Spatial Dynamics of Orai1-STIM1 Coupling for Calcium Entry in Antigen-Stimulated Mast Cells

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Abstract. Dynamics of Orai1-GFP diffusion on live mast cell membranes was studied using Total Internal Reflection Fluorescence (TIRF) microscopy. In resting RBL-2H3 cells, most Orai1 are highly mobile. After ER calcium stores release is stimulated by FcεRI crosslinking or thapsigargin, the mobile Orai1 fraction drops significantly. Experiments performed in the presence and absence of extracellular calcium show that the immobilization of Orai1 is strongly linked to the filling state of the store. We developed a whole cell mathematical model of calcium modeling, that includes STIM1 aggregation upon ER depletion, capture of Orai1 by STIM1 tetramers at ER-plasma membrane junctions and the resulting Orai1-mediated calcium influx. The model includes a rule-based representation of the aggregation of calcium-free STIM1, resulting in a highly sensitive ER calcium sensor whose setpoint is not solely based upon the affinity of the EF-hand calcium-binding domain. The model supports the conclusion that the kinetics of Orai1 diffusion/immobilization are in rapid equilibrium with the dynamics of ER depletion and refilling.

***Keywords* — Calcium Modeling; STIM; Orai; Mast cell**

I. Purpose

Symptomatic asthma and allergic responses occur through an antigen-triggered FcεRI pathway. Essential to the mast cell response is the ability to control time, concentration and localization of calcium influx. Allergen challenge initiates a tyrosine kinase cascade that results in activation of phospholipase C, producing Inositol (1,4,5) Trisphosphate (IP3). IP3 then binds to IP3 receptors in the endoplasmic reticulum, mobilizing calcium from these important stores. The second phase of calcium transport is initiated when the ER sensor, STIM1, responds to the drop in luminal ER by aggregating and moving by directed transport to the tips of ER processes that adjoin the plasma membrane.[1,2] A close up of these junctions is shown in Figure 1.[3] STIM1 aggregates form complexes with the tetrameric calcium influx channel, Orai1. Our goal was to develop a whole cell mathematical model of calcium transport, that includes STIM1 aggregation upon ER depletion, capture of Orai1 by STIM1 tetramers at ER-plasma membrane junctions and the stimulation of Orai1-mediated calcium influx. Our goal was to capture the dynamics of this process through quantitative imaging, providing novel parameters sets for mathematical modeling.

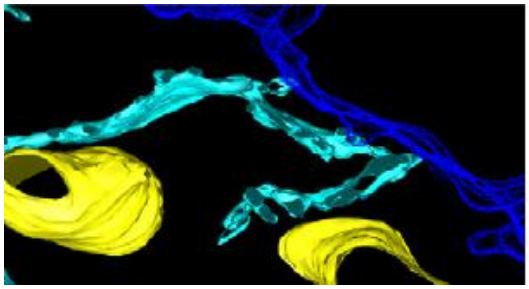

Figure 1. 3D view of the contact between the endoplasmic reticulum (cyan) and the plasma membrane (blue). Mitochondria are shown in yellow. Adapted from ref. 3. Bar = 500 nm

II. RESULTS

We used Total Internal Reflection Fluorescence (TIRF) microscopy to study real time dynamics of Orai1-GFP on the surface of RBL-2H3 cells. In the resting state, ~80% of Orai1 were mobile on the cell surface with an estimated diffusion coefficient of 0.2 ± 0.1 μm^2/sec. The fraction of immobile Orai1-GFP dramatically increased after activation, reaching levels as high as 65 % in cells doubly transfected with Orai1-GFP and STIM1-mOrange. Most Orai1-GFP resumed motion within 5 minutes of antigen addition in the presence of extracellular calcium or within 1 minute of calcium add-back to a calcium free buffer. These parameters have been incorporated into mathematical models of Orai-STIM coupling and calcium transport. Stim1 oligomerization, which is triggered by the loss of calcium from EF-Hand motifs, was evaluated employing the network-free modeling platform NFsim. Results point to the remarkable sensitivity of this ER sensor based upon cooperativity. Results in the whole cell model show that ER stores refilling is rapidly sensed by STIM1, limiting the duration and extent of Orai1-mediated influx.

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III.REFERENCES

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