

Analysis of STIM1 and Orai1 in distal caps and puncta in activated T cells using FRET, FRAP and FLIP

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Engagement of the T cell antigen receptor (TCR) induces the sustained influx of Ca^{+2} through Ca^{+2} release-activated Ca^{+2} channels. Two key components of this influx have been identified; the channel subunit Orai1 and the Ca^{+2} sensor, stromal interaction molecule-1 (STIM1) [1]. Following TCR engagement, STIM1 and Orai1 colocalize both in puncta near the site of stimulation and in a cap-like structure on the opposite side of the T cell. While Ca^{+2} store depletion is sufficient to induce puncta containing STIM1 and Orai1, activation of the TCR is required for cap formation [2].

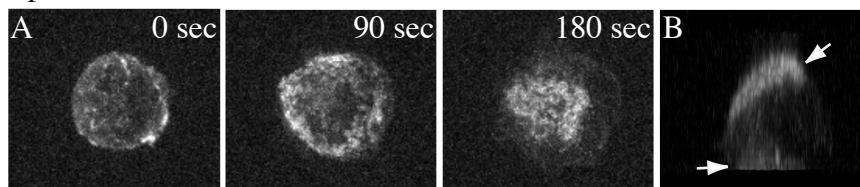


Figure 1: (A) Formation of a STIM1-CFP cap following activation of a Jurkat T cell (B) 3D projection of last timepoint showing cap at top and punta at the contact surface (arrows).

Förster resonance energy transfer (FRET) measurements indicated close interaction of STIM1-CFP and Orai1-YFP in cells fixed at 2 minutes after activation when only puncta were present and in cells fixed 13 minutes later when both caps and puncta were present. There was little interaction between these proteins in unstimulated cells. In cells that contained both puncta and caps, the relative FRET efficiency was consistently higher in the caps than in the puncta. Similar results were obtained in live cells.

Analysis of the recovery of fluorescence after photobleaching (FRAP), showed that the mobility of STIM1-CFP and Orai1-YFP decreased when the proteins were incorporated into a cap. In unstimulated cells, photobleaching of STIM1 in the endoplasmic reticulum was followed by a recovery of about half of the initial fluorescence indicating that 50% of the protein was immobile. STIM1-CFP in caps showed a decreased fluorescent recovery corresponding to an immobile fraction of 75%. Similarly, the immobile fraction of Orai-YFP increased from 40% in unstimulated cells to 60% in caps. When fluorescence loss was induced by repeated photobleaching (FLIP), there was no evidence of transfer of STIM1-CFP between the puncta and caps. Also, there was little exchange of STIM1-CFP molecules within a cap.

Taken together, these data suggest that the distal caps containing STIM1 and Orai1 are tightly packed, stable structures and that there is little movement of molecules within the cap.

[1] M Oh-hora, A Rao, "Calcium signaling in lymphocytes", *Curr Opin Immunol.*, **20**, 250-258 (2008).

[2] VA Barr, KM Bernot, S Srikanth, Y Gwack, L Balagopalan, CK Regan, DJ Helman, CL Sommers M Oh-hora, A Rao, LE Samelson, "Dynamic movement of the calcium sensor STIM1 and the calcium channel Orai1 in activated T cells: Puncta and distal caps", *Mol Biol Cell*, **19**, 2802-2817 (2008).