Reciprocal regulation of expression of STIM1 and Orai1 proteins

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Two proteins, stromal interaction molecule 1 (STIM1) and Orai1 constitute the minimum molecular components of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel (Liou *et al.*, 2005; Roos, *et al.*, 2005; Vig, *et al.*, 2006). STIM1 is predominantly located in the membrane of the endoplasmic reticulum (ER) and functions as a molecular sensor of free ER Ca^{2+}, whereas Orai1 is located on the plasma membrane and when activated by STIM1 forms the Ca^{2+} selective pore of the channel (Yeromin *et al.*, 2006). While activation of CRAC channels uniquely depends on the free Ca^{2+} concentration in the ER lumen, its inactivation is regulated by both the free ER [Ca^{2+}] and the cytosolic [Ca^{2+}]. Fast Ca^{2+}-depend inactivation (FCDI) is a feedback mechanisms which limits Ca^{2+} entry through these channels at negative potentials and is regulated by Ca^{2+} binding to surface composed of residues from both Orai1 and STIM1 (Mullins *et al.*, 2009; Lee *et al.*, 2009). Previously we identified that FCDI of I_{CRAC} depends on the relative expression levels of the STIM1 and Orai1 proteins (Scrimgeour *et al.*, 2009). Herein we present data that suggests the presence of another Ca^{2+}-dependent mechanism which regulates the activity of CRAC channels. Specifically, the expression of STIM1 and Orai1 are interdependent and also [Ca^{2+}]-dependent.

Heterologous expression of STIM1 and Orai1 was conducted in HEK293T cells using the plasmid/DNA vectors pEX-GFP-Myc-Orai1, pCMV-Sport6-STIM1, pCMV-Sport6-Orai1, Sport6-Orai1Δ70-88 and pCIneo-hClC-1 which were co-transfected at different ratios (between 1:8 and 8:1 of Orai1:STIM1) using PolyFect transfection reagent (Qiagen). The relative expression of STIM1 and Orai1-GFP proteins was determined using quantitative western blot analysis using anti-STIM1 and anti-GFP antibodies. GAPDH was used as an internal loading control.

Increasing the amount of Orai1 containing plasmid in the transfection mixture resulted in a significant decrease in STIM1 expression. In contrast, control experiments using expression of either, non-functional Orai1Δ70-88 or the unrelated ClC-1 protein had no effect on the expression levels of STIM1, identifying that the Orai1-STIM1 interaction was not a non-specific effect of competition in co-transfection. Depletion of intracellular Ca^{2+} stores, using thapsigargin, which activates Ca^{2+} entry through CRAC channels, increased the dependence of STIM1 expression on the Orai1. In contrast, inhibition of Ca^{2+} entry by 2-aminoethoxy-diphenyl borate (2-APB) or La^{3+} virtually abolished the interdependence of STIM1 and Orai1 expression.

These data indicate that the expression of STIM1 and Orai1 proteins is interdependent and is regulated in a Ca^{2+}-dependent manner which may provide an important cellular feedback mechanism to enable medium to long term regulation of ER Ca^{2+} homeostasis.

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