CALCIUM SIGNALING

Focus Issue: The ins and outs of ORAI in immune cells

Nancy R. Gough*

This Focus Issue highlights research into cell-specific regulation of store-operated calcium entry through the ORAI/STIM channel complex. Understanding the properties of these channels and how ORAI activity is regulated will lead to a better molecular view of immune cell function and diseases involving the immune system.

Calcium (Ca^{2+}) is a critical mediator of cellular signaling. Cells interpret both global and local Ca^{2+} signals. Furthermore, the pattern and source of the signal also encode information that enables cells to produce different responses to changes in intracellular Ca^{2+} concentrations. Store-operated calcium entry (SOCE) is a local Ca^{2+} signal that occurs at sites where the endoplasmic reticulum (ER) and plasma membrane are juxtaposed. This makes sense because the activating signal for SOCE is depletion of Ca^{2+} from the ER, which triggers a conformational change in and oligomerization of the ER transmembrane protein STIM such that STIM forms complexes with ORAI, resulting in the activation of the ORAI Ca^{2+} channel, the mediator of SOCE (see Wang *et al.*). This SOCE produces a local Ca^{2+} signal, can shape global Ca^{2+} patterns, and serves as a source of Ca^{2+} for refilling the ER store.

Immune cells rely on the Ca^{2+} signal produced by SOCE to control diverse functions, depending on the type of immune cell. As Saul *et al.* show in this issue, monocytes (phagocytic cells that are in the circulation and become macrophages when they enter tissues in response to injury or infection) rely on ORAI/STIM-mediated SOCE to stimulate a massive production of reactive oxygen species (ROS), called the oxidative burst, which is important for the ability of these cells to kill pathogens. ORAI channels are made up of six subunits, which can be all of one isoform (homomeric) or a mixture of isoforms (heteromeric). Bogeski *et al.* found that ORAI channels composed of only ORAI1 subunits are inactivated by ROS, but ORAI channels containing a single ORAI3 subunit in the hexamer are resistant to redox-mediated inactivation. Furthermore, as naïve human T lymphocytes differentiated into effector T lymphocytes, the abundance of mRNA encoding the ROS-insensitive ORAI3 protein increased, and redox-mediated inhibition of SOCE decreased. Saul *et al.* explored how monocytes maintain SOCE despite this oxidative stress and found that monocytes shift the ratio of ORAI3 to ORAI1 subunits so that more channels resistant to redox-mediated inactivation are present in response to encountering bacterial peptides or bacteria. Thus, effector T cells and monocytes rely on regulating the subunit composition to produce the optimal SOCE signal.

Macrophages contribute to various chronic inflammatory diseases, including inflammation associated with obesity. Velmurugan *et al.* found that macrophages in the adipose tissue of obese mice had decreased amounts of the gaseous signaling molecule hydrogen sulfide (H_2S) compared with macrophages in the adipose tissue of lean mice. Furthermore, unlike ORAI1, which is inhibited by ROS, ORAI3 was inhibited by H_2S, which reduced calcium entry into macrophages. Thus, in the obese condition, macrophages in the adipose tissue have less of this inhibitory regulatory gas and, consequently, exhibit increased SOCE and production of inflammatory cytokines.

Whereas monocytes enter tissues to become macrophages, mast cells travel from the bone marrow through the circulation and enter tissues where they respond to antigens by releasing inflammatory mediators through a process called degranulation. Derler *et al.* examined the regulation of ORAI/STIM-mediated SOCE in mast cell degranulation (see Hooper *et al.*). They identified that ORAI1 bound to cholesterol, which reduced Ca^{2+} influx through the channel. These results provide a potential molecular explanation for the increased degranulation of mast cells from patients with Smith-Lemli-Opitz syndrome, a type of hypocholesterolemia.

Many immune cells migrate throughout the body, experiencing different concentrations of Ca^{2+}, depending on the environment. In the circulation, Ca^{2+} concentrations tend to be higher than those in the tissues, and regions of tissues closer to the vasculature tend to be higher in Ca^{2+} compared with regions that are farther away. This variation in extracellular Ca^{2+} concentrations presents challenges for a Ca^{2+}-conducting channel. Frischauf *et al.* generated a three-dimensional model of human ORAI1, using the crystal structure of the *Drosophila* Orai channel as a starting point (see Jha and Muallem), and then performed molecular dynamics simulations to understand ion interactions and channel dynamics. This computational approach revealed the presence of a region in the extracellular opening of the channel that bound Ca^{2+}, effectively increasing the local Ca^{2+} concentration above the pore. The authors called this region the CAR (calcium-accumulating region). Experiments with a cultured mast cell line showed that a functional CAR was needed for these cells to activate the transcription factor NFAT under conditions of low extracellular Ca^{2+}. However, mast cells also travel through the circulation, where the Ca^{2+} concentration is high. Compromised NFAT activation also occurred in melanoma cells, which are normally found in the basal epidermal layer, when an essential residue in the CAR was mutated.

Among the three ORAI forms in humans, ORAI1 is posttranslationally modified by N-glycosylation, a modification that can affect protein function and protein-protein interactions. Indeed, entire families of carbohydrate-binding proteins, called lectins, exist that recognize specific glycosylation patterns on their glycosylated protein or lipid partners. Siglecs are one such family of lectins that specifically recognize sulfated glycans, and several of these proteins inhibit immune cell function. Dörr *et al.* examined the cell-specific effect of glycosylation of ORAI1 on SOCE in primary $CD4^+$ and $CD8^+$ T cells and several immune cell lines. Their results showed that the patterns of glycosylation were different among the cells. For all of the immune cells examined, N-glycosylation reduced ORAI1/STIM-mediated SOCE. Blocking the sulfation of glycans, and thus their ability to interact with members of the Siglec family, had different effects in the Jurkat T cell line and a mast cell line. Whereas blocking sulfation reduced SOCE in the Jurkat cells; it increased SOCE in the mast cell line, which also exhibited

Editor, Science Signaling, AAAS, Washington, DC 20005, USA.

***Corresponding author. E-mail: ngough@aaas.org**

increased SOCE when Siglec-8 was knocked down. Altered protein glycosylation can affect immune cell function and occurs in cancer; thus, the work of Dörr *et al*. has implications for several human diseases.

ORAI1 is also distinct in that the gene encoding this protein in mammals can produce long and short forms through the use of alternative translation initiation start sites. Desai *et al.* showed that these long and short forms produce Ca^{2+} channels with distinct properties. Both forms generated ORAI/STIM complexes that mediate SOCE, but only the long form contributes to a channel that is activated by arachidonic acid and leukotriene C_4, lipids that promote inflammation. Although this study was not performed with immune cells, the data suggest that altered SOCE may not be the only effect of knocking out *Orai1* in mice or knocking down ORAI1 in cells.

These studies reveal that, although the molecular composition of the SOCE-mediating channel may be known, regulatory mechanisms controlling ORAI/STIM complexes and the cell-specific complexity in channel composition and posttranslational modifications continue to emerge. Knowledge of how these channels control Ca^{2+} signals will lead to a better molecular understanding of immune cell function and pathology, which will enable the development of strategies to either enhance or inhibit immune responses.

Related Resources

Research Articles

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