# Store-operated calcium entry in disease: Beyond STIM/Orai expression levels

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**CRAC channels, Orai regulatory proteins, pH, glycosylation**

**Abstract**

Precise intracellular calcium signaling is crucial to numerous cellular functions. In non-excitable cells, store-operated calcium entry (SOCE) is a key step in the generation of intracellular calcium signals. Tight regulation of SOCE is important, and dysregulation is involved in several pathophysiological cellular malfunctions. The current underlying SOCE, calcium release-activated calcium current ($I_{CRAC}$), was first discovered almost three decades ago. Since its discovery, the molecular components of $I_{CRAC}$, Orai1 and stromal interaction molecule 1 (STIM1), have been extensively investigated. Several regulatory mechanisms and proteins contribute to alterations in SOCE and cellular malfunctions in cancer, immune and neurodegenerative diseases, inflammation, and neuronal disorders. This review summarizes these regulatory mechanisms, including glycosylation, pH sensing, and the regulatory proteins golli, α-SNAP, SARAF, ORMDL3, CRACR2A, and TRPM4 channels.

## 1. Introduction

One central nodal point in cell signaling is the production of the second messenger inositol 1,4,5-trisphosphate ($IP_3$) due to a broad range of membrane receptor stimuli [1]. Upon binding of $IP_3$, $IP_3$ receptors localized in the membrane of intracellular $Ca^{2+}$ stores, the endoplasmic reticulum (ER), release $Ca^{2+}$. The decrease in ER luminal $Ca^{2+}$ results in clustering of stromal interaction molecule 1 (STIM1) $Ca^{2+}$ sensor proteins and Orai1 $Ca^{2+}$ channel recruitment. Upon activation, Orai1 channels mediate store-operated $Ca^{2+}$ entry (SOCE), contributing to the spatiotemporal shape of intracellular $Ca^{2+}$ signaling. In addition to STIM1 and Orai1, STIM2, Orai2, and Orai3 contribute to intracellular $Ca^{2+}$ signaling and alter the SOCE response in different ways. Each Orai subunit has four plasma membrane-spanning helices, an intracellular N- and C-terminus, and two extracellular loops. $Ca^{2+}$ release activated $Ca^{2+}$ (CRAC) channels are presumably formed by six Orai subunits, and CRAC channel composition adds to different characteristics of SOCE (all reviewed in [2]).

SOCE signaling controls numerous cellular functions, including migration, proliferation, gene expression, and apoptosis [3], as well as very specific cellular responses, such as activation of T cells during an immune response [4,5]. Several groups have shown that SOCE is altered in different types of cancer and immune disease [6–8]. In addition, SOCE malfunction causes or adds to pathophysiological cellular functions, such as enhanced migration and the inability to undergo apoptosis in cancer cells [9,10]. Dysregulation of STIM and Orai protein expression levels contribute to different pathophysiological conditions and are described in corresponding reviews and this special feature.

Besides expression levels, numerous regulatory mechanisms alter $Ca^{2+}$ signaling within the SOCE pathway. Intracellular $Ca^{2+}$ is an important negative feedback regulator for SOCE and mitochondria play an important role in buffering intracellular $Ca^{2+}$ via $Ca^{2+}$ uptake by mitochondrial $Ca^{2+}$ uniporters (MCU) [11]. Moreover, posttranslational modifications including glycosylation [12], phosphorylation [13,14] and nitrosylation [15] regulate SOCE. Inhibition of SOCE by microRNA, targeting components responsible for T-cell fate, including Orai3 and STIM1, has recently been reported [16]. In addition, membrane trafficking and alternative splicing of SOCE's molecular components have been reported to regulate SOCE [17–20]. Several models for transient receptor potential canonical (TRPC) channels within the SOCE pathway have been proposed including TRPC as SOC channel, as interaction partners for STIM1, as membrane anchors or via depolarization of the membrane potential [21].

This review focusses on regulatory mechanisms of SOCE that contribute to cellular malfunction and disease. Increased reactive oxygen species are under investigation in the context of impaired SOCE and diseases [22–24], but were recently reviewed [25–27]. Thus, we here focus on altered glycosylation of Orai1 and regulation of SOCE by pH and, several regulatory proteins within the STIM/Orai signaling pathway.

## 2. Regulation of the STIM/Orai machinery

### 2.1. Glycosylation

N-linked glycosylation has previously been reported to modify ion channel function by, for example, affecting ion channel subunit multimerization or ion transport of transient receptor potential (TRP) and voltage-gated channels [28,29]. STIM1 is a core-glycosylated protein located in the ER membrane. Glycosylation of STIM1 seems to change STIM1 oligomerization rates [30], and enhance STIM1-mediated SOCE via structural changes [31]. Here, we focus on complex glycosylation of Orai1, as alterations of complex glycosylation observed in aging, cancer, and immune disease can impair plasma membrane protein function [32–34].

#### 2.1.1. Absence of Orai1's glycan

Orai1 is glycosylated at asparagine 223 (N223) in its second extracellular loop. Gwack et al. demonstrated that the glycodeficient Orai1 mutant Orai1N223A, in which the glycosylated asparagine is replaced with alanine, can restore SOCE in fibroblasts from patients with severe combined immunodeficiency initially caused by a mutation in Orai1. In these cells, SOCE is elevated when reconstituted with Orai1N223A compared to Orai1 wild type [35]. Each of the six Orai1 subunits forming the functional store-operated channel is N-glycosylated at asparagine 223, and the glycosylation pattern of Orai1 strongly depends on the cell type [12]. In T cells, expression of Orai1N223A increased the store-operated $Ca^{2+}$ entry rate by approximately 40%, and treatment with the antibiotic tunicamycin that removes N-glycosylation from all proteins amplifies SOCE by up to 100%. Comparing SOCE elevation upon tunicamycin treatment, when either Orai1 or Orai1N223A is expressed, demonstrates that the main part of the tunicamycin-induced enhancement is Orai1-specific. This is in very good agreement with data from a glycodeficient $CHO_{Lec}$ cell line generated by P. Stanley [36]. This cell line lacks several glycosyltransferases, and glycosylation of Orai1 is almost completely aberrant. In $CHO_{Lec}$ cells, expression of Orai1N223A does not increase SOCE or the underlying $Ca^{2+}$release-activated calcium current ($I_{CRAC}$) compared to Orai1 [12].

#### 2.1.2. Alteration of Orai1's glycan by β-galactoside α-2,6-sialyltransferase 1 in human T cells

β-Galactoside α-2,6-sialyltransferase 1 (ST6GAL1) is elevated in different types of cancer and is associated with cancer invasiveness, metastatic spread, and multidrug resistance, as well as differentiation of dendritic cells, endocytosis, and protection of cells from tumor necrosis factor 1 (TNF1)– or Fas receptor–mediated apoptosis [37,38]. ST6GAL1 adds α-2,6-linked sialic acids to the glycan of Orai1 [12]. Knockdown of ST6GAL1 in Jurkat T cells reduces α-2,6-linked sialic end structures in the glycan of Orai1 and increases Orai1-specific SOCE. These alterations are less dramatic compared to the expression of Orai1N223A and may be due to smaller changes in the glycan, i.e., loss of sialic acid end structures compared to complete removal of N-glycosylation, or residual expression of ST6GAL1 upon siRNA-based knockdown, as knockdown efficiency was approximately 80%. Future

investigations may show whether pathophysiological glycosylation of Orai1 adds to cellular malfunctions in cancer and immune disease associated with ST6Gal1 dysregulation.

### 2.1.3. Alteration of Orai1 by glycan-binding protein Siglec-8 in human mast cells

Three different types of glycan-binding proteins can bind to and alter the function of glycosylated plasma membrane proteins: soluble galactines, selectines (cell adhesion molecules), and sialic acid-binding immunoglobulin-type lectins (Siglecs) [34]. Siglecs bind to specific end structures in the glycan of surface proteins including α-2,6 sialic acids and, in most cases, diminish intracellular signaling via their cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs).

Though human T cells exhibit low or no detectable expression of Siglecs, human mast cells express Siglec-8, among other family members [12]. Siglec-8 binds to sulfated glycans and α-2,6-linked sialic acids. Siglec-8 is of special interest because its role has been investigated in mast cell- and eosinophil-associated disease [39], especially as selective targeting of the Siglec-8 endocytic pathway leads to apoptosis specific to malignant mast cells and eosinophils [40]. Interestingly, Orai channels are being investigated as therapeutic targets in mast cell-related diseases, including asthma, nasal polyposis, and house dust allergy [41–44]. Desulfation of Orai1 results in increased Orai1-specific $Ca^{2+}$ signaling, and downregulation of Siglec-8 slightly but significantly increases SOCE [12], pointing to a regulatory role for Siglec-8 within the SOCE pathway. Thus, targeting Siglec-8 may very well alter SOCE signaling, and SOCE signaling may be unleashed, contributing to apoptosis of targeted cells.

### 2.1.4. Effects of glycosylation on Orai1 in different cell types

Figure 1 summarizes these findings on Orai1 glycosylation in human T cells and mast cells. ST6GAL1 decreases $Ca^{2+}$ signaling via Orai1 by adding α-2,6 sialic acids to the glycan of Orai1 in human T cells. In addition, Siglec-8 may regulate Orai1 in mast cells. However, further investigations are needed to understand the mechanism underlying the tuning of Orai1 by N-linked glycosylation, as well as the physiology and pathophysiology of Orai1 modification by its glycan, galectins, selectins, and/or Siglecs.

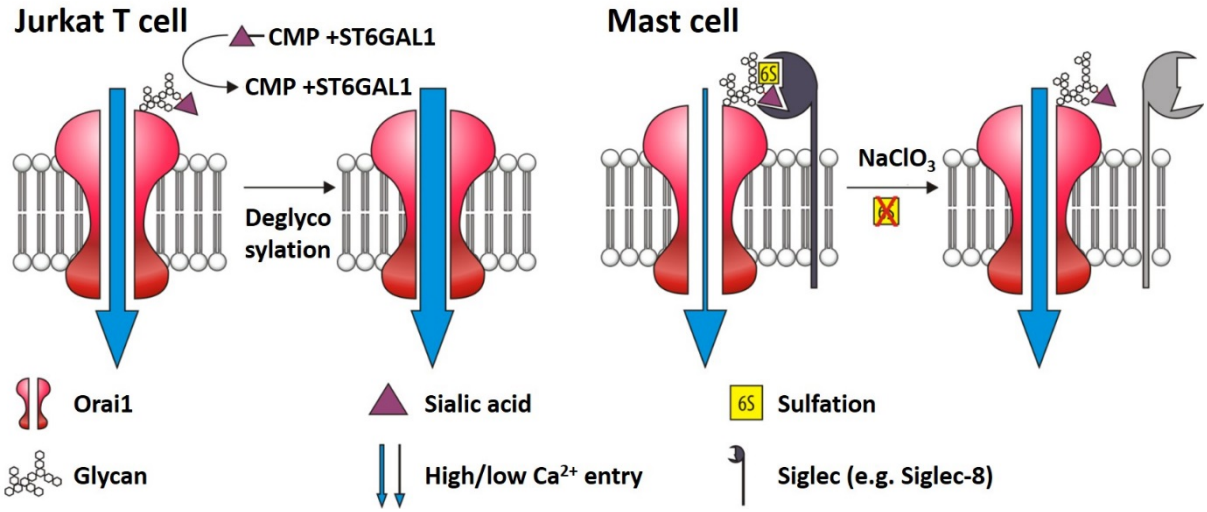

**Figure 1**: In T cells, ST6Gal1 adds α-2,6-linked sialic acid to the glycan of Orai1, resulting in reduced $Ca^{2+}$ entry via Orai1. In mast cells, both desulfation or down-regulation of Siglec-8 lead to elevated $Ca^{2+}$ signaling. This working model suggests that Siglec-8 directly binds to the sulfated glycan of Orai1. By removing either sulfation or Siglec-8, a direct interaction between Siglec-8 and Orai1 can be impaired and $Ca^{2+}$ signaling elevated.

### 2.2. Acidosis

Acidosis in tissues and blood is caused by different physiological and pathophysiological conditions. During inflammation, metabolic changes can acidify inflamed tissue and impact immune cell responses [45]. Acidosis plays an important role in the tumor microenvironment, contributing to tumor cell migration, metastasis, tumorigenesis, invasion [46], and tumor immunity, suggesting new therapeutic strategies targeting cancer [47,48]. $I_{CRAC}$ from both endogenous and heterologous expression is blocked at low extracellular pH [49]. Consequently, this possibly results in impaired SOCE in inflammation and cancer with several pathophysiological consequences.

#### 2.2.1. Amino acids involved in pH sensing

$I_{CRAC}$ is blocked when the extracellular pH is acidic based on several pH-sensing amino acids in STIM and Orai1 molecules. The glutamine residue at position 106 (Orai1E106) has been identified as the key residue for $I_{CRAC}$ selectivity [2] and is localized in the extracellular opening of the CRAC channel pore [50]. When the glutamic acid 106 is exchanged with aspartate, the pH-dependence of Orai1 is reversed. Low extracellular pH results in increased $I_{CRAC}$, as E106 acts as a pH sensor for Orai1 [51]. In addition, mutation of two aspartate residues to uncharged alanines (Orai1D110/112A) in the first extracellular loop of Orai1 reduces the inhibition of $I_{CRAC}$ at low extracellular pH [49]. Mutations of each of these residues to asparagine (Orai1D110N and Orai1D112/114N) does not result in a changed pH phenotype compared to wild type Orai1 [52]. Glutamic acid in position E190 confers pH sensitivity of the $Na^+$ current via CRAC channels in the absence of divalent ions [52,53]. Inhibition of $I_{CRAC}$ by internal pH can be caused by uncoupling of the STIM1/Orai1 complex under hypoxic conditions [54]. In addition, several residues in Orai1 [52,55] and STIM1 [55] have been identified as sensors for internal pH, including H155 on Orai1 and D475, D476, E482, E483 on STIM1. These residues may be involved in SOCE activation upon depletion of acidic dense granules found in human platelets [56].

#### 2.2.2. Pathophysiological implications of SOCE in acidosis

Many physiological and pathophysiological processes have been described as acidosis-dependent due to inhibition of SOCE, including blood platelet aggregation [57], endothelial dysfunction [58], and P2Y receptor-mediated $Ca^{2+}$ signaling [59]. During lactate acidosis, a low pH may diminish $Ca^{2+}$ refilling of the sarcoplasmic reticulum in skeletal muscle fibers [60]. SOCE controls ameloblast cell function and enamel development [61–63]. Signaling pathways induced by high pH produced by dental materials,

such as calcium hydroxide, involves SOCE in odontoblasts [64]. Orai channels differentially regulate NFAT signaling [65]. Recently, by mutation of D110 in Orai1 to alanine, Schindl's group showed the involvement of D110 in the $Ca^{2+}$-accumulating region (CAR) of Orai1 in NFAT-dependent gene expression [66]. Remarkably, NFAT signaling depends on acidosis [67], and further investigations may show whether this dependence is mediated, at least in part, via Orai channels.

The pathophysiological implications of endogenous CRAC channels in the acidic tumor microenvironment are less clear. Acidosis promotes migration via the production of metalloproteases, as has been shown for MMP-9 in a Lewis lung carcinoma model [68]. On the other hand, blocking of Orai1 reduces MMP-9 in the plasma membrane of melanoma cells, reducing the degradation of the extracellular matrix [69]. These findings may contradict a potential role for Orai1 in promoting migration in an acidic environment. One explanation could be that Orai proteins form heteromeric CRAC channels [23,24,70,71] and heteromeric CRAC channels are differentially regulated. The ratio of Orai1 to Orai3 in heteromeric CRAC channels determines the properties of $I_{CRAC}$ such as the pharmacological profile upon application of 2-APB [71,72] and the ROS induced block [23,24]. In consequence, heteromeric CRAC channels may be differentially regulated by an acidic tumor environment and further studies are needed to show the role of acidosis in CRAC channel regulation.

For immune conditions, one may also speculate that, though T cell immune function including the production of interferon-γ (IFN- γ) strongly depends on Orai1 [73], buffering of tumor acidosis leads to a rescue of IFN- γ and could involve rescue of SOCE activity. However, this effect may be restricted to natural killer cells [74]. In summary, regulation of SOCE channel activity is involved in several pathophysiological conditions that involve changes in pH. Nevertheless, further investigations will contribute to understanding the detailed role of SOCE channels under acidic conditions.

### 2.3. Modification by proteins

Recently, an increasing number of key elements were reported to play essential roles in the regulation of Orai1 and STIM1 and the process of SOCE. Here, we focus on golli proteins, SARAF, α-SNAP, ORMDL3, septins, CRACR2A, TRPC, and TRPM4, a few of the most important modifiers of SOCE, and their relevance in human diseases (summarized in Fig. 2).

#### 2.3.1. Golli

Golli protein, an isoform of myelin basic protein (MBP), was the first protein to be described as a negative regulator of SOCE [75]. The MBP gene generates two families of proteins via its three transcription start sites. The first transcript produces golli protein, whereas the second and third sites generate the classic MBPs. Golli proteins are expressed in various human tissues and are abundantly expressed in the immune system, as well as the brain [76,77]. Golli has been linked to multiple sclerosis [78], and a polymorphism in the golli-MBP gene is associated with lymphoma in humans [79]. Golli

proteins negatively regulate T cell activation and modulate the induction of experimental autoimmune encephalomyelitis (EAE) [77]. In addition to their role in T cell activation, golli proteins have been shown to directly interact with STIM1 and negatively regulate SOCE [75,80]. T cells from golli-deficient mice have been reported to exhibit increased $Ca^{2+}$ influx compared to wild type control cells. In addition, SOCE has been shown to be inhibited in Jurkat T cells after golli overexpression [75]. Thus, golli exhibits negative regulatory function on SOCE activity. Moreover, following an *in vitro* binding assay, golli proteins were confirmed to interact with the C-terminal domain of STIM1. However, no interaction with Orai1 has been reported [80].

In a recent study, golli expression levels were shown to correlate with oral lichen planus (OLP), a T cell-mediated chronic inflammatory oral mucosal disease. Both golli and STIM1 mRNA expression were reported to be significantly elevated in OLP patients compared to healthy controls. In addition, the intracellular $Ca^{2+}$ levels were lower in OLP patients than the control group, indicating a potential role of golli and STIM1 in the pathogenesis of OLP [81].

#### 2.3.2. SARAF

SOCE-associated regulatory factor (SARAF) is an ER membrane-bound protein recently described to interact with STIM1, thereby preventing STIM1 clustering and spontaneous activation [82]. SARAF was previously considered to be an ER-resident protein only, but a recent study reported that SARAF is also present in the plasma membrane of SH-SY5Y neuroblastoma cells [83].

Jha et al. showed that the C-terminal inhibitory domain (CTID) of STIM1 is required for interaction with SARAF [84]. The cytosolic region of SARAF is responsible for the STIM1-Orai1 interaction and is needed for the regulation of SOCE [82]. Upon store depletion, SARAF disassociates from the STIM-Orai activating region, thereby allowing STIM1 to interact with Orai1, leading to full activation and SOCE. A truncated version of STIM1 lacking the CTID was reported to lead to spontaneous clustering of STIM1, leading to full activation of Orai1 independent of store depletion and increased intracellular $Ca^{2+}$. Under resting conditions, SARAF binds to the STIM1-Orai-activating region (SOAR) and inhibits spontaneous activation of Orai1 by STIM1 [84]. More recently, SARAF was reported to transiently interact with Orai1 in cells with low STIM1 expression. This interaction was shown to activate Orai1 upon stimulation with the physiological agonist ATP [85]. As intrinsic inhibitor of STIM1/Orai1 signaling pathway, SARAF could prevent cardiac hypertrophy that is mediated via SOCE signaling [86].

#### 2.3.3. α-SNAP

Soluble N-ethylmaleimide-Sensitive Factor (NSF) attachment protein (SNAP) was first reported to be involved in SOCE in *Drosophila melanogaster* kc cells. SNAP is ubiquitously expressed [87], and alterations in its expression have been reported in cancer, type 2 diabetes, and neurological disorders

[88]. In knockdown experiments in *Drosophila* kc cells using double-stranded RNA sequences specific for SNAP, Miao et al. recorded strongly reduced SOCE. In a follow-up experiment in which the mammalian version of SNAP, α-SNAP and β-SNAP, was silenced using a lentiviral RNAi technique, HEK293, U2SOS, and Jurkat T cells exhibited strongly reduced SOCE activity compared to control cells. α- and β-SNAP, but not γ-SNAP, overexpression were able to rescue the decrease in SOCE recorded during the knockout experiments. Moreover, α-SNAP was shown to bind and interact directly with STIM1 and Orai1. α-SNAP co-localizes with Orai1 and STIM1 at ER-PM junctions. Importantly, STIM1-Orai clustering still occurs without α-SNAP. However, through the interaction within the CRAC channel cluster, α-SNAP enables the optimal STIM1:Orai1 ratio needed for SOCE activation [87]. Recent work suggests that changes in intracellular $Ca^{2+}$ signaling are linked to secretion of signal transducer of activators of transcription (STAT)-activating cytokines by α-Snap and NSF [89], a central pathway in immunity, associated with a plethora of cellular malfunctions [90].

#### 2.3.4. ORMDL3

Orosomucoid-like 3 (ORMDL3) is a member of the *ORMDL* gene family ubiquitously expressed in human fetal and adult tissue, encoding transmembrane proteins anchored in the ER [91]. Protein homologs of ORMDL in yeast (Orm) have been identified as homeostatic regulators of sphingolipid metabolism [92]. In addition to the structural importance of sphingolipids, the biosynthetic intermediates ceramide and sphingosine and their phosphorylated derivatives represent signaling molecules that are crucial in physiological and pathophysiological processes [93,94]. Therefore, tight regulation of sphingolipids is needed. Single nucleotide polymorphisms (SNPs) near *ORMDL3* have been linked to Crohn's disease [95], ulcerative colitis [96], type I diabetes [97], and primary biliary cirrhosis [98]. Furthermore, genetic variants controlling ORMDL3 expression are strongly linked to childhood asthma [99,100].

Though it is unable to interact directly, ORDML3 co-localizes with STIM1 in unstimulated cells and moves together with STIM1 into punctae upon activation of Jurkat T cells [101]. Moreover, an interaction between ORDML3 and SERCA2b has been reported and overexpression reported to alter ER calcium homeostasis and the unfolded protein response (UPR), which is considered an endogenous inducer of inflammation [102]. Overexpression of ORMDL3 impairs mitochondrial calcium uptake, increasing slow calcium-dependent inactivation of CRAC currents and reducing store-operated calcium entry. These results linking the genetic associations of *ORMDL3* with inflammatory diseases to ORMDL3-mediated changes in calcium signaling [101].

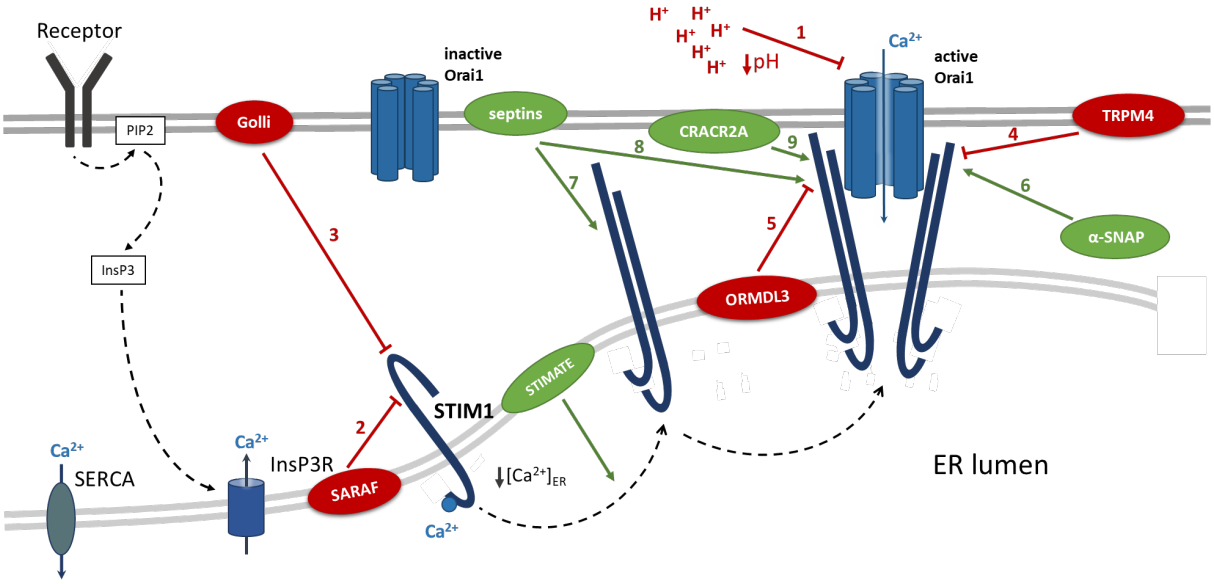


**Figure 2:** Overview of key players in SOCE regulation. Negative regulators (red ellipses): $I_{CRAC}$ is blocked by the decrease in extracellular pH (1). SARAF is an ER membrane-bound protein shown to interact with STIM1, thereby preventing it from clustering and activation (2). Golli proteins have been shown to reduce SOCE activity and to interact with the STIM1 C-terminus (3). TRPM4 negatively regulates SOCE by conducting $Na^+$ ions and increasing the positive charge in the intracellular space (4). ORMDL3 negatively modulates $I_{CRAC}$ and SOCE by enhancing slow $Ca^{2+}$-dependent inhibition (5). Positive regulators (green ellipses): α-SNAP co-localizes with Orai1 and STIM1 in the ER-PM junctions and enables the optimal STIM1:Orai1 ratio needed for SOCE activation (6). Septins have been shown to facilitate STIM1 targeting to the ER-PM junctions, leading to stable recruitment of Orai1 (7) and formation of a lipid microdomain that stabilizes the STIM-Orai complex (8). CRACR2A directly interacts with Orai1 and STIM1, facilitating the clustering of Orai1 and STIM1 at the ER-PM junctions (9). STIMATE regulates STIM1 transition [103,104]. We added STIMATE here to give a comprehensive overview. STIMATE has not been reported to be involved in the context of SOCE and pathophysiology.

### 2.3.5. Septins

Septins have been reported to maintain diffusion barriers, controlling protein localization and modulating exocytic membrane fusion [105–107]. Belonging to the same class of GTPases as the *RAS* oncogenes [108], the expression of septin is altered in cancer [109], including leukemia [110], epithelial carcinoma [111–113], melanoma [114], and glioma [115]. They have also been associated with neurodegenerative diseases [116–119], mental disorders [120], male infertility [121], and blood [122] and neuromuscular disorders [123].

Recently, Sharma et al. found septins to be coordinators of store-operated entry. Depletion of septin 2, 4,and 5 by siRNA decreased nuclear translocation of NFAT, a process activated by sustained calcium

signals via Orai1 [124–126]. Septins have been shown to bind phosphoinositides in the plasma membrane [127,128], thereby facilitating STIM1 targeting to ER-PM junctions and leading to stable recruitment of Orai1. In addition, septins form a lipid microdomain that correlates with the stability of the STIM-Orai complex [124]. In drosophila, septin 7 expression levels alter neuronal circuit function and SOCE, however, further investigation is needed to link altered septin 7 expression to impaired SOCE in human neurodegenerative diseases [129,130].

#### 2.3.6. CRACR2A

Links between polymorphisms in the CRAC channel regulator 2A gene (*CRACR2A*) and human diseases, including chronic HIV type 1 infection, have been made by various genome-wide association studies [131–133]. CRACR2A is a large GTPase that belongs to the Rab family. In T cells, two isoforms of CRACR2A are expressed. The short isoform, CRACR2A-c, interacts directly with Orai1 and STIM1, thereby facilitating the clustering of Orai1 and STIM1 at PM-ER junctions [134]. In addition to the N-terminal CRAC-regulating domain of CRACR2A-c, the large isoform CRACR2A-a contains a proline-rich domain and a C-terminal GTPase domain. CRACR2A-a is located at the proximal Golgi area and in vesicles associated with the trans-Golgi network, in contrast to the cytosolic short isoform [135]. Mechanistically, both isoforms act as a signal transmitter between stimulation of the T cell receptor (TCR) and the $Ca^{2+}$/NFAT pathway, whereas the contribution to the JNK/AP1 signaling pathway is exclusive to CRACR2A-a [136,137]. As the strength of the TCR signals is crucial in T cell fate determination, strong TCR signaling induces preferential differentiation of Th1 cells, whereas weak signals favor differentiation to Th2 cells [137]. CRACR2A-mediated signaling has been reported to play a key role in Th1 cell differentiation. In addition, CRACR2A deficiency impairs T cell transition into pathogenic Th17 cells in EAE [137]. This is of particular interest because CRACR2A-a is degraded by statins [135], which are used to suppress autoimmune diseases of the central nervous system [138,139]. Accordingly, CRACR2A-a may serve as a target for novel drugs with fewer side effects [137].

#### 2.3.7. TRPM4

Transient receptor potential melastatin 4 channel (TRPM4) is a calcium-activated monovalent cation channel [140,141]. TRPM4 plays an important role as a negative regulator of SOCE. TRPM4-mediated $Na^+$ conductance leads to the accumulation of positive charges inside the cell, depolarizing the membrane potential and reducing the driving force for SOCE. TRPM4 is widely expressed in a variety of tissues, though its expression is pronounced in prostate, colon, and heart tissue [142,143]. TRPM4 has been linked to a variety of human diseases and pathological and physiological processes. For example, TRPM4 plays a central role in neuronal degeneration in multiple sclerosis [144] and in several cardiac conduction disorders [145–148]. TRPM4 is associated with poor outcome in B cell lymphoma [149] and promotes cervical cancer cell proliferation [150]. TRPM4 was described as a cancer-driver

gene [151] in androgen independent prostate cancer cells and contributes to proliferation, migration and invasion of prostate cancer cells [152–155]. While in DU145 prostate cancer cells knockdown of TRPM4 increases SOCE, in PC3 prostate cancer cells TRPM4 depletion does not impair SOCE. Further studies using specific small molecule inhibitors [156] will reveal the underlying mechanism for cellular malfunctions: $Na^+$ conductivity, negative feedback regulation of SOCE or other mechanisms of action that have been described such as localization in the focal adhesome or interaction with SUR-1 [157–159].

**Conclusions**

Though SOCE strongly depends on the expression of STIM and Orai proteins, regulatory mechanisms, including glycosylation of Orai1, low pH in acidosis, and several regulatory proteins, add to the complexity of $Ca^{2+}$ signaling. The rising number of studies and the increasing knowledge about these regulatory mechanisms opens up future possibilities for fine-tuning of intracellular calcium signaling in the treatment of physiological malfunctions and diseases. As many of these regulatory mechanisms are putative drug targets, they may serve as a basis in the development of novel pharmacological strategies.

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