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Gating and permeation of Orai channels

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1. ABSTRACT

Ca^{2+} release-activated Ca^{2+} channels mediate a sustained Ca^{2+} influx following depletion of endoplasmic reticulum stores. This signalling cascade that triggers T-cell activation or mast cell degranulation involves STIM1, the Ca^{2+} sensor in the endoplasmic reticulum, and the Ca^{2+} selective Orai channel in the plasma membrane. This review describes the molecular mechanism (s) governing the STIM1/Orai signalling machinery. Moreover, we provide an overview on additional proteins modulating or interacting with the STIM1/Orai1 system. A structure-function relationship highlights regions within STIM1/Orai proteins contributing to activation, permeation and inactivation of CRAC currents.

2. STORE-OPERATED CA^{2+} CHANNELS

Changes in cytosolic Ca^{2+} levels control a variety of cellular processes ranging from short-term to long-term responses of immune cells, like mast cells, T-, B-cells and lymphocytes (1). Activation of immune receptors such as T-, B-cell- or Fc-receptors initiates robust Ca^{2+} influx into the cell. A main calcium entry pathway is represented by the so called store-operated calcium channels (SOCs) among which the calcium release-activated Ca^{2+} (CRAC) channel is best characterized (1-5). The CRAC channel is activated by depletion of intracellular Ca^{2+} stores which is caused by the second messenger inositol 1,4,5-trisphosphate (IP_3) (2-6). Biophysical properties of the CRAC channels include a high Ca^{2+} selectivity, very low

single channel conductance and a pronounced inwardly rectifying current/voltage relationship (2). Function-based genetic screens by systematic RNA interference (RNAi) has revealed solid evidence that the stromal interaction molecule (STIM) and the Orai (also termed CRACM) family are key components of the CRAC channels (7-11).

2.1. STIM1, the Ca^{2+} sensor in the ER membrane

STIM1 has been identified as an ER-located Ca^{2+} sensor (7, 8) containing a pair of luminal EF-hands and a sterile-alpha motif (SAM) in its N-terminus followed by a single transmembrane domain (12). The cytosolic C-terminus includes three coiled-coil domains (13), a CRAC modulatory domain (CMD) and a serine-/proline- and lysine-rich region (7, 14-17). Within the STIM1 C-terminus, a region including the second coiled-coil domain and the following ~55 amino acids has been recently identified as the smallest fragment binding to and activating Orai1 (18-21). The second STIM protein, i.e. STIM2, is structurally 61% homologous to STIM1. The sequences of both proteins diverge significantly in their short N-terminal region (22) and their C-terminus subsequent to the ERM/coiled-coil region (23).

2.2. Orai proteins, the pore forming subunits of CRAC channels

RNAi based screens and analysis of single nucleotide polymorphism arrays of patients with severe combined immune deficieny (SCID) syndrome who exhibit a defect in CRAC channel function, have led to the identification of Orai1 (9-11). Orai1 is located in the plasma membrane and functions as a Ca^{2+}-selective ion channel (9-11). Since co-expression of STIM1 and Orai1 has revealed currents with biophysical and pharmacological properties similar to endogenous CRAC currents in RBL mast or Jurkat T cells (24), both proteins are supposed to manifest the main components of CRAC channels. The Orai family includes two further human homologs, Orai2 and Orai3, all of which contain four transmembrane segments and cytosolic N- and C-termini (10). While a proline-/arginine-rich region is only present in the Orai1 N-terminus (25), a cluster of positively charged amino acids close to the first transmembrane region is fully conserved within all three Orai channels. The C-terminus of each Orai protein contains a putative coiled-coil domain (26-28). All three Orai channels are highly selective for Ca^{2+} over monovalent ions and can be activated following store depletion via coupling to STIM1. Furthermore the respective Orai channels exhibit distinct inactivation profiles and 2-aminoethyldiphenyl borate (2-APB) sensitivities (29).

2.3. Stoichiometry of Orai channels and their assembly

Orai proteins display no known homology to other calcium channels which led to speculations concerning the subunit stoichiometry of the channel. Primarily biochemical assays have revealed the existence of Orai1 dimers; while tetramers have been found after addition of a chemical cross-linking agent (30). By applying disulfide cross-linking assays Orai1 assembles as a tetramer or as a higher order oligomer (31). Electron microscopy studies have determined a tetrameric stoichiometry of purified Orai1 proteins (32). In functional assays, currents derived from a tetrameric Orai1 concatamer remained unaffected by co-expression with dominant-negative Orai1 monomers indicating the Orai channels represent a tetrameric assembly (33). Single molecule imaging approaches (34, 35) have allowed for monitoring single molecule photo-bleaching to measure the stoichiometry of each CRAC channel complex formed by co-localized GFP-tagged Orai1 subunits. While Ji *et al.* (34) have observed exclusively Orai1 tetramers in fixed HEK-293 cells, Penna *et al.* (35) have demonstrated Orai dimers in resting *Xenopus laevis* oocytes that associate to tetramers only upon co-expression of the STIM1 C-terminus. The latter data may argue for a stimulatory role of STIM1 in the tetrameric assembly of Orai proteins. A constraint of these two studies may arise from analysis to only immobile or immobilized proteins. Orai proteins are expected less mobile upon potential pre-association with STIM1. We have recently focused on the mobile fraction of Orai1 proteins from resting cells, and primarily tetrameric assemblies have been observed (36).

In addition to the homomeric assembly of Orai proteins, they are also able to form heteromeric channels (29, 30, 37). Besides tetrameric Orai protein aggregation, Shuttleworth's lab has reported that Orai1 and Orai3 subunits are able to form pentameric aggregates which function as arachidonate regulated Ca^{2+} (ARC) channels. A multimer including three Orai1 and two Orai3 subunits represents the functional ARC channel pore (33, 38-40). It has been shown that the Orai3 N-terminal domain accounts for the switch from a predominantly store-operated to an exclusively arachidonic acid activated channel (41).

Orai subunits oligomerize mainly via their transmembrane regions as deletion of the cytosolic strands does not impair aggregation of Orai proteins (28, 42). Nevertheless Orai1 N-terminus has still been suggested to play a role in Orai assembly as it acts in a dominant negative manner on SOC (25) in line with our results (43) (R. Schindl, C. Romanin, unpublished results). However, a detailed analysis of domains relevant for Orai subunit multimerization is still lacking.

3. SIGNALLING MACHINERY OF STIM AND ORAI

STIM1 and Orai1 represent the two pivotal molecular components that link Ca^{2+} store-depletion of the ER with CRAC channel activation (7-12, 44). STIM1 proteins are diffusely located in the ER membrane and sense with their N-terminal EF-hands the luminal Ca^{2+} content (7, 8). Store-depletion triggers oligomerization of the STIM1 proteins which redistribute into puncta at junctional ER sites close to the plasma membrane (20nm) (7, 14, 44-48). Thereby STIM1 couples to Orai channels in the plasma membrane forming co-clustered puncta (7, 45, 48, 49). Orai channels are activated by this association and Ca^{2+} flows into the cell. Recently, Korzeniowski *et al.* (50) have demonstrated that a basic amino acid stretch within the second coiled-coil domain of STIM1 interacts with an acidic domain in the first coiled-coil domain. This intramolecular clamp is abrogated upon store-depletion and

allows the coupling of the basic segment in the second coiled-coil domain of STIM1 with acidic residues within the C-terminal coiled-coil region of Orai1 (50). Furthermore, Muik *et al* have (51) visualized the intramolecular transition switching the cytosolic portion of STIM1 into an extended conformation and revealed the additional involvement of hydrophobic amino acids contributing to this conformational rearrangement. The stoichiometry of STIM1 proteins required for Orai1 channel opening has been recently estimated with eight STIM1 molecules interacting with one CRAC channel to induced maximal activation (52). Thus, CRAC channel activation is not mediated in an "all-or-one" fashion but occurs via a graded process involving up to eight STIM1 molecules (52). Overall, these findings delineate a complex signaling cascade with the co-clustering of STIM1 and Orai1 thereby activating CRAC channels. Within this signaling process a series of interaction domains that mediate STIM1 oligomerization, Orai multimerization as well as STIM1/Orai coupling are involved.

3.1. Domains mediating STIM1 oligomerization

At resting state, STIM1 is uniformly distributed within the ER (28, 47) and exhibits tubular structures. It binds to the microtubule-plus-end-tracking protein EB1 at those sites where microtubule ends come in close contact with the ER (53). Moreover, STIM1 co-localizes with endogenous α-tubulin (54). The STIM1 EF-hand senses the decrease in Ca^{2+} concentrations in the ER lumen which under resting cell conditions is approximately 300–500 μM (55, 56). Subsequently, STIM1 forms oligomers before it redistributes at an EC_{50} of 210 μM Ca^{2+} (57) into punctuate clusters close to the plasma-membrane (7, 14, 44-48). STIM1 oligomerization has been reported to be sufficient to drive puncta formation and CRAC channel activation (58). STIM1 redistribution and CRAC activation share a steep dependence on the Ca^{2+} level in the ER and defines input-output relationship of the CRAC channel (58). The initial trigger for STIM1 oligomerization is represented by its N-terminal EF-hand. Upon store-depletion the EF-hand looses bound Ca^{2+} and consequently allows for aggregation of STIM1 proteins. In line, an N-terminal STIM1 fragment including the EF-hand and the SAM domain oligomerizes in the absence of Ca^{2} (59, 60). In between the EF-hand and the SAM domain lies a "hidden" EF-hand that is unable to bind Ca^{2+} (59). Both EF-hands together mediate interactions via hydrophobic residues with the SAM domain (59). Specific mutations of these residues disrupt Ca^{2+} sensitivity and oligomerization via destabilization of the entire EF-SAM entity consequently resulting in puncta formation and constitutive activation of SOC (59). In accordance, a STIM1 deletion mutant lacking the whole C-terminus also oligomerizes via the N-terminal EF-SAM domain, but forms unstable aggregates (61). However, a functional EF-hand-SAM domain is not sufficient to trigger, via oligomerization, the activation of Orai channels. Therefore, STIM1 cytosolic regions are indispensable for CRAC channel activation as described in the following passage.

Within the cytosolic region of STIM1, the first coiled-coil domain has been reported to support formation of resting STIM1 oligomers (61). However, complexes formed by the interaction of the first coiled-coil domain are still unstable as observed by STIM1 C-terminal deletion mutants truncated after this region (61). A short C-terminal STIM1 fragment (233-420) which includes only the first and second coiled-coil domains and lacks the STIM1 N-terminus and the transmembrane domain are also unable to form higher order aggregates than monomers (18). The formation of stable store-dependent oligomers is enabled when the first coiled-coil domain together with the Orai1 activation domain (CAD, 342-448) is present within the STIM1 protein (61). Similarly, STIM1 C-terminal fragments (233-450, 233-474) including the first, second and third coiled-coil domains together with ~30 additional amino acids are sufficient for aggregation (18). Within CAD, the second and third coiled-coil domains as well as ~20 C-terminal residues have been suggested to contribute to oligomer formation (61). Employing short STIM1 C-terminal fragment, Muik *et al* (18, 43) have narrowed down the domain contributing to STIM1 oligomerization to the region between amino acid 420-450, located C-terminal to the coiled-coil regions.

3.2. Domains mediating STIM1/Orai coupling

Among the STIM1 C-terminal coiled-coil domains, the second is indispensable for the coupling to Orai1 (27). Short STIM1 C-terminal fragments (CAD (aa342-448), SOAR (aa344-442), OASF (aa233-450), Ccb9 (aa339-444)) including mainly the second and third coiled-coil domains together with some additional residues downstream have emerged as sufficient for coupling to and activation of Orai proteins (18-21). Park *et al.* (20) have further demonstrated that the GST-tagged 107 residue STIM1 C-terminal fragment CAD co-precipitates and co-elutes with Orai1.

The potential coupling site for the second coiled-coil domain of STIM1 is the coiled-coil domain in the C-terminus of Orai proteins. All Orai proteins contain a single, putative coiled-coil domain in their C-terminus, estimated with a 15-17 fold higher probability in Orai2 and Orai3 compared to Orai1 (27). While a coiled-coil single point mutation in Orai1 C-terminus (L273S, L276D) has abrogated communication with STIM1 C-terminus (27, 28, 62), the analogous exchange in Orai2 and Orai3 still allows for moderate STIM1 C-terminal coupling and current activation (27). Introduction of a second point mutation in the C-terminus of either Orai2 or Orai3 fully disrupts coupling to STIM1. Decreasing the probability of the putative, second coiled-coil domain of STIM1 C-terminus by a single mutation retained partial stimulation of Orai2 and Orai3 channels while Orai1 currents are inhibited. A double mutation within the second coiled-coil motif of STIM1 C-terminus fully disrupts communication with all three Orai channels. Thus, coiled-coil domains represent pivotal structures mediating coupling of STIM1 and Orai (27). The coiled-coil domain of Orai1 includes a series of acidic residues, while the second coiled-coil domain of STIM1 overlaps with a highly conserved cluster of basic

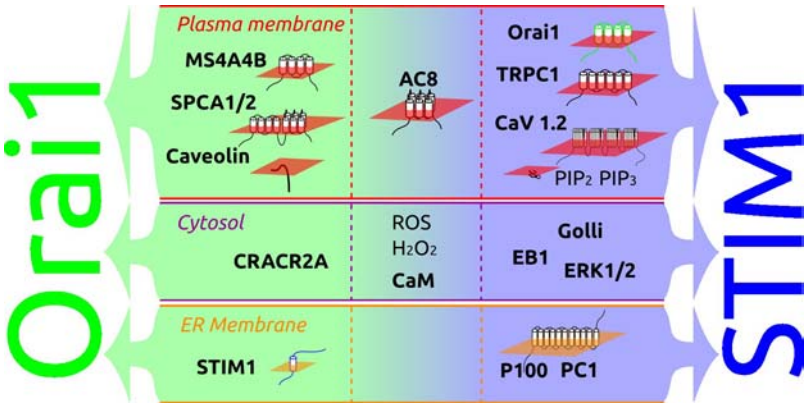

Figure 1. Structure-function relationship of the STIM1/Orai1 proteins depicting regions contributing to activation (yellow), permeation (pink) and inactivation (blue) of Orai1/CRAC channels. Activation: STIM1 – CAD/SOAR, Orai – C-terminus, N-terminus; Permeation: Orai – E106 in TM1, negative residues in the first loop, 2-APB and Orai3; Inactivation: STIM1 – negative residues 474-485; Orai – negative residues in C-terminus, second. intracellular loop, N-terminus/CaM/Ca^{2+}.

residues (KIKKKR – aa 382-387 of human STIM1; KLRKKR – aa 380-385 of BmSTIM1). Recent publications (63-65) have demonstrated that these charged amino acids enable STIM1/Orai coupling. However, a charge swap within coiled-coil domains of STIM1 and Orai C-termini disrupted coupling of these mutants (64). Hence, additional structural features have been hypothesized to be involved in the coupling process. Besides the requirement of hydrophobic amino acids for lipophilic interactions within a coiled-coil structure, charged residues additionally contribute to stable coiled-coil formation or heteromerization (66, 67). Thus, deletion or mutation of these charged amino acids might disrupt coiled-coil structure or heteromerization and consequently impair STIM1/Orai coupling.

3.3. Regions modulating STIM1-Orai coupling

While coiled-coiled domains are indispensable for STIM1/Orai coupling, several regions within both proteins exhibit a modulatory role on Orai activation. The coupling of STIM1 to Orai1 does not only include the C-terminal coiled-coil domain but also the N-terminal region of Orai1 (20, 28, 42). The CAD fragment interacts with Orai1 C-terminus and to a weaker extent with the N-terminus (20). Park *et al.* (20) as well as Hogan *et al.* have shown direct binding of STIM1 C-terminal fragments to both N- and C-terminus of Orai1, thereby triggering channel gating. Deletion of the whole N-terminus of Orai1 abolishes SOCE, while its partial truncation up to amino acid 73, with the conserved region retained, preserves Orai1 channel activity (42, 68). This observation suggests that the Orai1 N-terminal region between amino acid 74 and 90 conserved among all Orai proteins (Figure 1) is indispensable for Orai activation. Within this region a CaM binding domain has been predicted (69). Mutation of specific amino acids, i.e. A73, W76 and Y80, results in the loss of CaM binding which is accompanied by elimination of fast inactivation (see below section on inactivation). Besides hydrophobic residues, it contains five positively charged amino acids. This series of basic re see whether PIP_2 depletion modulates processes of the STIM1/Orai signaling machinery. In any case, this N-terminal polybasic region, which is fully conserved in all Orai proteins, is expected to play an important role in the activation by STIM1. While all Orai proteins exhibit this polybasic domain within the N-terminus, an arginine-/proline-rich region occurs only in Orai1 (43). A series of Orai1 mutants lacking the proline-/arginine-rich region, i.e. Orai1 ΔN_{1-73} (19, 42), an Orai1 chimera with the N-terminus of Orai2 (25) or Orai1 P3,5A and Orai1 P39,40A (19) exhibit significantly reduced store-operated Ca^{2+} currents upon store depletion. Together these observations suggest that the extent of maximum current density of Orai channels is at least partially determined by the presence of these prolines within the N-terminus. sidues partially resembles the consensus sequence of a PH-domain (66), suggesting that it binds PIP_2. Thus, it will be interesting to

Recently, a point mutation approach has revealed that a highly conserved amino acid (K85 in CRACM1 and K60 in CRACM3) in the N-terminal region close to the first transmembrane domain represents a critical element for STIM1-dependent gating of CRAC channels (68). These respective single point mutations (K85E and K60E) have abolished store-dependent currents and have diminished store-independent gating by 2-APB (68). Nevertheless, co-immunoprecipitation studies have shown still retained but weaker binding of CAD to an N-terminal peptide of Orai3 K60E N-terminus (68). In aggregate, these results have revelaed a single amino acid in the N terminus of CRAC channels essential for their store-operated gating.

A polybasic cluster at the very end of the STIM1 C-terminus - the lysine-rich domain - represents another regulatory region. It is required for the redistribution of STIM1 as its deletion eliminates puncta formation despite preserved STIM1 homomerization (7, 20). Nevertheless, this deletion mutant is still able to activate Orai1 channels in a store-operated manner, yet with a slightly delayed response (42). The polybasic region represents a putative

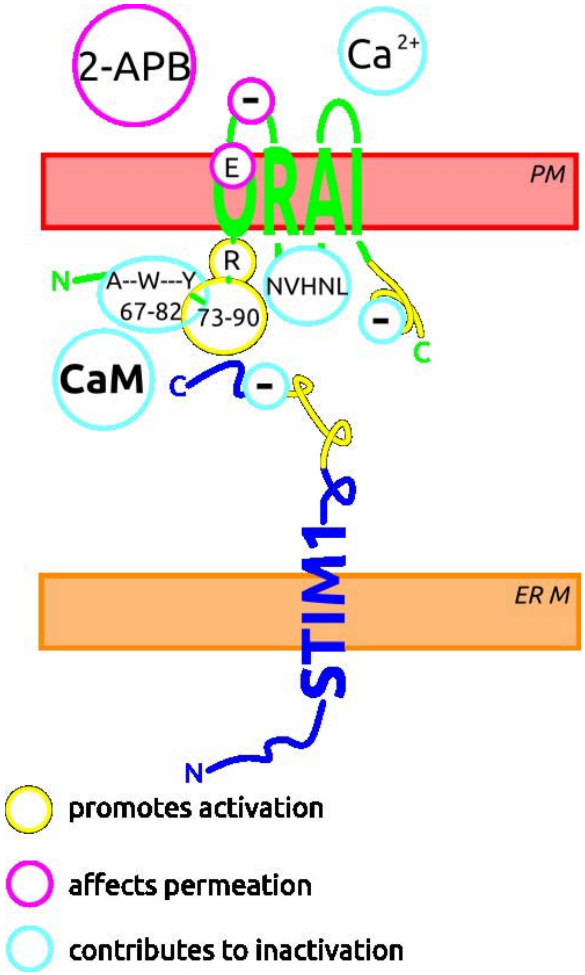

Figure 2. Overview on proteins and lipids interacting with STIM1/Orai1

phosphoinositide binding domain. Hence, it has been assumed that puncta formation is forced by phosphoinositides in the plasma membrane (47). The role of phosphoinositides will be further discussed below.

While STIM1 aggregation is influenced by the lysine-rich region, STIM1/Orai coupling is regulated by the CRAC modulatory domain, termed CMD (aa 474-485). This region comprises seven negatively charged residues and is located in the C-terminus of STIM1. Mutation of at least four of these amino acids or their deletion leads to a strongly enhanced coupling to Orai1 together with 2-3 fold increased Ca^{2+} inward currents (17, 69, 70). These enhanced Ca^{2+} currents are accompanied by a loss of fast inactivation, as detailed below. In aggregate, it may be assumed that CMD acts as an inhibitory region on STIM1/Orai coupling, current activation as well as inactivation.

3.4. Direct interaction of STIM1 and Orai1 and modulatory proteins

Whether the coupling of STIM1 and Orai1 occurs directly or requires additional components has been a controversial issue for a long time. While Yeromin *et al.* (71) have first observed STIM1-Orai1 co-immunoprecipitation, this has not been observed by Gwack *et al.* (30). Chemically inducible bridge formation between the plasma and ER membranes to estimate the distance of the STIM1/Orai1 has revealed that Orai1 is part of a much larger complex than represented solely by coupled STIM1 and Orai1 (49). One hypothesis favors a Ca^{2+} influx factor (CIF), which may be generated via ER store depletion to activate STIM1/Orai1 signaling machinery (72). However, a number of laboratories have demonstrated that STIM1 and Orai1 couple close to each other upon store-depletion employing fluorescence resonance energy transfer as well as biochemistry (20, 28, 62, 63, 73) as mentioned in detail in a previous chapter (19-21, 27, 28). Employing a yeast expression system and purified STIM1 proteins, Zhou *et al.* (74) have demonstrated that STIM1 and Orai1 are sufficient for CRAC channel activation without the requirement of additional proteins. Hence, there is clear evidence that STIM1 and Orai1 represent the key proteins for Orai1 current activation. Additional components within the CRAC channel signaling complex may function in a modulatory manner on CRAC current regulation. These proteins/lipids comprise (overview in Figure 2): CaM (69, 75), CRACR2A/B (76), MS4A4B (77), Golli (78), adenylyl cyclase type 8 (AC8) (79), the polycystin-1 cleavage product P100 (80), caveolin (81), SPCA2 (82) and the L-type Ca^{2+} channel (Cav1.2) (83, 84) or the phospholipids PIP_2 and PIP_3 (85-87) which have been recently identified to be involved in the STIM1/Orai complex.

CaM represents an ubiquitous signaling molecule playing an essential role in diverse cellular processes ranging from muscle contraction by gene expression to cell growth as well as apoptosis (88). Employing bioinformatics (http://calcium.uhnres.utoronto.ca/ctdb/ctdb/sequence.html) a putative CaM binding domain has been identified within the C-terminus of STIM1 as well as the N-terminus of all Orai proteins. While the role of CaM on STIM1 has not been investigated so far, STIM2 mediated CRAC currents are inhibited by CaM (75). This blockage only occurs when intracellular Ca^{2+} levels are elevated, suggesting that only Ca^{2+}-bound CaM but not apo-CaM is required (75). Yet, functional effects coupled to a direct interaction of CaM with one of the pivotal players of CRAC channel signaling machinery have only been reported for Orai1 (69). (see section on inactivation).

Another EF-hand-containing protein, CRACR2A (76), has been recently reported to stabilize the interaction between STIM1 and Orai1. These proteins are suggested to form a ternary complex which dissociates at higher Ca^{2+} concentrations. A mutation in the EF hand of CRACR2A leads to enhanced STIM1 clustering and elevated cytoplasmic Ca^{2+} concentrations causing cell death in T-cells. CRACR2A interacts with the N-terminus of Orai1, and requires the positively charged amino acids K85 and K87 in the conserved region close to the first transmembrane domain (76). The homologue CRACR2B seems to have a distinct role to that of CRACR2A. Although its knock-down by siRNA has reduced SOC in HEK and Jurkat cells, its co-expression with Orai1 does not

increase the store-operated Ca^{2+} entry to a similar extent as in the presence of CRACR2A. Additionally, an interaction of CRACR2B with Orai1 N-terminus is lacking. Functional electrophysiological studies demonstrating an effect of CRACR2A on CRAC channel currents are not yet available.

MS4A4B is a GITR (glucocorticoid-induced TNFR- (Tumor Necrosis Factor Receptor) related protein) associated membrane adapter which is expressed in regulatory T cells and modulates T cell activation. It has been demonstrated to interact in addition to GITR with diverse surface proteins, and also with Orai1 (77).

Golli, a member of the myelin basic protein (MBP) family has been discovered to directly interact with the STIM1 C-terminus, which is modulated by intracellular Ca^{2+} concentrations. An over-expression of Golli reduces SOCE in HeLa cells which can be overcome by additional over-expression of STIM1 (78).

AC8, which generates cAMP via Ca^{2+}-bound CaM, has been found to co-localize with STIM1 and Orai1 in lipid rafts (79). A cytosolic FRET-based cAMP sensor has enabled monitoring the increased cAMP production upon elevation of Ca^{2+} levels via STIM1/Orai1 coupling. Interference with STIM1 translocation markedly suppresses Ca^{2+}-dependent cAMP formation (79). Thus, AC8 forms aggregates with STIM1 and Orai proteins and stimulates down-stream processes following the Ca^{2+} influx.

The protein polycystin 1 (PC1), a member of the TRP ion channel family represents an essential regulator of intracellular Ca^{2+} in renal tubules of the kidney (80). The cleavage product P100 of PC1 is a 100 kDa fragment that down-regulates store-operated Ca^{2+} entry by physical interaction with STIM1 and interference with STIM1 puncta formation. A disease-caused mutation in PC1, has been demonstrated not to inhibit SOCE. Thus, store-dependent Ca^{2+} entry might play a role in kidney diseases.

Cav, a member of integral membrane proteins has been shown to bind to an Orai1 Cav consensus-binding site in the Orai1 N-terminus (81). This interaction together with a dynamin-dependent endocytic pathway has been shown to mediate internalization of Orai1 during meiosis in oocytes. At resting state, a significant amount of total Orai1 localizes to intracellular compartments while store depletion completely shifts endosomal Orai1 to the cell membrane. Thus a vesicular trafficking mechanisms controls Orai1 subcellular localization in the oocyte at steady-state, during meiosis, and after store depletion.

SPCA2, a member of the secretory pathway Ca^{2+}-ATPases (SPCA), has been reported to constitutively activate Ca^{2+} entry (82). This process is dependent on Orai1, however, independent of store-depletion, STIM1 and STIM2. Moreover, it is uncoupled from the Ca^{2+}-ATPase activity of SPCA2. The amino terminus of SPCA2 binds to the C-terminus of Orai1, which then enables SPCA2 C-terminus to activate Ca^{2+} influx. In this context, SPC2 and Orai1 have been suggested to promote tumorigenesis of breast cancer derived cells.

Besides SOC channels, voltage-gated Ca^{2+} channels provide another major Ca^{2+} entry pathway into the cell. Two laboratories (83, 84) have recently shown that STIM1 suppresses Ca^{2+} currents of the L-type Ca^{2+} channel $Ca_V1.2$. This inhibition is similarly seen with the short STIM-Orai activating region (SOAR) of STIM1 (84). The C-terminus of $Ca_V1.2$ interacts with STIM1 leading to an internalization of $Ca_V1.2$ (83). STIM1 co-localizes with both $Ca_V1.2$ and Orai1 channels in discrete endoplasmic reticulum/plasma membrane junctions (84). Thus, STIM1 interacts with and reciprocally regulates two major calcium channels hitherto thought to operate independently (84).

In summary, STIM1/Orai mediated Ca^{2+} entry is apparently fine-tuned by the expression of the above proteins in different tissues (see Figure 2).

3.5. STIM1/Orai and lipid rafts

Lipid raft domains have been reported to provide an adequate environment for the interaction of different SOCE-associated proteins (89-91). They contain twice the amount of cholesterol than the surrounding bilayer (92). Due to the rigid nature of the sterol groups, cholesterol partitions preferentially into the lipid rafts where acyl chains of the lipids tend to be more rigid and in a less fluid state (93). One of the first studies focused particularly on the role of lipid rafts in the clustering of STIM1 as well as co-regulation of STIM1 with TRPC1, a member of the canonical transient receptor (TRPC) potential channels (94). Upon sequestration of membrane cholesterol in human salivary gland and HEK293 cells, thapsigargin-induced clustering and puncta formation of STIM1 is significantly attenuated. Both STIM1 and TRPC1 partition to plasma membrane lipid raft domains and their degree of association is dynamically regulated by the status of the ER Ca^{2+} content. Disruption of lipid raft domains has decreased plasma membrane recruitment and association with TRPC1 as well as activation of SOC. Together these results suggest that a functional interaction of STIM1 with TRPC1 is facilitated by intact lipid raft domains (90).

Further evidence that STIM1 and Orai1 co-localize in lipid rafts has been obtained of studies on AC8 and TRP as well as Orai channels. Both proteins have been identified in lipid rafts together with STIM1 and Orai1 (79, 94, 95). Disturbance of lipid rafts, employing methyl-beta-cyclodextrin, has reduced association of endogenously expressed Orai1 and both STIM1 and TRPC1 upon depletion of the intracellular Ca^{2+} stores and has attenuated thapsigargin-evoked Ca^{2+} entry (96). Galan *et al.* have additionally shown that disturbance of lipid rafts, employing methyl-beta-cyclodextrin, reduces association of endogenously expressed Orai1 and both STIM1 and TRPC1 upon depletion of the intracellular Ca^{2+} stores and attenuates thapsigargin-evoked Ca^{2+} entry (96). Moreover, cholesterol sequestration by methyl-beta-cyclodextrin prevents store-operated Ca^{2+} influx (96). In contrast, cholesterol depletion after store-depletion has further

enhanced thapsigargin-induced Ca^{2+} entry. Hence, it has been hypothesized that lipid rafts are important for the activation but not for the maintenance of SOCE.

In summary, TRPC1 and AC8, etc have been identified to co-localize with STIM1 and Orai1 in lipid rafts, even if they are not necessarily required for CRAC current activation. However, they might act in a modulatory manner and lipid rafts probably facilitate their interplay.

3.6. STIM1/Orai1 and plasma membrane phosphoinositides

STIM1 contains a lysine-rich domain at the very end of its C-terminus which resembles a PIP_2 binding domain. An interaction with PIP_2 has been hypothesized to modulate STIM1 puncta formation (47). Various recent studies have focused on the role of phosphoinositides on STIM1 localization as well as SOC activation. ATP depletion which is accompanied by decrease in plasma membrane PIP_2 has been reported to induce puncta formation of STIM1-Orai1 clusters (85). However, ATP also affects a series of other proteins and signaling cascades within the cell. Walsh *et al.* (86) have reported that PIP_2 or PIP_3 depletion alone hardly reduce STIM1 puncta formation, whereas depletion of both phospholipids PIP_2 and PIP_3 together significantly reduces targeting of STIM1 to ER-PM junctions (86). Concerning the effect of PIP_2 on SOCE contradictory results have been obtained (86, 87). Korzeniowski *et al.* (87) have reported that endogenous as well as STIM1/Orai1 mediated SOCE remained unaffected upon PIP_2 depletion. In contrast, Walsh *et al.* (78) have observed that PIP_2 depletion reduces endogenous SOCE by about 60%, while over-expression of Orai1 partially reversed this Ca^{2+} entry. Moreover it has been reported that PIP_2 contributes to the stabilization of STIM1-PM interaction rather than to STIM1 translocation (87). One reason for these distinct findings may be seen in the fact that depletion of phosphoinositides is carried out before (78) or after (87) stimulation of puncta formation. Ecran *et al* (97) have demonstrated preferential binding of STIM1 to liposomes containing PIP_2 suggesting a specific contribution of PIP_2 for recruitment of STIM1 C-terminus to the plasma membrane.

Contrary to the phosphoinositides, down-regulation of phosphatidylinositol-4-kinases (PI_4K) under conditions in which PIP_2 levels remained constant has not affected STIM1 movements, although STIM1/Orai1-mediated or CRAC currents are strongly inhibited (87).

In summary, phosphoinositides exhibit a regulatory impact on the STIM1/Orai signaling cascade, however, other components besides STIM1 might be additionally involved.

3.7. Phosphorylation sites in STIM1 and Orai proteins

STIM1 has been defined as a phosphor-protein as it contains a series of phosphorylation sites in its C-terminus (98). Recently, Smyth *et al* (99) have identified that STIM1 phosphorylation suppresses store-operated Ca^{2+} entry during mitosis in accordance to earlier findings (100). Here, two serines S486 and S668 represent the mitosis-specific phosphorylation sites, and their mutation to alanines rescues mitotic SOCE (99). Additionally STIM1 has been found to function as a probable target of the extracellular-signal-regulated kinase 1 and 2 (ERK1/2) (101). Phosphorylation of ERK1/2 target sites on STIM1 has been shown to modulate store-operated calcium entry. Mutation of these specific sites S575A/S608A/S621A does not alter thapsigargin-induced aggregation or re-localization of STIM1 but does decrease the binding to Orai1. Hence, the authors suggest that the decreased STIM1/Orai1 coupling is responsible for reduced SOC entry.

Store-operated currents are inactive during meiosis, due to an internalization of Orai1 proteins, and diminished STIM1 cluster formation in response to store-depletion (102). Similarly, store-operated currents are suppressed during mitosis which underlies the inability of STIM1 to form puncta near the plasma membrane upon store-depletion. Two residues Ser 486 and Ser 668 have been identified as mitosis-specific phosphorylation sites with their mutation significantly rescuing store-operated Ca^{2+} entry (99)..

Orai1 is also phosphorylated at N-terminal residues S27 and S30 by PKC (103). Substitution of these serines by alanines has enhanced store-operated Ca^{2+} entry as well as CRAC channel currents suggesting that PKC suppresses SOC and CRAC channel function by phosphorylation of Orai1 at these residues.

Thus, phosphorylation of the two pivotal players of the STIM1/Orai signaling cascade exerts a modulatory role on CRAC channels.

3.8. Regulation of STIM1/Orai signaling by oxidative stress

Reactive oxygen species (ROS) are important mediators of many physiological and patho-physiological processes (104, 105) and are generated in the intracellular as well as extracellular space by redox-active proteins (104, 106, 107). Antioxidants clear ROS and preserve the physiological redox state of cells (104, 108). Among the ~20 types of ROS, hydrogen peroxide (H_2O_2) appears biologically most relevant (104). H_2O_2 is relatively stable and can diffuse across the cell membrane. It primarily acts by oxidizing cysteine residues in target proteins (104). In several recent studies a molecular link between oxidative stress and STIM1/Orai regulation has been reported (109-111).

Two independent studies have recently shown that native CRAC currents are stimulated by oxidative stress employing micromolar concentrations of H_2O_2 (110, 111). Grupe *et al* (110) have suggested that STIM1 is required for activation of CRAC currents by oxidant stress. Another study has attributed the stimulating effect of oxidative stress on CRAC currents to a decrease in the Ca^{2+}-binding affinity of STIM1. The cysteine 56 near the EF-hand within STIM1 N-terminus is S-glutathionylated upon addition of ROS which then triggers STIM1 oligomerization (111). Hence, CRAC channels are

constitutively activated which facilitates an enhancement in Ca^{2+} levels.

In contrast, Niemeyer's lab (109) has demonstrated that Orai1 channels are inhibited by oxidation via H_2O_2. Contrary to Orai1, Orai3 remained unaffected upon oxidative stress. The differential redox sensitivity of Orai1 and Orai3 channels depends mainly on an extracellularly located reactive cysteine, which is absent in Orai3. The authors hypothesize that oxidation of cysteines may lock the pore in the closed conformation. Moreover redox sensitivity in T_H cells depends on their differentiation state. CRAC currents in naïve cells are blocked by H_2O_2, while those in effector cells remain unaffected. This difference has been attributed to an enhanced level of Orai3 expression. Thus, the controversial stimulatory or inhibitory effect of oxidative stress on CRAC currents may occur due to a distinct expression ratio of the three Orai homologues compared to the sole Orai1 expression in HEK cells. Moreover, different concentrations of H_2O_2 have been employed in these studies (109-111). Alternatively, these results may indicate several target sites on STIM1 and Orai proteins within the CRAC channel signaling cascade.

These findings demonstrate the influence of oxidative stress on STIM1/Orai activation reveal new perspectives of a cross talk between Ca^{2+} homeostasis and ROS.

4. PERMEATION OF ORAI AND CRAC CHANNELS

The discovery of the three Orai proteins has enabled comparison of their electrophysiological properties with those of endogenous, expressed CRAC currents. Whole cell recordings of CRAC currents with Ca^{2+} as a charge carrier yield a current-voltage relationship with a prominent inward rectification and a high reversal potential of > +60mV (112). The Ca^{2+} selectivity of CRAC channels has been estimated more than 1000 times higher for Ca^{2+} over Na^+ at physiological conditions (113). Hence CRAC channels conduct one of the most Ca^{2+} selective currents, and all three Orai channels exhibit similar permeation properties (29, 114). Currents of CRAC and Orai1 channels are transiently increased when Ba^{2+} is substituted for Ca^{2+} as charge carrier, suggesting a preferred conductance for Ba^{2+} over Ca^{2+} (115-117). Subsequent steady-state Ba^{2+} currents are diminished (29, 118). Omission of Ca^{2+} in the presence of extracellular Na^+ and Mg^{2+} abolishes inward current (24, 112). However, in the absence of divalent ions, CRAC and Orai channels become permeable for monovalent ions, like Na^+ and Li^+ (29, 113, 118, 119). A Na^+ divalent-free solution yields increased currents due to a removal of Ca^{2+} from a binding site within the pore (117, 120). Na^+ currents of CRAC channels, Orai1 and Orai2 subsequently depotentiate (121), while Orai3 channels exhibit a significantly slower depotentiation (118). The monovalent cesium ion is almost impermeant for both CRAC and Orai channels, while other Ca^{2+} selective channels, such as TRPV6 or L-type Ca^{2+} channels are highly Cs^+ permeable in a divalent-free solution (122, 123). The diameter of a cesium ion covered by a hydration shell in solution is 3.8 Å. To estimate the pore size of CRAC/Orai channels, methylated derivates of ammonium with known diameters have been analysed. The size of these ions has been calculated with the Corey-Pauling-Koltun space-filling models (124). The narrowest region of the pore of CRAC/Orai channels has been determined as ~ 3.8 to 3.9 Å (117, 125, 126), suggesting that Cs^+ ions are small enough to enter the selectivity filter but may be sterically hindered. In addition, CRAC and Orai channels exhibit an extremely small unitary conductance of 9-24 fS, and 6 fS respectively, in a 2-110 mM Ca^{2+} solution (117, 121, 127).

Hence, these permeation properties and the amino acid sequence of the Orai proteins which is unrelated to other ion channels suggest a unique selectivity filter for these channels. The CRAC channel is formed by a tetrameric assembly of Orai proteins (33-35) that either contains the same isoforms or any other heteromeric combination (29, 30, 37). Ca^{2+} selectivity is expected to be mediated by negatively charged amino acids within or close to the transmembrane regions that interact with and partially dehydrate Ca^{2+} ions. Analysis of negatively charged residues reveals altered permeation profiles when glutamates/aspartates within the first and third transmembrane (TM) segment and the first loop (71, 117, 126, 128, 129) are mutated. Orai1 or Orai3 point mutants with a glutamate to aspartate substitution in TM1 (human: E106 in Orai1, E80 in Orai2, E81 in Orai3, drosophila: E180 Orai) results in a strongly reduced Ca^{2+} selectivity, but increased monovalent cation permeability (71, 117, 126, 128, 129). E106 substitution to a glutamine or alanine in Orai1 produces a non-permeant channel that acts in a dominant negative manner on all three Orai-mediated (29, 71, 129) and native CRAC currents in T-cells (30, 128). Within TM3 (human: E190 in Orai1, E164 in Orai2, E165 in Orai3 and drosophila: E262 Orai), the glutamate to glutamine substitution affects Ca^{2+} selectivity while mutation to an aspartate or alanine retains pore properties of Orai1 and Orai3 (126, 128, 129). The reduced Ca^{2+} selectivity of Orai1-E106D and Orai1-E190Q correlates with an increase of the minimum pore size to 5.3 and 7 Å, respectively (117). The enlargement of the narrow Orai1 channel pore comes along with further relief of the steric hindrance for Cs^+ permeation (117).

In an alternative approach, single residues within the first and third transmembrane segments were systematically substituted to cysteines (130, 131). Hence, accessible cysteine residues are able to interact with applied thiol-specific reagents or Cd^{2+}, leading to a rapid channel block. Cd^{2+} with a similar size as Ca^{2+} ions, strongly blocks several of the mutants with a cysteine introduced in the first TM1 domain, in contrast to methanethiosulfate (MTS) derived reagents. Hence, the bulky MTS reagents are unable to access the deep pore regions (130). Cross-linking of Orai1 mutants in TM1 with cysteines at position 88, 95, 102 and 106 results in dimerisation, demonstrating that TM1 is centrally located between or among Orai1 subunits (31). A weaker dimerization has been observed with several other positions in the N-terminal half of TM1, but not in the C-terminal half (131). The close packing of the

N-terminal segment of TM1 may contribute to the low single-channel conductance and help to coordinate Ca^{2+} binding of E106 in Orai1. This glutamate side-chain contributes to limit the maximum size of ions up to 3.8 Å, allowing only small cations such as Na^+ or Ca^{2+} to pass. In line, this constriction is reduced by a substitution to an aspartate that includes a shorter side-chain in comparison to the glutamate.

All cysteine mutants in the third transmembrane domain and especially Orai1-E190C lack any significant changes in ion-selectivity, thus excluding it as a high affinity Ca^{2+} binding site (130). These mutants also fail to crosslink, suggesting that TM3 helices are distant from each other in the Orai1 channel (31). Hence, these results have been taken as indication that the TM3 segment with the E190 therein do not flank the ion-conduction pathway (130), suggesting an allosteric effect of E190Q mutant on the pore.

A second important site in the permeation pathway is formed by the first extracellular loop. In contrast to the conserved glutamates in the TM segments of all three Orai proteins, key residues within this loop consist either of glutamates, glutamines, aspartates or asparagines (D110/112/114 for Orai1; E84/Q86/Q88 for Orai2; E85/D87/E89 for Orai3, D182/D184/N186 for drosophila Orai). None of the aspartate to alanine single point mutations in the first loop of Orai1 alters Ca^{2+} selectivity. Moreover, a systematic substitution of single amino acids to cysteins within the first loop retained the high Ca^{2+} selectivity of wild-type Orai1 (130). However, channel block by lanthanides is reduced for each of the aspartate to alanine loop mutants as well as the Orai1-Q108C mutant in comparison to wild-type Orai1 (71, 130). Alanine substitutions of two aspartates in the first loop of Orai1 (Orai1-D110A-D112A) drastically increase outward currents, while inward currents remain preserved (10). Therefore, at least these two acidic residues together are required to attract Ca^{2+} ions towards the pore (71), while a single substitution is silent and probably compensated by the other two aspartates. Alternatively, these mutants allo-sterically affect the pore and modulate the channels' selectivity. A triple alanine mutant (Orai1-D110A-D112A-D114A) increases Cs^+ permeability concomitant with an increased minimum pore size of 4.4 Å (117). The cysteine scanning methods reveals several additional properties. Application of 6 to > 8 Å large MTS reagents blocked several of the cysteine loop mutants, suggesting that these residues flank a wide outer vestibule (130). However, it is of note that several cysteine mutants form spontaneous disulfide bonds, suggesting close proximity of residues in the first loops of two Orai1 proteins. These findings can be reconciled if the first loop behaves more like a flexible domain (130) that undergoes a conformational change .

In a heteromeric Orai channel the first loops of different proteins apparently get in close contact during Ca^{2+} permeation and may affect pore properties. Indeed, co-expression of Orai1 and Orai3 or an Orai1/Orai3 tandem construct result in a diminished Ca^{2+} selectivity and robust Cs^+ permeation in contrast to homomeric isoforms (132). Substituting glutamates to aspartates in the first loop of Orai3 mimicking the outer vestibule of Orai1 yield highly Ca^{2+} selective currents similar to wild-type Orai channels. However, co-expression with either wild-type Orai3 or Orai1 decreases or recovers Ca^{2+} selectivity (132). These experiments suggest that an asymmetric arrangement of aspartate/glutamate within the first loops result in altered pore conformation, probably via an allosteric mechanism. Other reports have presented heteromeric Orai1/Orai3 channels as the molecular basis of the ARC channel that responds to arachidonic acid rather than store-depletion (39, 133). Hence, analysis of native heteromeric Orai1/Orai3 channels (134-136) may help to determine the intrinsic function of the proteins.

Summarizing, the Ca^{2+} ions initially enter through a flexible outer pore vestibule and permeate through the narrow pore that is surrounded by residues of helical first transmembrane segments (130). The Ca^{2+} ions need to pass the selectivity filter mainly formed by glutamate residues within TM1. A second constriction site arises through the smaller N-terminal packing of TM1 segment. The hydrophilic side-chain of arginine 91 in Orai1, directly located at the interface between TM1 and the N-terminus, is required to allow Ca^{2+} passage into the cytosol (137), which is inhibited by mutations to hydrophobic residues.

4.1. Distinct effects of 2-APB on the three Orai isoforms

Several compounds that block CRAC currents have been re-examined for their effects on STIM1/Orai mediated channels as recently reviewed (*138, 139)*. The best pharmacologically characterised modulator of CRAC/Orai currents is 2-aminoethoxydiphenyl borate (2-APB). The effects of 2-APB are complex and depend on the Orai isoform. Orai1 and Orai2, when co-expressed with STIM1, are stimulated by low concentration of 2-APB and inhibited by high concentrations, while Orai3 currents are exclusively increased (140). The stimulatory effect of 2-APB is attributed to an increased association of STIM1 with Orai channels as monitored by FRET microscopy (62, 141). The inhibitory effect of 2-APB may result from a reversal of STIM1 puncta formation in store-depleted cells (114, 140). However, co-expression of STIM1 and Orai1 diminishes this effect (114). Alternatively, 2-APB alters the FRET between two labelled Orai proteins, pointing to a conformational change within the channel (62).

In addition, 2-APB is able to modulate the permeation profile of Orai3 channels, suggesting an interaction with the channels' pore. Independent of STIM1 or store-depletion, Orai3 is robustly stimulated by 2-APB (37, 118, 126, 140) and Orai1 to a minor extent (140). While store-operated Orai3 channels exhibit a Ca^{2+}-selective inward-rectifying current, those stimulated by 2-APB permeate both Ca^{2+} and monovalent cations, yielding a double-rectifying current-voltage relationship (126, 140). A decrease in Ca^{2+} selectivity correlates with an increase in pore dimension to more than 5 Å (126). Interaction of 2-APB is mapped to the second as well as third transmembrane segment and the second loop of Orai3 (37). Consistently, we have observed a dramatically decreased sensitivity to 2-APB for an Orai3 chimera with a

substituted 2nd loop of Orai1 (unpublished results; R. Schindl, I. Frischauf, C. Romanin). In addition, heteromeric Orai1/Orai3 concatemers, which have been established by direct linkage of the two proteins exhibit substantially reduced 2-APB activation (132). Hence, the homomeric Orai3 channel architecture contains unique structures for 2-APB sensitivity (37). Surprisingly, 2-APB stimulated Orai3 currents represent a similar current-voltage profile as a glutamate to glutamine single point mutant within of the third TM in either Orai1 (Orai1-E190Q) or Orai3 (Orai3-E165Q) (126, 128, 129). Therefore these mutants may adopt a similar conformation in the selectivity filter as Orai3 in the presence of 2-APB. Indeed, store-operated Orai3-E165Q currents are only marginally stimulated by 2-APB (126), suggesting that 2-APB is unable to further enhance currents in this altered pore configuration. An alanine point mutation at a corresponding site in Orai1 (Orai1-E190A) robustly but transiently activated currents upon 2-APB addition, yielding a similar double-rectifying current-voltage relationship (140). Hence, this glutamate located in the third transmembrane domain of Orai3 together with the Orai3 subtype-specific regions is involved in 2-APB interaction.

4.2. The Orai1 R91W mutant linked to Severe Combined Immune Deficiency (SCID)

SCID represents a subgroup of immune disorders leading to defective T cell signaling and arises amongst other gene defects by the single point mutation R91W in Orai1 (142-144). This Orai1 R91W mutant displays complete loss of function upon store depletion (9, 137, 145). The arginine at position 91 in Orai1 is fully conserved in Orai2 and Orai3, located at the beginning of the first transmembrane domain (146). Point mutations at respective positions R65W in Orai2 and R66W in Orai3 cause analogous loss of function as with Orai1 R91W (126, 137). Whether the Orai SCID mutant acts in a dominant negative manner has so far remained controversial. While Muik *et al* (28) have reported that Orai1 R91W causes a noticeable delay of activation but no significant reduction of maximum STIM1/Orai1 currents, other studies (147, 148) have determined a dominant negative effect of Orai1 R91W on SOCE in HEK cells. A series of concatenated tetramers of Orai1 proteins that contain increasing numbers of mutant Orai1 R91W linked to wild-type Orai1 proteins exhibit a gradual reduction of Ca^{2+} currents (40). Hence the diverse observations may arise from distinct channel stoichiometries due to different expression levels of wild-type Orai1 and its R91W mutant.

Despite a functional defect of Orai1 R91W its coupling to STIM1 is still largely preserved. A series of point mutants substituting the arginine at position 91 by various other amino acids ranging from charged to hydrophobic residues, has revealed that the increase in hydrophobicity at the N-terminus/transmembrane interface represents the major cause for yielding non-functional Orai channels (137). Structurally, this enhanced hydrophobicity may alter Orai channel conformation by altering the orientation of the first transmembrane helix in the plasma membrane thus resulting in loss of channel function. As Orai channels with an increased pore size (117) such as Orai1 E106D or Orai3 in the presence of 2-APB (126) that additionally include the R91W or R66W mutation, respectively, also remain non-functional, we have suggested a markedly disrupted permeation/gating for the Orai1 R91W mutant (137).

Navarro-Borelly *et al* (62) report that store depletion triggers molecular rearrangements in Orai1 which are possibly reflected by a decline in Orai1–Orai1 homomeric FRET. Such a reduction has been similarly detected for Orai1 R91W–Orai1 R91W homomers. They attributed this decrease in FRET to a conformational coupling induced by the coupling of STIM1, which occurs both for wild-type Orai1 as well as its R91W mutant. Hence, these authors (62) suggest a defect in permeation rather than channel gating of Orai1 R91W. Nevertheless, a potential constriction or collapse of the pore appears unlikely, as Orai mutants with increased pore sizes do not recover function. As permeation is somehow coupled to gating (117), both processes are probably defective in the Orai1 R91W mutant. The findings that an increased hydrophobicity at the N-terminus/transmembrane interface leads to disrupted channel function by disturbed permeation/gating offers a mechanistic interpretation of SCID-linked Orai1 R91W non-functionality. However, an ultimate proof for the suggested defect in permeation / gating structures awaits resolution at the atomic level by crystallizing the respective Orai1 proteins.

5. INACTIVATION OF ORAI AND CRAC CHANNELS

Accurate control of activation and inactivation of Ca^{2+} channels is a prerequisite for correct cell function. Fast inactivation of CRAC channels limits Ca^{2+} influx into the cell and thus represents an important feedback mechanism. It occurs over tens of milliseconds during hyperpolarizing voltage steps and results from feedback inhibition of channel activity by high cytoplasmic Ca^{2+} concentrations close to the channels pore mouth (113, 149). However, the resolution of the underlying mechanisms of rapid inactivation is only starting to emerge.

All store-operated Orai channels display fast inactivation within the first 100 ms of a voltage step, with that of Orai3 three times stronger compared to Orai1 or Orai2 (29, 70, 132). Subsequently Orai1 channels reactivate, while Orai2 and Orai3 currents exhibit a slow inactivation phase over 1 second (29, 132).

For native CRAC channels, Zweifach *et al.* (149) have demonstrated that fast inactivation is strongly reduced in the presence of Ba^{2+} compared to Ca^{2+}. It is of note that inactivation of native CRAC currents in RBL cells (17) as well as Jurkat T lymphocytes (149) is much more pronounced than that observed for Orai1 currents in HEK 293 cells. Moreover the characteristic reactivation phase of Orai1 currents is lacking for native CRAC currents. Apparently, additional factors contribute to inactivation of native CRAC currents, among which a potential involvement of other Orai proteins may be considered. Orai2 and Orai3 proteins when expressed together with

STIM1 in HEK293 cells exhibit indeed a stronger, fast inactivation than Orai1, and at least Orai2 expression is reported for RBL cells (29). Thus heteromeric assemblies with Orai1 possibly increasing the extent of inactivation are conceivable. Alternatively the distinct amount of inactivation in native tissue compared to over-expression systems might result from a distinct stoichiometry within a STIM1-Orai1 complex (150). Recently it has been reported that low Orai1 to STIM1 ratios produce CRAC currents with strong fast Ca^{2+}-dependent inactivation, while cells expressing high ratios yield attenuated inactivation (150). The results suggest that several key properties of Ca^{2+} channels formed by Orai1 depend on its interaction with STIM1. Consequently, the stoichiometry of this interaction may vary depending on the relative expression levels of these proteins and thus result in varying fast inactivation.

Further molecular determinants for inactivation of Orai channels are represented by cytosolic regions of the Orai channels as well as C-terminal regions within STIM1 (for an overview see Figure 1).

Regarding the role of Orai N-terminus in fast inactivation, an interaction of calmodulin (CaM) with a membrane proximal N-terminal domain in Orai1 has been identified (69). Specific mutations within this CaM binding domain (A73E, W76E, Y80E) which abrogate CaM binding have reduced fast inactivation (69). However, further detailed analysis is still required to elucidate how STIM1, Orai and CaM interplay. A potential effect of other Orai N-terminal regions like the arginine-/proline-rich domain within Orai1 or the conserved region close to the first transmembrane sequence on inactivation is still unresolved.

Transfer of the C-terminus of Orai1 onto Orai2 or Orai3 results in chimeric channels exhibiting diminished Orai1-type fast inactivation (70). Fast inactivation of Orai2 and Orai3 channels has been attributed to three conserved glutamates in their C-termini (70). Moreover a central region within the intracellular loop of Orai1 between TM2 and TM3 is essential for fast inactivation of Orai1 (151). Mutations within this loop region have decreased fast inactivation. Since fast inactivation of a concatamer containing three loop mutants and one wild-type Orai1 subunit is still present, it has been concluded that a single functional loop domain is sufficient for inactivation probably by blocking the pore. Expression of a peptide comprising the second loop inhibited Orai1 channel activity, suggesting a key role of the second loop as inactivation particle (151).

Yamashita *et al.* (117) have demonstrated that fast inactivation is additionally controlled by negatively charged residues within the outer pore vestibule of Orai1 channels. Their mutation to alanines has diminished Ca^{2+}-mediated fast inactivation concomitant to an alteration of ion permeation properties.

Hence, multiple domains within Orai channels have been identified contributing to current inactivation.

Within STIM1, an acidic cluster (aa 475-483) is indispensable for fast inactivation of all Orai channels (17, 69, 70). Mutations of all the negatively charged residues within this CRAC modulatory domain (CMD) to alanines inhibited or reduced fast inactivation of all Orai1-3 channels. Interestingly, partial D/E to alanine mutations revealed that four negative residues (aa475, 476, 478, 479) within CMD are sufficient for Orai1 (17) but not Orai3 (70) to accomplish fast inactivation. Since Orai1 displays besides fast inactivation, a unique slow reactivation phase, we further examined if it is influenced by the negative residues. However, CMD mutations or deletions in full-length STIM1 as well as STIM1 C-terminal fragments did not affect the extent of reactivation (data not shown). Similarly, fast inactivation of CRAC currents in RBL cells was reduced by over-expressing STIM1 C-terminus with the seven negatively charged residues mutated to alanines (STIM1 C-terminus 7xA) (17) compared to those expressing wild-type STIM1 C-terminus while the second phase of the inactivation profile remained unaffected.

As mutation of negative residues within Orai2 and Orai3 C-termini have been additionally shown to inhibit fast inactivation, Muallems group has proposed a model where the negative residues of both STIM1 and Orai C-termini act as Ca^{2+} sensor to reduce channel activity (70). Concomitant mutations of anionic amino acids in either C-terminus substantially but not fully inhibited fast inactivation. Hence, additional domains/components may contribute to this negative feedback mechanism.

5.1. 2-APB alters inactivation of Orai/CRAC channels

The dual 2-APB action on native CRAC currents of T-lymphocytes is also accompanied by alterations in the kinetics of fast Ca2+-dependent inactivation. Activation of these currents by concentrations of 1-5 μM 2-APB enhances the extent of fast inactivation. In contrast, inhibition by high concentrations of 2-APB is accompanied by the loss of fast Ca2+-dependent inactivation (115, 125, 149, 152). Based on the concomitant reduction of both CRAC current amplitude and inactivation, it has been hypothesized that these effects are possibly linked (125). Prakriya et al. (125) have hypothesized that 2-APB may interrupt the coupling between the CRAC channel and components mediating activation and fast inactivation. The hypothesized component mediating inactivation of CRAC currents (125) may be represented by STIM1. Whether 2-APB disrupts coupling of STIM1 to Orai is still a matter of debate. It seems that the mechanism of inhibition is more complex than simple pore blockade, particularly with the Orai3 isoform. The intriguing strong activation of Orai3 currents by high concentrations of 2-APB occurs along with a loss of fast inactivation and the induction of a robust reactivation phase (data not shown). In this case, 2-APB affects both activation and inactivation, but also the selectivity of the pore. Based on the findings that 2-APB stimulated Orai3 channels display an increased pore size, it appears likely that the pore is somewhat linked to the inactivation gate.

6. CONCLUSIONS

Store-operated Ca^{2+} channels have been extensively studied over the last 20 years before Orai1 and STIM1 has been identified as the two pivotal key players in the CRAC channel signaling machinery of T-cells (9). They are involved in a broad range of cellular processes together with their pharmacological, physiological and pathophysiological roles and an important role in autoimmune and inflammatory immune disorders (2). The identification of STIM1 and Orai1-3 and their role in calcium signaling through SOC/CRAC channels opens the repertoire for targeting immune diseases, like rheumatoid arthritis, inflammatory disorders, allograft rejection (138). In lymphocytes, mast cells as well as platelets, novel drug development is additionally clear in light of the uniquely important roles of the Orai proteins (138).

Various proteins have been already identified which additionally couple to and/or modulate the STIM1/Orai signaling machinery (Figure 2). Their overall interplay expected to be tissue-specific awaits detailed characterization. Although these additional proteins are dispensable for basic STIM1/Orai function, they may still have a modulatory impact fine-tuning the CRAC channel machinery.

The identification of STIM1 and Orai proteins has further allowed characterizing molecular events and structure-function relationships governing CRAC current activation, permeation and inactivation (Figure 1). However, within this signaling cascade various processes still require more detailed elucidation. Particularly, the activation step (s) transforming STIM1 interaction with Orai1 into the opening of CRAC channels is only partially understood. FRET microscopy studies have provided evidence for a conformational change of the intracellular N- and C-termini of Orai1 upon binding to STIM1 (62). These changes may be coupled to movements of the gate and provide the energy required for CRAC channel opening. This process may be similarly mirrored in conformational rearrangement within STIM1 which has been recently suggested to involve an intramolecular switching mechanism (50).

While the Orai1 C-terminus is an essential region for coupling to the CAD/SOAR domain in STIM1, an important role of the Orai1 N-terminus for the gating has recently been reported (153). The proposed bridging of Orai N- and C-termini via STIM1 together with the identification of the respective interactions sites on both proteins has still to be demonstrated.

While several Orai domains contribute to the in-/reactivation behaviour, those that are responsible for the distinct subtype-specific gating profiles have so far remained elusive. A chimeric approach with single to multiple domain exchange may reveal a potential interdependence of domains in controlling inactivation/reactivation.

A 3D atomic resolution of Orai and STIM1 proteins, preferentially as complex, will reveal ultimate insight not only in the gating process, but also on the unusual selectivity filter of this ligand-gated Ca^{2+} channel, and pave the way for rational drug design.

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8. REFERENCES

1. M. J. Berridge, M. D. Bootman and H. L. Roderick: Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*, 4(7), 517-29 (2003)

2. A. B. Parekh and J. W. Putney, Jr.: Store-operated calcium channels. *Physiol Rev*, 85(2), 757-810 (2005)

3. M. A. Spassova, J. Soboloff, L. P. He, T. Hewavitharana, W. Xu, K. Venkatachalam, D. B. van Rossum, R. L. Patterson and D. L. Gill: Calcium entry mediated by SOCs and TRP channels: variations and enigma. *Biochim Biophys Acta*, 1742(1-3), 9-20 (2004)

4. D. Dutta: Mechanism of store-operated calcium entry. *J Biosci*, 25(4), 397-404 (2000)

5. R. Chakrabarti: Calcium signaling in non-excitable cells: Ca2+ release and influx are independent events linked to two plasma membrane Ca2+ entry channels. *J Cell Biochem*, 99(6), 1503-16 (2006)

6. J. Liou, M. L. Kim, W. D. Heo, J. T. Jones, J. W. Myers, J. E. Ferrell, Jr. and T. Meyer: STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol*, 15(13), 1235-41 (2005)

7. J. Roos, P. J. DiGregorio, A. V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J. A. Kozak, S. L. Wagner, M. D. Cahalan, G. Velicelebi and K. A. Stauderman: STIM1, an essential and conserved component of store-operated Ca2+ channel function. *J Cell Biol*, 169(3), 435-45 (2005)

8. S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S. H. Puppel, B. Tanasa, P. G. Hogan, R. S. Lewis, M. Daly and A. Rao: A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*, 441(7090), 179-85 (2006)

9. M. Vig, C. Peinelt, A. Beck, D. L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner and J. P. Kinet: CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. *Science*, 312(5777), 1220-3 (2006)

10. S. L. Zhang, A. V. Yeromin, X. H. Zhang, Y. Yu, O. Safrina, A. Penna, J. Roos, K. A. Stauderman and M. D. Cahalan: Genome-wide RNAi screen of Ca(2+) influx

identifies genes that regulate Ca(2+) release-activated Ca(2+) channel activity. *Proc Natl Acad Sci U S A*, 103(24), 9357-62 (2006)

11. M. A. Spassova, J. Soboloff, L. P. He, W. Xu, M. A. Dziadek and D. L. Gill: STIM1 has a plasma membrane role in the activation of store-operated Ca(2+) channels. *Proc Natl Acad Sci U S A*, 103(11), 4040-5 (2006)

12. P. G. Hogan, R. S. Lewis and A. Rao: Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol*, 28, 491-533 (2010)

13. Y. Baba, K. Hayashi, Y. Fujii, A. Mizushima, H. Watarai, M. Wakamori, T. Numaga, Y. Mori, M. Iino, M. Hikida and T. Kurosaki: Coupling of STIM1 to store-operated Ca2+ entry through its constitutive and inducible movement in the endoplasmic reticulum. *Proc Natl Acad Sci U S A*, 103(45), 16704-9 (2006)

14. J. T. Smyth, W. I. Dehaven, B. F. Jones, J. C. Mercer, M. Trebak, G. Vazquez and J. W. Putney, Jr.: Emerging perspectives in store-operated Ca2+ entry: roles of Orai, Stim and TRP. *Biochim Biophys Acta*, 1763(11), 1147-60 (2006)

15. G. N. Huang, W. Zeng, J. Y. Kim, J. P. Yuan, L. Han, S. Muallem and P. F. Worley: STIM1 carboxyl-terminus activates native SOC, I(crac) and TRPC1 channels. *Nat Cell Biol*, 8(9), 1003-10 (2006)

16. I. Derler, M. Fahrner, M. Muik, B. Lackner, R. Schindl, K. Groschner and C. Romanin: A Ca2(+)release-activated Ca2(+) (CRAC) modulatory domain (CMD) within STIM1 mediates fast Ca2(+)-dependent inactivation of ORAI1 channels. *J Biol Chem*, 284(37), 24933-8 (2009)

17. M. Muik, M. Fahrner, I. Derler, R. Schindl, J. Bergsmann, I. Frischauf, K. Groschner and C. Romanin: A Cytosolic Homomerization and a Modulatory Domain within STIM1 C Terminus Determine Coupling to ORAI1 Channels. *J Biol Chem*, 284(13), 8421-6 (2009)

18. J. P. Yuan, W. Zeng, M. R. Dorwart, Y. J. Choi, P. F. Worley and S. Muallem: SOAR and the polybasic STIM1 domains gate and regulate Orai channels. *Nat Cell Biol*, 11(3), 337-43 (2009)

19. C. Y. Park, P. J. Hoover, F. M. Mullins, P. Bachhawat, E. D. Covington, S. Raunser, T. Walz, K. C. Garcia, R. E. Dolmetsch and R. S. Lewis: STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell*, 136(5), 876-90 (2009)

20. T. Kawasaki, I. Lange and S. Feske: A minimal regulatory domain in the C terminus of STIM1 binds to and activates ORAI1 CRAC channels. *Biochem Biophys Res Commun*, 385(1), 49-54 (2009)

21. Y. Zhou, S. Mancarella, Y. Wang, C. Yue, M. Ritchie, D. L. Gill and J. Soboloff: The short N-terminal domains of STIM1 and STIM2 control the activation kinetics of Orai1 channels. *J Biol Chem*, 284(29), 19164-8 (2009)

22. L. Zheng, P. B. Stathopulos, G. Y. Li and M. Ikura: Biophysical characterization of the EF-hand and SAM domain containing Ca2+ sensory region of STIM1 and STIM2. *Biochem Biophys Res Commun*, 369(1), 240-6 (2008)

23. C. Peinelt, M. Vig, D. L. Koomoa, A. Beck, M. J. Nadler, M. Koblan-Huberson, A. Lis, A. Fleig, R. Penner and J. P. Kinet: Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat Cell Biol*, 8(7), 771-3 (2006)

24. Y. Takahashi, M. Murakami, H. Watanabe, H. Hasegawa, T. Ohba, Y. Munehisa, K. Nobori, K. Ono, T. Iijima and H. Ito: Essential role of the N-terminus of murine Orai1 in store-operated Ca2+ entry. *Biochem Biophys Res Commun*, 356(1), 45-52 (2007)

25. M. D. Cahalan, S. L. Zhang, A. V. Yeromin, K. Ohlsen, J. Roos and K. A. Stauderman: Molecular basis of the CRAC channel. *Cell Calcium*, 42(2), 133-44 (2007)

26. I. Frischauf, M. Muik, I. Derler, J. Bergsmann, M. Fahrner, R. Schindl, K. Groschner and C. Romanin: Molecular determinants of the coupling between STIM1 and Orai channels: differential activation of Orai1-3 channels by a STIM1 coiled-coil mutant. *J Biol Chem*, 284(32), 21696-706 (2009)

27. M. Muik, I. Frischauf, I. Derler, M. Fahrner, J. Bergsmann, P. Eder, R. Schindl, C. Hesch, B. Polzinger, R. Fritsch, H. Kahr, J. Madl, H. Gruber, K. Groschner and C. Romanin: Dynamic Coupling of the Putative Coiled-coil Domain of ORAI1 with STIM1 Mediates ORAI1 Channel Activation. *J Biol Chem*, 283(12), 8014-22 (2008)

28. A. Lis, C. Peinelt, A. Beck, S. Parvez, M. Monteilh-Zoller, A. Fleig and R. Penner: CRACM1, CRACM2, and CRACM3 are store-operated Ca2+ channels with distinct functional properties. *Curr Biol*, 17(9), 794-800 (2007)

29. Y. Gwack, S. Srikanth, S. Feske, F. Cruz-Guilloty, M. Oh-hora, D. S. Neems, P. G. Hogan and A. Rao: Biochemical and functional characterization of Orai proteins. J Biol Chem, 282(22), 16232-43 (2007)

30. Y. Zhou, S. Ramachandran, M. Oh-Hora, A. Rao and P. G. Hogan: Pore architecture of the ORAI1 store-operated calcium channel. Proc Natl Acad Sci U S A, 107(11), 4896-901 (2010)

31. Y. Maruyama, T. Ogura, K. Mio, K. Kato, T. Kaneko, S. Kiyonaka, Y. Mori and C. Sato: Tetrameric Orai1 is a teardrop-shaped molecule with a long, tapered cytoplasmic domain. J Biol Chem (2009)

32. O. Mignen, J. L. Thompson and T. J. Shuttleworth: Orai1 subunit stoichiometry of the mammalian CRAC channel pore. J Physiol, 586(2), 419-25 (2008)

33. W. Ji, P. Xu, Z. Li, J. Lu, L. Liu, Y. Zhan, Y. Chen, B. Hille, T. Xu and L. Chen: Functional stoichiometry of the unitary calcium-release-activated calcium channel. *Proc Natl Acad Sci U S A*, 105(36), 13668-73 (2008)

34. A. Penna, A. Demuro, A. V. Yeromin, S. L. Zhang, O. Safrina, I. Parker and M. D. Cahalan: The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. *Nature*, 456(7218), 116-20 (2008)

35. J. Madl, J. Weghuber, R. Fritsch, I. Derler, M. Fahrner, I. Frischauf, B. Lackner, C. Romanin and G. J. Schutz: Resting state Orai1 diffuses as homotetramer in the plasma membrane of live mammalian cells. *The Journal of biological chemistry*, 285(52), 41135-42 (2010)

36. S. L. Zhang, J. A. Kozak, W. Jiang, A. V. Yeromin, J. Chen, Y. Yu, A. Penna, W. Shen, V. Chi and M. D. Cahalan: Store-dependent and -independent modes regulating Ca2+ release-activated Ca2+ channel activity of human Orai1 and Orai3. *J Biol Chem*, 283(25), 17662-71 (2008)

37. O. Mignen, J. L. Thompson and T. J. Shuttleworth: STIM1 regulates Ca2+ entry via arachidonate-regulated Ca2+-selective (ARC) channels without store depletion or translocation to the plasma membrane. *J Physiol*, 579(Pt 3), 703-15 (2007)

38. O. Mignen, J. L. Thompson and T. J. Shuttleworth: Both Orai1 and Orai3 are essential components of the arachidonate-regulated Ca2+-selective (ARC) channels. *J Physiol*, 586(1), 185-95 (2008)

39. J. L. Thompson, O. Mignen and T. J. Shuttleworth: The Orai1 severe combined immune deficiency mutation and calcium release-activated Ca2+ channel function in the heterozygous condition. *J Biol Chem*, 284(11), 6620-6 (2009)

40. J. Thompson, O. Mignen and T. J. Shuttleworth: The N-terminal domain of Orai3 determines selectivity for activation of the store-independent ARC channel by arachidonic acid. *Channels*, 4(5), 398-410 (2010)

41. Z. Li, J. Lu, P. Xu, X. Xie, L. Chen and T. Xu: Mapping the interacting domains of STIM1 and Orai1 in Ca2+ release-activated Ca2+ channel activation. *J Biol Chem*, 282(40), 29448-56 (2007)

42. M. Fahrner, M. Muik, I. Derler, R. Schindl, R. Fritsch, I. Frischauf and C. Romanin: Mechanistic view on domains mediating STIM1-Orai coupling. *Immunol Rev*, 231(1), 99-112 (2009)

43. S. L. Zhang, Y. Yu, J. Roos, J. A. Kozak, T. J. Deerinck, M. H. Ellisman, K. A. Stauderman and M. D. Cahalan: STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. *Nature*, 437(7060), 902-5 (2005)

44. R. M. Luik, M. M. Wu, J. Buchanan and R. S. Lewis: The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions. *J Cell Biol*, 174(6), 815-25 (2006)

45. P. Xu, J. Lu, Z. Li, X. Yu, L. Chen and T. Xu: Aggregation of STIM1 underneath the plasma membrane induces clustering of Orai1. *Biochem Biophys Res Commun*, 350(4), 969-76 (2006)

46. J. Liou, M. Fivaz, T. Inoue and T. Meyer: Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca2+ store depletion. *Proc Natl Acad Sci U S A*, 104(22), 9301-6 (2007)

47. M. M. Wu, J. Buchanan, R. M. Luik and R. S. Lewis: Ca2+ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J Cell Biol*, 174(6), 803-13 (2006)

48. P. Varnai, B. Toth, D. J. Toth, L. Hunyady and T. Balla: Visualization and manipulation of plasma membrane-endoplasmic reticulum contact sites indicates the presence of additional molecular components within the STIM1-Orai1 Complex. *J Biol Chem*, 282(40), 29678-90 (2007)

49. M. K. Korzeniowski, I. M. Manjarres, P. Varnai and T. Balla: Activation of STIM1-Orai1 Involves an Intramolecular Switching Mechanism. *Sci Signal*, 3(148), ra82 (2010) doi:3/148/ra82 [pii]

50. M. Muik, M. Fahrner, R. Schindl, P. Stathopulos, I. Frischauf, I. Derler, P. Plenk, B. Lackner, K. Groschner, M. Ikura and C. Romanin: STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. *The EMBO journal* (2011)

51. Z. Li, L. Liu, Y. Deng, W. Ji, W. Du, P. Xu, L. Chen and T. Xu: Graded activation of CRAC channel by binding of different numbers of STIM1 to Orai1 subunits. *Cell research*, 21(2), 305-15 (2011)

52. I. Grigoriev, S. M. Gouveia, B. van der Vaart, J. Demmers, J. T. Smyth, S. Honnappa, D. Splinter, M. O. Steinmetz, J. W. Putney, Jr., C. C. Hoogenraad and A. Akhmanova: STIM1 Is a MT-Plus-End-Tracking Protein Involved in Remodeling of the ER. *Curr Biol*, 18(3), 177-182 (2008)

53. J. T. Smyth, W. I. DeHaven, G. S. Bird and J. W. Putney, Jr.: Role of the microtubule cytoskeleton in the function of the store-operated Ca2+ channel activator STIM1. *J Cell Sci*, 120(Pt 21), 3762-71 (2007)

54. M. Brini, P. Pinton, T. Pozzan and R. Rizzuto: Targeted recombinant aequorins: tools for monitoring [Ca2+] in the various compartments of a living cell. *Microsc Res Tech*, 46(6), 380-9 (1999)

55. R. Yu and P. M. Hinkle: Rapid turnover of calcium in the endoplasmic reticulum during signaling. Studies with cameleon calcium indicators. *J Biol Chem*, 275(31), 23648-53 (2000)

56. O. Brandman, J. Liou, W. S. Park and T. Meyer: STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. *Cell*, 131(7), 1327-39 (2007)

57. R. M. Luik, B. Wang, M. Prakriya, M. M. Wu and R. S. Lewis: Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature*, 454(7203), 538-42 (2008)

58. P. B. Stathopulos, L. Zheng, G. Y. Li, M. J. Plevin and M. Ikura: Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell*, 135(1), 110-22 (2008)

59. P. B. Stathopulos, G. Y. Li, M. J. Plevin, J. B. Ames and M. Ikura: Stored Ca2+ depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: An initiation mechanism for capacitive Ca2+ entry. *J Biol Chem*, 281(47), 35855-62 (2006)

60. E. D. Covington, M. M. Wu and R. S. Lewis: Essential role for the CRAC activation domain in store-dependent oligomerization of STIM1. *Molecular biology of the cell*, 21(11), 1897-907 (2010)

61. L. Navarro-Borelly, A. Somasundaram, M. Yamashita, D. Ren, R. J. Miller and M. Prakriya: STIM1-Orai1 interactions and Orai1 conformational changes revealed by live-cell FRET microscopy. *J Physiol*, 586(Pt 22), 5383-401 (2008)

62. N. Calloway, M. Vig, J. P. Kinet, D. Holowka and B. Baird: Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca2+ stores and on electrostatic interactions. *Mol Biol Cell*, 20(1), 389-99 (2009)

63. N. Calloway, D. Holowka and B. Baird: A basic sequence in STIM1 promotes Ca2+ influx by interacting with the C-terminal acidic coiled coil of Orai1. *Biochemistry*, 49(6), 1067-71

64. J. J. Hull, J. M. Lee, R. Kajigaya and S. Matsumoto: Bombyx mori homologs of STIM1 and Orai1 are essential components of the signal transduction cascade that regulates sex pheromone production. *J Biol Chem*, 284(45), 31200-13 (2009)

65. D. N. Woolfson: The design of coiled-coil structures and assemblies. *Adv Protein Chem*, 70, 79-112 (2005)

66. R. Fairman, H. G. Chao, T. B. Lavoie, J. J. Villafranca, G. R. Matsueda and J. Novotny: Design of heterotetrameric coiled coils: evidence for increased stabilization by Glu(-)-Lys(+) ion pair interactions. *Biochemistry*, 35(9), 2824-9 (1996)

67. A. Lis, S. Zierler, C. Peinelt, A. Fleig and R. Penner: A single lysine in the N-terminal region of store-operated channels is critical for STIM1-mediated gating. *The Journal of general physiology*, 136(6), 673-86 (2010)

68. F. M. Mullins, C. Y. Park, R. E. Dolmetsch and R. S. Lewis: STIM1 and calmodulin interact with Orai1 to induce Ca2+-dependent inactivation of CRAC channels. *Proc Natl Acad Sci U S A*, 106(36), 15495-500 (2009)

69. K. P. Lee, J. P. Yuan, W. Zeng, I. So, P. F. Worley and S. Muallem: Molecular determinants of fast Ca2+-dependent inactivation and gating of the Orai channels. *Proc Natl Acad Sci U S A*, 106(34), 14687-92 (2009)

70. A. V. Yeromin, S. L. Zhang, W. Jiang, Y. Yu, O. Safrina and M. D. Cahalan: Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature*, 443(7108), 226-9 (2006)

71. P. Csutora, K. Peter, H. Kilic, K. M. Park, V. Zarayskiy, T. Gwozdz and V. M. Bolotina: Novel role for STIM1 as a trigger for calcium influx factor production. *J Biol Chem*, 283(21), 14524-31 (2008)

72. V. A. Barr, K. M. Bernot, S. Srikanth, Y. Gwack, L. Balagopalan, C. K. Regan, D. J. Helman, C. L. Sommers, M. Oh-Hora, A. Rao and L. E. 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(7), 2802-17 (2008)

73. Y. Zhou, P. Meraner, H. T. Kwon, D. Machnes, M. Oh-hora, J. Zimmer, Y. Huang, A. Stura, A. Rao and P. G. Hogan: STIM1 gates the store-operated calcium channel ORAI1 *in vitro*. *Nat Struct Mol Biol*, 17(1), 112-6 (2010)

74. S. Parvez, A. Beck, C. Peinelt, J. Soboloff, A. Lis, M. Monteilh-Zoller, D. L. Gill, A. Fleig and R. Penner: STIM2 protein mediates distinct store-dependent and store-independent modes of CRAC channel activation. *Faseb J*, 22(3), 752-61 (2008)

75. S. Srikanth, H. J. Jung, K. D. Kim, P. Souda, J. Whitelegge and Y. Gwack: A novel EF-hand protein, CRACR2A, is a cytosolic Ca2+ sensor that stabilizes CRAC channels in T cells. *Nat Cell Biol*, 12(5), 436-46 (2010)

76. D. Howie, K. F. Nolan, S. Daley, E. Butterfield, E. Adams, H. Garcia-Rueda, C. Thompson, N. J. Saunders, S. P. Cobbold, Y. Tone, M. Tone and H. Waldmann: MS4A4B is a GITR-associated membrane adapter, expressed by regulatory T cells, which modulates T cell activation. *J Immunol*, 183(7), 4197-204 (2009)

77. C. M. Walsh, M. K. Doherty, A. V. Tepikin and R. D. Burgoyne: Evidence for an interaction between Golli and

STIM1 in store-operated calcium entry. *The Biochemical journal*, 430(3), 453-60 (2010)

78. A. C. Martin, D. Willoughby, A. Ciruela, L. J. Ayling, M. Pagano, S. Wachten, A. Tengholm and D. M. Cooper: Capacitative Ca2+ entry via Orai1 and stromal interacting molecule 1 (STIM1) regulates adenylyl cyclase type 8. *Mol Pharmacol*, 75(4), 830-42 (2009)

79. O. M. Woodward, Y. Li, S. Yu, P. Greenwell, C. Wodarczyk, A. Boletta, W. B. Guggino and F. Qian: Identification of a polycystin-1 cleavage product, P100, that regulates store operated Ca entry through interactions with STIM1. *PLoS One*, 5(8), e12305 (2010)

80. F. Yu, L. Sun and K. Machaca: Constitutive recycling of the store-operated Ca2+ channel Orai1 and its internalization during meiosis. *J Cell Biol*, 191(3), 523-35 (2010)

81. M. Feng, D. M. Grice, H. M. Faddy, N. Nguyen, S. Leitch, Y. Wang, S. Muend, P. A. Kenny, S. Sukumar, S. J. Roberts-Thomson, G. R. Monteith and R. Rao: Store-independent activation of Orai1 by SPCA2 in mammary tumors. *Cell*, 143(1), 84-98 (2010)

82. C. Y. Park, A. Shcheglovitov and R. Dolmetsch: The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science*, 330(6000), 101-5 (2010)

83. Y. Wang, X. Deng, S. Mancarella, E. Hendron, S. Eguchi, J. Soboloff, X. D. Tang and D. L. Gill: The calcium store sensor, STIM1, reciprocally controls Orai and CaV1.2 channels. *Science*, 330(6000), 105-9 (2010)

84. M. Chvanov, C. M. Walsh, L. P. Haynes, S. G. Voronina, G. Lur, O. V. Gerasimenko, R. Barraclough, P. S. Rudland, O. H. Petersen, R. D. Burgoyne and A. V. Tepikin: ATP depletion induces translocation of STIM1 to puncta and formation of STIM1-ORAI1 clusters: translocation and re-translocation of STIM1 does not require ATP. *Pflugers Arch*, 457(2), 505-17 (2008)

85. C. M. Walsh, M. Chvanov, L. P. Haynes, O. H. Petersen, A. V. Tepikin and R. D. Burgoyne: Role of phosphoinositides in STIM1 dynamics and store-operated calcium entry. *The Biochemical journal*, 425(1), 159-68 (2010)

86. M. K. Korzeniowski, M. A. Popovic, Z. Szentpetery, P. Varnai, S. S. Stojilkovic and T. Balla: Dependence of STIM1/Orai1-mediated calcium entry on plasma membrane phosphoinositides. *J Biol Chem*, 284(31), 21027-35 (2009)

87. Y. Saimi and C. Kung: Calmodulin as an ion channel subunit. *Annu Rev Physiol*, 64, 289-311 (2002)

88. L. Vaca: SOCIC: the store-operated calcium influx complex. *Cell Calcium*, 47(3), 199-209 (2010)

89. B. Pani, H. L. Ong, X. Liu, K. Rauser, I. S. Ambudkar and B. B. Singh: Lipid rafts determine clustering of STIM1 in endoplasmic reticulum-plasma membrane junctions and regulation of store-operated Ca2+ entry (SOCE). *J Biol Chem*, 283(25), 17333-40 (2008)

90. I. Jardin, G. M. Salido and J. A. Rosado: Role of lipid rafts in the interaction between hTRPC1, Orai1 and STIM1. *Channels (Austin)*, 2(6), 401-3 (2008)

91. Z. Korade and A. K. Kenworthy: Lipid rafts, cholesterol, and the brain. *Neuropharmacology*, 55(8), 1265-73 (2008)

92. L. J. Pike: The challenge of lipid rafts. *J Lipid Res*, 50 Suppl, S323-8 (2009)

93. I. Jardin, G. M. Salido and J. A. Rosado: Role of lipid rafts in the interaction between hTRPC1, Orai1 and STIM1. *Channels (Austin)*, 2(6) (2008)

94. W. I. DeHaven, B. F. Jones, J. G. Petranka, J. T. Smyth, T. Tomita, G. S. Bird and J. W. Putney, Jr.: TRPC channels function independently of STIM1 and Orai1. *J Physiol*, 587(Pt 10), 2275-98 (2009)

95. C. Galan, G. E. Woodard, N. Dionisio, G. M. Salido and J. A. Rosado: Lipid rafts modulate the activation but not the maintenance of store-operated Ca(2+) entry. *Biochimica et biophysica acta*, 1803(9), 1083-93 (2010)

96. E. Ercan, F. Momburg, U. Engel, K. Temmerman, W. Nickel and M. Seedorf: A conserved, lipid-mediated sorting mechanism of yeast Ist2 and mammalian STIM proteins to the peripheral ER. *Traffic*, 10(12), 1802-18 (2009)

97. S. S. Manji, N. J. Parker, R. T. Williams, L. van Stekelenburg, R. B. Pearson, M. Dziadek and P. J. Smith: STIM1: a novel phosphoprotein located at the cell surface. *Biochim Biophys Acta*, 1481(1), 147-55 (2000)

98. J. T. Smyth, J. G. Petranka, R. R. Boyles, W. I. DeHaven, M. Fukushima, K. L. Johnson, J. G. Williams and J. W. Putney, Jr.: Phosphorylation of STIM1 underlies suppression of store-operated calcium entry during mitosis. *Nat Cell Biol*, 11(12), 1465-72 (2009)

99. S. F. Preston, R. I. Sha'afi and R. D. Berlin: Regulation of Ca2+ influx during mitosis: Ca2+ influx and depletion of intracellular Ca2+ stores are coupled in interphase but not mitosis. *Cell Regul*, 2(11), 915-25 (1991)

100. E. Pozo-Guisado, D. G. Campbell, M. Deak, A. Alvarez-Barrientos, N. A. Morrice, I. S. Alvarez, D. R. Alessi and F. J. Martin-Romero: Phosphorylation of STIM1 at ERK1/2 target sites modulates store-operated calcium entry. *Journal of cell science*, 123(Pt 18), 3084-93 (2010)

101. F. Yu, L. Sun and K. Machaca: Orai1 internalization and STIM1 clustering inhibition modulate SOCE inactivation

during meiosis. *Proc Natl Acad Sci U S A*, 106(41), 17401-6 (2009)

102. T. Kawasaki, T. Ueyama, I. Lange, S. Feske and N. Saito: Protein kinase C-induced phosphorylation of Orai1 regulates the intracellular Ca2+ level via the store-operated Ca2+ channel. *The Journal of biological chemistry*, 285(33), 25720-30 (2010)

103. W. Droge: Free radicals in the physiological control of cell function. *Physiol Rev*, 82(1), 47-95 (2002)

104. S. G. Rhee: Cell signaling. H2O2, a necessary evil for cell signaling. *Science*, 312(5782), 1882-3 (2006)

105. J. D. Lambeth: NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol*, 4(3), 181-9 (2004)

106. A. A. Starkov: The role of mitochondria in reactive oxygen species metabolism and signaling. *Ann N Y Acad Sci*, 1147, 37-52 (2008)

107. I. Fridovich: Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? *Ann N Y Acad Sci*, 893, 13-8 (1999)

108. I. Bogeski, C. Kummerow, D. Al-Ansary, E. C. Schwarz, R. Koehler, D. Kozai, N. Takahashi, C. Peinelt, D. Griesemer, M. Bozem, Y. Mori, M. Hoth and B. A. Niemeyer: Differential redox regulation of ORAI ion channels: a mechanism to tune cellular calcium signaling. *Sci Signal*, 3(115), ra24 (2010)

109. M. Grupe, G. Myers, R. Penner and A. Fleig: Activation of store-operated I(CRAC) by hydrogen peroxide. *Cell Calcium*, 48(1), 1-9 (2010)

110. B. J. Hawkins, K. M. Irrinki, K. Mallilankaraman, Y. C. Lien, Y. Wang, C. D. Bhanumathy, R. Subbiah, M. F. Ritchie, J. Soboloff, Y. Baba, T. Kurosaki, S. K. Joseph, D. L. Gill and M. Madesh: S-glutathionylation activates STIM1 and alters mitochondrial homeostasis. *The Journal of cell biology*, 190(3), 391-405 (2010)

111. M. Hoth and R. Penner: Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature*, 355(6358), 353-6 (1992)

112. M. Hoth and R. Penner: Calcium release-activated calcium current in rat mast cells. *J Physiol*, 465, 359-86 (1993)

113. W. I. DeHaven, J. T. Smyth, R. R. Boyles, G. S. Bird and J. W. Putney, Jr.: Complex actions of 2-aminoethyldiphenyl borate on store-operated calcium entry. *J Biol Chem*, 283(28), 19265-73 (2008)

114. M. Prakriya: The molecular physiology of CRAC channels. *Immunol Rev*, 231(1), 88-98 (2009)

115. Z. Su, R. L. Shoemaker, R. B. Marchase and J. E. Blalock: Ca2+ modulation of Ca2+ release-activated Ca2+ channels is responsible for the inactivation of its monovalent cation current. *Biophys J*, 86(2), 805-14 (2004)

116. M. Yamashita, L. Navarro-Borelly, B. A. McNally and M. Prakriya: Orai1 mutations alter ion permeation and Ca2+-dependent fast inactivation of CRAC channels: evidence for coupling of permeation and gating. *J Gen Physiol*, 130(5), 525-40 (2007)

117. W. I. DeHaven, J. T. Smyth, R. R. Boyles and J. W. Putney, Jr.: Calcium inhibition and calcium potentiation of Orai1, Orai2, and Orai3 calcium release-activated calcium channels. *J Biol Chem*, 282(24), 17548-56 (2007)

118. A. Lepple-Wienhues and M. D. Cahalan: Conductance and permeation of monovalent cations through depletion-activated Ca2+ channels (ICRAC) in Jurkat T cells. *Biophys J*, 71(2), 787-94 (1996)

119. M. Prakriya and R. S. Lewis: Regulation of CRAC channel activity by recruitment of silent channels to a high open-probability gating mode. *J Gen Physiol*, 128(3), 373-86 (2006)

120. M. Prakriya and R. S. Lewis: Separation and characterization of currents through store-operated CRAC channels and Mg2+-inhibited cation (MIC) channels. *J Gen Physiol*, 119(5), 487-507 (2002)

121. T. Voets, J. Prenen, A. Fleig, R. Vennekens, H. Watanabe, J. G. Hoenderop, R. J. Bindels, G. Droogmans, R. Penner and B. Nilius: CaT1 and the calcium release-activated calcium channel manifest distinct pore properties. *J Biol Chem*, 276(51), 47767-70 (2001)

122. P. Hess, J. B. Lansman and R. W. Tsien: Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. *J Gen Physiol*, 88(3), 293-319 (1986)

123. D. M. Liu and D. J. Adams: Ionic selectivity of native ATP-activated (P2X) receptor channels in dissociated neurones from rat parasympathetic ganglia. *J Physiol*, 534(Pt. 2), 423-35 (2001)

124. M. Prakriya and R. S. Lewis: Potentiation and inhibition of Ca(2+) release-activated Ca(2+) channels by 2-aminoethyldiphenyl borate (2-APB) occurs independently of IP(3) receptors. *J Physiol*, 536(Pt 1), 3-19 (2001)

125. R. Schindl, J. Bergsmann, I. Frischauf, I. Derler, M. Fahrner, M. Muik, R. Fritsch, K. Groschner and C. Romanin: 2-aminoethoxydiphenyl borate alters selectivity of Orai3 channels by increasing their pore size. *J Biol Chem*, 283(29), 20261-7 (2008)

126. A. Zweifach and R. S. Lewis: Mitogen-regulated Ca2+ current of T lymphocytes is activated by depletion

of intracellular Ca2+ stores. *Proc Natl Acad Sci U S A*, 90(13), 6295-9 (1993)

127. M. Prakriya, S. Feske, Y. Gwack, S. Srikanth, A. Rao and P. G. Hogan: Orai1 is an essential pore subunit of the CRAC channel. *Nature*, 443(7108), 230-3 (2006)

128. M. Vig, A. Beck, J. M. Billingsley, A. Lis, S. Parvez, C. Peinelt, D. L. Koomoa, J. Soboloff, D. L. Gill, A. Fleig, J. P. Kinet and R. Penner: CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr Biol*, 16(20), 2073-9 (2006)

129. B. A. McNally, M. Yamashita, A. Engh and M. Prakriya: Structural determinants of ion permeation in CRAC channels. *Proc Natl Acad Sci U S A*, 106(52), 22516-21 (2009)

130. Y. Zhou, S. Ramachandran, M. Oh-Hora, A. Rao and P. G. Hogan: Pore architecture of the ORAI1 store-operated calcium channel. *Proc Natl Acad Sci U S A*, 107(11), 4896-901

131. R. Schindl, I. Frischauf, J. Bergsmann, M. Muik, I. Derler, B. Lackner, K. Groschner and C. Romanin: Plasticity in Ca2+ selectivity of Orai1/Orai3 heteromeric channel. *Proc Natl Acad Sci U S A*, 106(46), 19623-8 (2009)

132. O. Mignen, J. L. Thompson and T. J. Shuttleworth: The molecular architecture of the arachidonate-regulated Ca2+-selective ARC channel is a pentameric assembly of Orai1 and Orai3 subunits. *J Physiol*, 587(Pt 17), 4181-97 (2009)

133. R. K. Motiani, I. F. Abdullaev and M. Trebak: A novel native store-operated calcium channel encoded by Orai3: selective requirement of Orai3 versus Orai1 in estrogen receptor-positive versus estrogen receptor-negative breast cancer cells. *The Journal of biological chemistry*, 285(25), 19173-83 (2010)

134. B. F. Jones, R. R. Boyles, S. Y. Hwang, G. S. Bird and J. W. Putney: Calcium influx mechanisms underlying calcium oscillations in rat hepatocytes. *Hepatology*, 48(4), 1273-81 (2008)

135. S. E. Peel, B. Liu and I. P. Hall: ORAI and store-operated calcium influx in human airway smooth muscle cells. *Am J Respir Cell Mol Biol*, 38(6), 744-9 (2008)

136. I. Derler, M. Fahrner, O. Carugo, M. Muik, J. Bergsmann, R. Schindl, I. Frischauf, S. Eshaghi and C. Romanin: Increased hydrophobicity at the N terminus/membrane interface impairs gating of the severe combined immunodeficiency-related ORAI1 mutant. *J Biol Chem*, 284(23), 15903-15 (2009)

137. I. Derler, R. Fritsch, R. Schindl and C. Romanin: CRAC inhibitors: identification and potential. *Expert Opinion on Drug Discovery*, 3(7), 787-800 (2008)

138. J. W. Putney: Pharmacology of Store-operated Calcium Channels. *Mol Interv*, 10(4), 209-18 (2010)

139. C. Peinelt, A. Lis, A. Beck, A. Fleig and R. Penner: 2-Aminoethoxydiphenyl borate directly facilitates and indirectly inhibits STIM1-dependent gating of CRAC channels. *J Physiol*, 586(13), 3061-73 (2008)

140. Y. Wang, X. Deng, Y. Zhou, E. Hendron, S. Mancarella, M. F. Ritchie, X. D. Tang, Y. Baba, T. Kurosaki, Y. Mori, J. Soboloff and D. L. Gill: STIM protein coupling in the activation of Orai channels. *Proc Natl Acad Sci U S A*, 106(18), 7391-6 (2009)

141. R. H. Buckley: The multiple causes of human SCID. *J Clin Invest*, 114(10), 1409-11 (2004)

142. H. P. Carroll, B. B. McNaull and M. Gadina: Immunodeficiency is a tough nut to CRAC: the importance of calcium flux in T cell activation. *Mol Interv*, 6(5), 253-6 (2006)

143. H. B. Gaspar and A. J. Thrasher: Gene therapy for severe combined immunodeficiencies. *Expert Opin Biol Ther*, 5(9), 1175-82 (2005)

144. S. Feske: A severe defect in CRAC Ca^{2+} channel activation and altered K^+ channel gating in T cells from immunodeficient patients. *J Exp Med.*, 202(5), 651-62 (2005)

145. J. Soboloff, M. A. Spassova, M. A. Dziadek and D. L. Gill: Calcium signals mediated by STIM and Orai proteins--a new paradigm in inter-organelle communication. *Biochim Biophys Acta*, 1763(11), 1161-8 (2006)

146. Y. Liao, C. Erxleben, E. Yildirim, J. Abramowitz, D. L. Armstrong and L. Birnbaumer: Orai proteins interact with TRPC channels and confer responsiveness to store depletion. *Proc Natl Acad Sci U S A*, 104(11), 4682-7 (2007)

147. K. T. Cheng, X. Liu, H. L. Ong and I. S. Ambudkar: Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels. *J Biol Chem*, 283(19), 12935-40 (2008)

148. A. Zweifach and R. S. Lewis: Rapid inactivation of depletion-activated calcium current (ICRAC) due to local calcium feedback. *J Gen Physiol*, 105(2), 209-26 (1995)

149. N. Scrimgeour, T. Litjens, L. Ma, G. J. Barritt and G. Y. Rychkov: Properties of Orai1 mediated store-operated current depend on the expression levels of STIM1 and Orai1 proteins. *J Physiol* (2009)

150. S. Srikanth, H. J. Jung, B. Ribalet and Y. Gwack: The intracellular loop of Orai1 plays a central role in fast inactivation of Ca2+ release-activated Ca2+ channels. *J Biol Chem*, 285(7), 5066-75 (2010)

151. M. Prakriya and R. S. Lewis: CRAC channels: activation, permeation, and the search for a molecular identity. *Cell Calcium*, 33(5-6), 311-21 (2003)

152. A. Lis, S. Zierler, C. Peinelt, A. Fleig and R. Penner: A single lysine in the N-terminal region of store-operated channels is critical for STIM1-mediated gating. *J Gen Physiol*, 136(6), 673-86 (2010)

Abbreviations: ARC: arachidonate regulated Ca^{2+}, 2-APB: 2-aminoethyldiphenyl borate, CIF: Ca^{2+} influx factor, CMD: CRAC modulatory domain, CRAC: Ca^{2+} release activated Ca^{2+}, IP_3: inositol 1,4,5-triphosphate, MTS: methanethiosulfate, RBL: rat basophilic leucemia; ROS: reative oxygen species, SAM: steril alpha motif, SCID: severe combined immune deficiency, SOC: store-operated Ca^{2+}, SOCE: store-operated Ca^{2+} entry, TRPC: canonical transient receptor potential

Key Words: STIM1, Orai, CRAC, Activation, Permeation, Gating, Inactivation, Review

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