment for microfluidic experiments to investigate calcium flux and the behavior of the CRAC channels. In T cells, the activation pathway can be artificially stimulated via the introduction of ionomycin and calcium into the microfluidic nanophysiometer device. Calcium flux can be viewed with an intracellular calcium dye and the use of a fluorescence microscope.

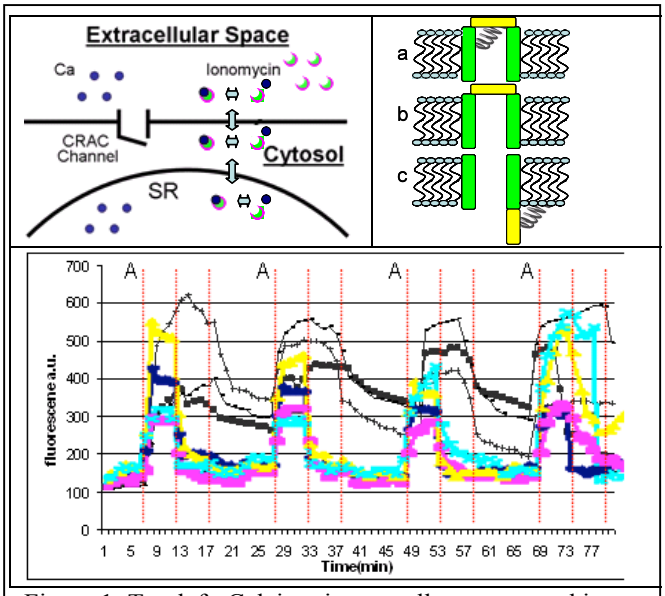

Figure 1: Top left: Calcium is normally sequestered in the SR, but ionomycin stimulates elevated cytosolic Ca causing opening of CRAC channels and flux of Ca into cells. Top Right: Three switch analogs of the CRAC channels. Bottom: cytosolic Ca fluorescence as a function of time with A-B-C, represented in color, and A-C-B, represented as black traces, switching in the multi-trap nanophysiometer vertical dashed line represent switches, A markers denote times after which treatments were common

II. Results

The T cells respond to different stimulation patterns switching between normal media (A) and media enriched with ionomycin (1μM) and calcium (1.8mM) (B) and media enriched with only calcium (C). Significant heterogeneity is observed during the resequestration of calcium when media with ionomycin and calcium is replaced by media with only calcium,. Further differences in the cyclic cell response are noted when the order of media switching is A-B-C versus A-C-B (see figure 1).

III. Conclusion

This experiment has demonstrated controlled calcium transients in single cells which vary from cell to cell and with the order of microenvironment switching. The interplay of the SERCA pump and CRAC channel in calcium release and resequestration can be studied in cells by experimentally varying the timing and sequence of microenvironmental changes in the MTNP.

References

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