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Store-Operated Ca^{2+} Channels: Mechanism, Function, Pharmacology, and Therapeutic Targets

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Abstract

Calcium (Ca^{2+}) release–activated Ca^{2+} (CRAC) channels are a major route for Ca^{2+} entry in eukaryotic cells. These channels are store operated, opening when the endoplasmic reticulum (ER) is depleted of Ca^{2+}, and are composed of the ER Ca^{2+} sensor protein STIM and the pore-forming plasma membrane subunit Orai. Recent years have heralded major strides in our understanding of the structure, gating, and function of the channels. Loss-of-function and gain-of-function mutants combined with RNAi knockdown strategies have revealed important roles for the channel in numerous human diseases, making the channel a clinically relevant target. Drugs targeting the channels generally lack specificity or exhibit poor efficacy in animal models. However, the landscape is changing, and CRAC channel blockers are now entering clinical trials. Here, we describe the key molecular and biological features of CRAC channels, consider various diseases associated with aberrant channel activity, and discuss targeting of the channels from a therapeutic perspective.

A BRIEF HISTORY OF STORE-OPERATED Ca^{2+} CHANNELS

A rise in cytosolic calcium (Ca^{2+}) concentration is a universal second messenger, regulating biological responses from sperm motility and egg fertilization to cell death via apoptosis and necrosis. Cytosolic Ca^{2+} controls myriad responses within the life-death cycle, including exocytosis, energy production, gene transcription, and cell motility. In metazoans, a highly conserved mechanism for raising cytosolic Ca^{2+} is through store-operated Ca^{2+} channels in the plasma membrane (PM). These channels are so named because they activate following a loss of Ca^{2+} from the endoplasmic reticulum (ER) Ca^{2+} store, which is accomplished physiologically through the stimulation of cell-surface receptors that engage the phospholipase C pathway via either G proteins or tyrosine kinase phosphorylation cascades. Activation of phospholipase C cleaves the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce the second messenger inositol 1,4,5-trisphosphate ($InsP_3$). $InsP_3$ directly gates ER Ca^{2+} release channels to deplete ER Ca^{2+} content and open store-operated Ca^{2+} channels.

The concept of store-operated Ca^{2+} entry originated in 1986 (1). Drawing from experiments on parotid acinar cells investigating the temporal relationship between Ca^{2+} release, Ca^{2+} influx, and Ca^{2+} store refilling and from an eclectic collection of observations in the literature, Putney (1, 2) put forward a radical hypothesis called capacitative Ca^{2+} entry. This hypothesis proposed that the Ca^{2+} content of the ER store per se dictated the extent of Ca^{2+} influx in nonexcitable cells: When stores were replete, Ca^{2+} influx did not occur, but as stores emptied, Ca^{2+} entry ensued. A direct prediction of this model was that store depletion independent of receptor stimulation should activate Ca^{2+} influx. The sesquiterpene lactone thapsigargin, a noncompetitive inhibitor of sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps in the ER extracted from the plant *Thapsia garganica* (3), provided such a tool. Thapsigargin shifts the pump-leak cycle across the ER in favor of Ca^{2+} efflux to cause store depletion and powerfully activated Ca^{2+} influx, confirming the central tenet of Putney's hypothesis (4). Ca^{2+} entry in response to thapsigargin challenge has been observed in many cell types, establishing the importance of store-operated Ca^{2+} influx (**Figure 1*a***). In 1995, capacitative Ca^{2+} entry was renamed store-operated Ca^{2+} entry to distinguish its mode of activation from other types of Ca^{2+} channels.

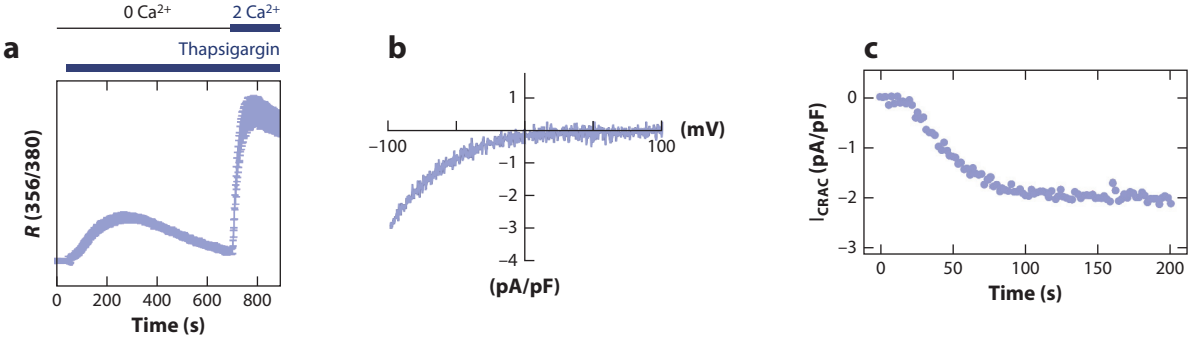

Figure 1

Measurements of store-operated calcium (Ca^{2+}) entry in RBL-2H3 cells. (*a*) Cytosolic Ca^{2+} measurement of Ca^{2+} influx is shown. Ca^{2+} stores were depleted with 2 μM of thapsigargin in nominally Ca^{2+}-free external solution, yielding a characteristic transient rise in cytosolic [Ca^{2+}], here reported by the ratiometric Ca^{2+}-sensitive dye Fura-2. On readmission of extracellular Ca^{2+}, Ca^{2+} influx through store-operated channels causes a secondary, sustained rise in cytosolic [Ca^{2+}]. (*b*) Characteristic whole-cell current-voltage relationship of Ca^{2+} release–activated Ca^{2+} current (I_{CRAC}) (measured at −80 mV) in RBL-2H3 cells, recorded using the whole-cell patch clamp technique. (*c*) Time course of development of I_{CRAC} following the onset of whole-cell recording. The current develops slowly, taking several tens of seconds to peak. In panels *b* and *c*, stores were depleted by dialysis with 30-μM $InsP_3$ plus 10-mM EGTA [ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid] in the pipette solution.

Which channels account for store-operated Ca^{2+} influx? In a seminal study, Hoth & Penner (5) elegantly demonstrated that store depletion by several independent means in mast cells activated a low-noise, nonvoltage-activated, inwardly rectifying Ca^{2+}-selective current, the Ca^{2+} release–activated Ca^{2+} (CRAC) current (I_{CRAC}), which arises from CRAC channels. Direct chelation of Ca^{2+} within the ER by a membrane-permeable Ca^{2+} buffer also activated I_{CRAC} (6). Therefore, it is Ca^{2+} store depletion itself rather than the identity of the stimulus that opens CRAC channels.

BIOPHYSICAL HALLMARKS OF THE CRAC CHANNEL

CRAC channels have several notable features (7, 8). I_{CRAC} is nonvoltage activated, is inwardly rectifying with a very positive reversal potential, and activates over several tens of seconds (**Figure 1*b*,*c***). CRAC channels are selective for Ca^{2+} (more so than for Sr^{2+} and Ba^{2+}), and their Ca^{2+} to Na^{+} permeability of more than 1,000:1 is similar to that of voltage-gated Ca^{2+} ($Ca_V1.2$) channels. CRAC channels become permeable to monovalent cations when extracellular divalent cations are removed. Measurements of monovalent conductivity estimate a minimum pore diameter of approximately 0.3 Å, almost twice as narrow as that seen in $Ca_V1.2$ channels. Unitary conductance is minuscule, approximately 10 fS from noise analysis estimates, which is three orders of magnitude smaller than most voltage-gated channels. Unitary conductance increases to approximately 0.2 pS when extracellular divalent cations are removed (9), still too small to measure directly. CRAC channels are partially inactivated by cytosolic Ca^{2+} through spatially and temporally distinct pathways. Fast Ca^{2+}-dependent inactivation occurs within milliseconds as Ca^{2+} ions bind to a channel site within 10 nm of the pore (10, 11), and slow Ca^{2+}-dependent inactivation develops over tens of seconds and requires a rise in global cytosolic Ca^{2+} (12, 13). Slow inactivation can be delayed by mitochondrial Ca^{2+} buffering (14, 15) and is thought to involve the ER-resident protein SARAF (16).

MOLECULAR COMPOSITION OF THE CRAC CHANNEL

Since the discovery of CRAC channels, various mechanisms have been offered to explain their coupling to ER Ca^{2+} content (reviewed in 17; see the **Supplemental Appendix**). However, data underpinning these models were mainly based on pharmacological tools with limited specificity, and studies often yielded conflicting results.

Supplemental Material >

The introduction of RNA interference (RNAi) technology combined with advances in high-throughput screening identified the two key protein components of the CRAC channel: the ER Ca^{2+} sensor STIM (stromal interaction molecule) and the pore-forming channel subunit Orai.

STIM PROTEINS ARE SENSORS OF THE ENDOPLASMIC RETICULUM Ca^{2+} CONTENT

In a *Drosophila* screen of approximately 150 double-stranded RNA constructs against genes with ion channel–like features or Ca^{2+} binding-motifs, Roos et al. (18) discovered that *Drosophila* STIM is a key component of store-operated Ca^{2+} entry. They demonstrated that knockdown of the mammalian homolog STIM1 suppressed store-operated Ca^{2+} influx in HEK293 cells and Jurkat T lymphocytes, while knockdown of the homolog STIM2 had little effect (18). Liou et al. (19) used a small interfering RNA strategy against 2,304 human proteins with known signaling domains and also identified STIM1 and STIM2 as key components of store-operated Ca^{2+} influx; again, STIM1 knockdown had a greater inhibitory effect on Ca^{2+} influx than did the loss of STIM2.

What was the role of STIM? Liou et al. showed that STIM was an ER-resident protein with a single-pass transmembrane domain (TM). Stimulation redistributed STIM1 into punctate-like

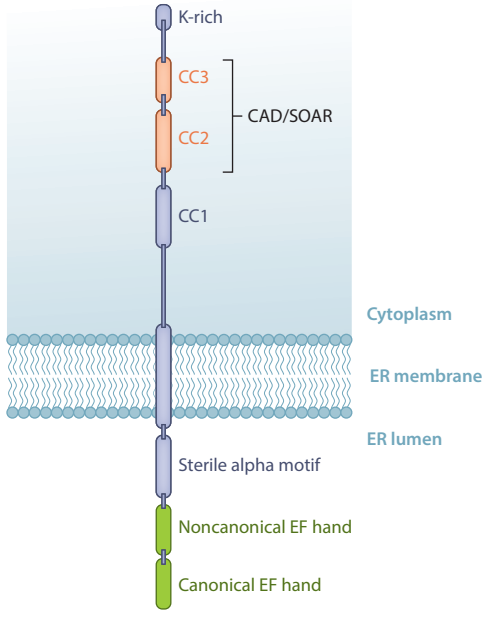

Figure 2

Summary of the domain architecture of STIM, including the coiled-coil domains CC1, CC2, and CC3.

clusters (19) just below the plasma membrane. They also noted that STIM possessed a Ca^{2+}-binding EF hand predicted to lie within the ER lumen, suggesting it might detect store Ca^{2+} content. Consistent with this, mutation of the EF hand to reduce Ca^{2+} affinity (D76A-STIM1) led to STIM1 puncta formation and store-operated Ca^{2+} entry in the absence of store depletion.

Distinct functional modules of STIM1 (**Figure 2**) sequentially activate store-operated Ca^{2+} entry (reviewed in 20). The protein comprises three regions: a luminal section containing the EF hand and a sterile alpha motif (SAM); a single-pass TM; and a large cytoplasmic region containing three coiled-coil (CC) domains (CC1–3), including the critical region that activates CRAC channels (CRAC activation domain/STIM1 Orai1-activating region; CAD/SOAR domain) as well as a polybasic section at the COOH terminus that interacts with negatively charged sites at the plasma membrane, including PIP_2.

STIM1 is thought to be dimeric at rest (21–23), stabilized by hydrophobic interactions and hydrogen bonding between CAD monomers on CC2 and CC3 (24). A decrease in ER Ca^{2+} causes Ca^{2+} to dissociate from the luminal EF hand, triggering unfolding of the EF-SAM and a conformational change in the cytosolic region. Unfolding of the EF-SAM domain exposes hydrophobic sections that, with CC2 and CC3, encourage the formation of STIM1 oligomers (25). Fluorescence resonance energy transfer (FRET) studies of coexpressed CFP- and YFP-STIM1 confirmed STIM-STIM interaction after store depletion (21, 26) and in regions away from the plasma membrane (27). Therefore, STIM1 oligomerization is an early step in the activation of store-operated Ca^{2+} entry. How many STIM1 molecules are in an oligomer and whether different agonists recruit different-sized oligomers are open questions.

How does Ca^{2+} dissociation from the luminal EF hand translate into the conformational changes in the cytosolic STIM that gate CRAC channels? Evidence favors the removal of a resting CC1-CAD/SOAR inhibitory brake (28, 29). FRET studies show that the cytosolic domain

is compact at rest but extends when activated (30). CC1 is monomeric in solution and bound to the CAD domain; crosslinking CC1 leads to the formation of a CC dimer at the expense of CAD interaction (23). Collectively, these results suggest that CC1 clamps CAD in the resting state and that EF-SAM dimerization on store depletion releases this clamp via CC1 dimerization, extending STIM1 and presenting the critical CAD region to gate Orai1 (30).

How does STIM1, an ER-resident protein, engage with CRAC channels in the plasma membrane? Following store depletion, STIM1 oligomers migrate to the cortical ER juxtaposed against the plasma membrane. Here, at these ER-PM junctions, the membrane separation of approximately 15 nm is short enough for direct interaction between STIM1 and Orai1 (31). This close distance is mediated by ribosome-free thin cortical ER (32).

Ca^{2+} AFFINITY, DISTRIBUTION, AND SPLICE VARIANTS

STIM1 and STIM2 share 54% sequence identity and have similar molecular weights (77 and 84 kDa). Their EF-SAM domains exhibit different apparent Ca^{2+} affinities (~200 μM for STIM1 and ~500 μM for STIM2) (25, 33, 34). Semiquantitative measurements place ER resting Ca^{2+} around 600–800 μM (35, 36), suggesting that modest falls in ER Ca^{2+} activate STIM2 but that a more precipitous drop is required to activate STIM1. Measuring ER Ca^{2+} concentration with a targeted Ca^{2+} indicator in Jurkat T cells revealed that STIM1 redistribution and I_{CRAC} amplitude both followed the same function of ER Ca^{2+}, exhibiting $K_{1/2}$s of ~200 μM (37). Substantial depletion of the ER Ca^{2+} content is required for I_{CRAC} to develop, consistent with earlier studies that demonstrated that significant ER Ca^{2+} loss occurred before CRAC channels activated (38, 39). Numerous studies have shown that knockdown of STIM1 suppresses I_{CRAC} and store-operated Ca^{2+} entry, whereas loss of STIM2 has a weaker impact, supporting the view that I_{CRAC} activation is a STIM1-driven process (18, 19, 40–42). STIM2 is thought to regulate both basal Ca^{2+} influx at rest and Ca^{2+} signals evoked by weak stimuli that only modestly reduce ER Ca^{2+} content (34). An interesting twist to this scheme has come from reports that STIM2 recruits STIM1 to ER-PM junctions following low levels of stimulation and triggers remodeling of STIM1 to expose the CAD/SOAR domain (43). How this finding reconciles with the strict dependence of CRAC channels on substantial store depletion, and thereby exclusively on STIM1-dependent activation (37), remains unclear.

STIM1 and STIM2 are widely expressed, although relative levels of expression can vary considerably (44). STIM protein function is further increased by alternative splice variants, including a long variant (STIML), which is robustly expressed in muscle (45), and an inhibitory STIM2 protein (46, 47), which reduces store-operated Ca^{2+} entry.

ORAI PROTEINS ARE STORE-OPERATED CRAC CHANNELS

Human genetic linkage analysis and RNAi screens in *Drosophila* S2 cells converged to identify the Orai family of proteins as the pore-forming subunits of the CRAC channel (48). Feske, Rao, and colleagues (48) had previously identified a small cohort of patients with a severe combined immunodeficiency (SCID) due to the absence of functional CRAC channels. One hit in a genome-wide RNAi screen, *olf186-F*, had a human homolog at a locus on chromosome 12 that they identified with linkage analysis; the protein, which they named Orai1, is expressed at the cell surface and has four TMs. Simultaneously, Penner and Kinet and colleagues (49) and Cahalan and colleagues (50) also identified *olf186-F* from RNAi screens. Whereas overexpression of Orai1 tended to reduce store-operated Ca^{2+} entry, coexpression with STIM1 resulted in large CRAC currents (50, 51). Importantly, expression of Orai1 rescued I_{CRAC} and downstream signaling in cells from SCID

patients (48). Detailed mutagenesis studies established Orai1 as the pore-forming subunit of the CRAC channel (52–54). The high Ca^{2+} selectivity of the pore is not immutable but is regulated by STIM1 binding (55).

Mammals also express the Orai1 homologs Orai2 and Orai3 (56), the latter apparently evolving from Orai1 through a gene duplication event. All three homologs exhibit a broad tissue distribution, but Orai3 in particular is enhanced in cancerous tissue, where it seems to promote proliferation and metastasis (57). All three function as store-operated Ca^{2+} channels when overexpressed in HEK293 cells, but they differ in their pharmacological sensitivity and the rate and extent of Ca^{2+}-dependent inactivation processes (58).

PORE ARCHITECTURE OF THE CRAC CHANNEL

Cysteine-scanning mutagenesis has identified key pore-lining residues in TM1 of human Orai1, including E106, V102, G98, L95, and R91 (59, 60). TM1 helices are positioned close to each other to form a narrow pore, consistent with the low single CRAC channel conductance and biophysical estimates of minimum pore diameter. The crystal structure of closed *Drosophila* Orai, determined at 3.35-Å resolution, revealed the channel to be a hexamer with a central narrow pore approximately 55 Å long (61) and composed of six TM1 helices, one from each subunit. The external selectivity filter is a ring of six negatively charged glutamate (E106) residues, with one provided by each subunit. Mutation of the glutamate to an asparagine abolishes Ca^{2+} permeation, and remarkably, a conservative mutation to aspartate reduces Ca^{2+} selectivity (53). The selectivity filter is followed by a hydrophobic section approximately 15 Å long, then a basic section that is also approximately 15 Å long, and finally a cytosolic portion. Ca^{2+} ions that pass through the selectivity filter are confronted with a central hydrophobic gate, wherein mutations result in leaky channels (62).

The crystal structure shows that TM2 and TM3 helices form a middle ring that envelopes TM1 (61); TM4 helices are arranged in an outer ring and have fewer contacts with other parts of the channel. The N terminus is an extension of the pore and protrudes into the cytosol, and C-terminal TM4 helices also reach into the cytosol, forming antiparallel CC helices that provide a strong binding site for the CAD/SOAR domain of STIM1.

OPENING OF THE CRAC CHANNEL

STIM1 binding to the C terminus of Orai1 gates the channel (**Figure 3**). Crystal structures of *Drosophila* Orai in the open state have recently been reported by two groups. In one study (63), the structure was obtained using a mutant H206A *Drosophila* Orai protein. The corresponding human H134A Orai1 mutation appears to rotate TM1 and realign F99 within the pore, permitting Ca^{2+} permeation in the absence of STIM1 binding (64). H206A-Orai exhibits an approximately 10-Å-wide dilated lower channel pore. Moreover, the antiparallel TM4 CC extension helices unpair, and TM4-TM3 helices unlatch, enabling TM4 to extend away from the rest of Orai1. STIM1 binding to the extended TM4 helix might result in unlatching and allosteric pore opening via readjustment of TM2-TM1 interaction.

X-ray crystallography and cryo-electron microscopy (cryo-EM) reconstructions of *Drosophila* P288L have also been reported recently, and a different mechanism for Ca^{2+} permeation has been proposed (65). *Drosophila* P288L is equivalent to human P245L, a constitutively active channel linked to Stormorken-like syndrome (66), discussed below. This mutant also shows straightened and extended unpaired TM4 helices (63) but a more modestly dilated pore than H134A. Dilation was largely restricted to a basic amino acid–rich region of the lower pore that includes R155,

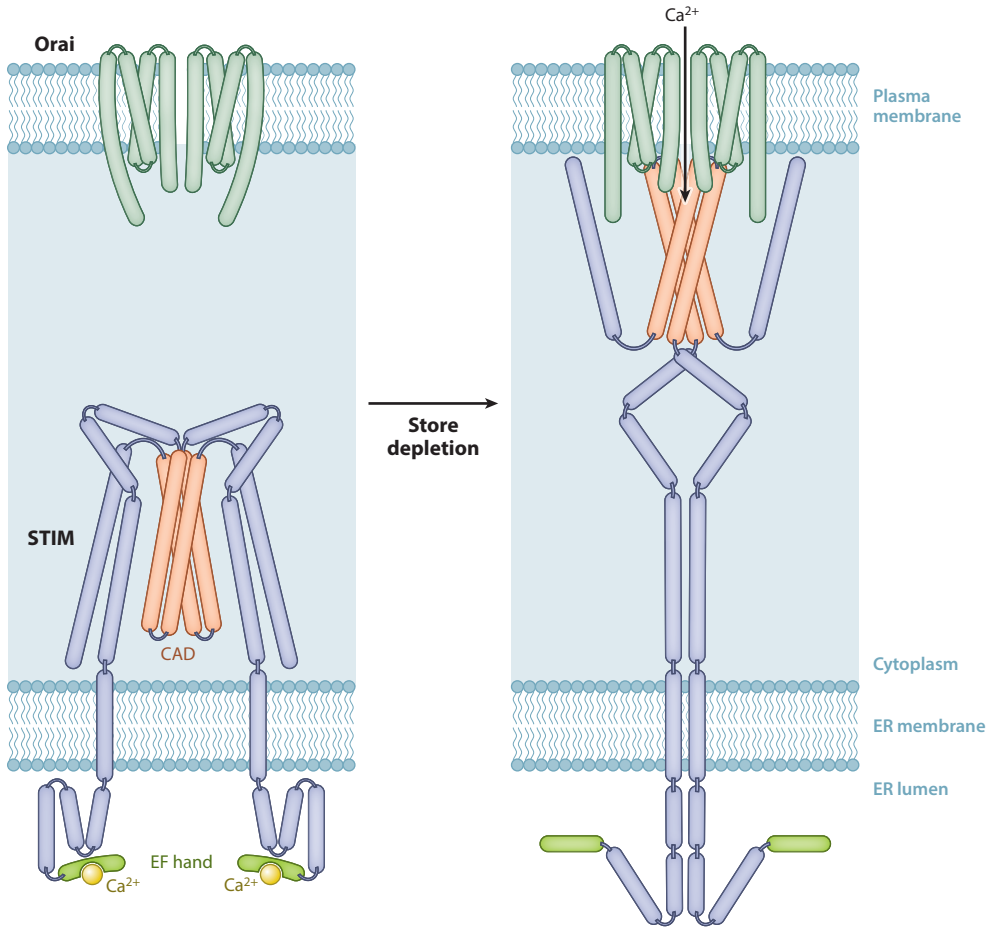

Figure 3

Diagram showing the gating of Orai channels by STIM. The depletion of endoplasmic reticulum (ER) [Ca^{2+}] is sensed by the luminal EF hand of a STIM1 dimer, leading to a conformational change that propagates across the ER membrane and promotes oligomerization and, ultimately, extension and presentation of the CAD/SOAR domain, which interacts with and activates Orai channels.

K159, and K163 and whose role in permeation is unclear. Elimination of these positively charged residues inhibits channel activity, arguing against a role for electrostatic repulsion (67). Liu et al. (65) proposed that the latched TM4 helix maintains the pore in the closed state and that positive-charge repulsion between the basic region and Ca^{2+} ions combined with anion plugs suppresses Ca^{2+} flux. In this model, the TM4 helix twists the basic section outward, dilating this section of the pore and recruiting more anions into the pore, establishing the open state. The anions are proposed to both neutralize R155, K159, and K163 and increase the potential gradient across the membrane, allowing Ca^{2+} permeation. One legitimate question is whether the structures of mutant Orai channels that are locked in the open state faithfully replicate the physiological activation by STIM1 binding. Crystallographic and cryo-EM studies of Orai1 bound to STIM1 will certainly resolve some of the current controversies. Nevertheless, what is apparent is that the gating of Orai1 by STIM1 is allosteric and involves a propagated conformational switch from the extended TM4 helices in the cytosol to the innermost TM1 helices that line the pore.

Table 1 Downstream targets of store-operated Ca^{2+} entry

Function	Target	Reference(s)
ER refilling	SERCA	140, 141
Enzymes	PMCA	142
	Calcineurin	143
	CaMKII	144
	$cPLA_2$	145
	MEK/ERK	146
	Adenylyl cyclase 8	68, 147
	PIP5K	148
	eNOS	149
	Pancreatic proteases	86
Immune function	Immunometabolism: metabolic reprogramming, nutrient sensing, mitochondrial function	138, 150, 151
	Phagosomal ROS production	152
	T cell autoimmunity	100
Secretion	Mast cell degranulation	153
	T cell degranulation	154
	Cytokine release (e.g., IL-2, IFN-γ, TNF-α)	153
	Leukotriene C_4	146, 155
	TSLP	156
	Gliotransmitter release	157
Cell migration	Chemotaxis	158
Ion channels and transporters	TRPC1	159
	Ca^{2+}-activated chloride channels	160, 161
	TRPM4	162
	NCX	163
	$K_{Ca}3.1$	164
Transcription factors	NFAT	165
	c-fos	166
	NF-κB	165
	STAT5	167
Lactation	NA	168

Abbreviations: CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; $cPLA_2$, cytosolic phospholipase A2; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; IL-2, interleukin-2; IFN-γ, interferon-γ; $K_{Ca}3.1$, intermediate-conductance Ca^{2+}-activated K^+ channel; MEK/ERK, mitogen-activated protein kinase kinase/extracellular signal–regulated kinase; NA, not applicable; NCX, Na^+-Ca^{2+} exchanger; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PIP5K, phosphatidylinositol-4-phosphate-5-kinase; PMCA, plasma membrane Ca^{2+} ATPase; ROS, reactive oxygen species; SERCA, sarcoplasmic-endoplasmic Ca^{2+} ATPase; STAT5, signal transducer and activator of transcription 5; TNF-α, tumor necrosis factor-α; TSLP, thymic stromal lymphopoietin.

DOWNSTREAM TARGETS ACTIVATED BY CRAC CHANNELS: CELLULAR STUDIES

The canonical function attributed to CRAC channels was to ensure adequate refilling of the ER with Ca^{2+}, which is necessary for protein folding, trafficking, and Ca^{2+} signaling. However, numerous studies have revealed that Ca^{2+} entry through CRAC channels directly activates downstream responses and without the need to refill the stores first. These functions are listed in **Table 1** and shown in **Figure 4**.

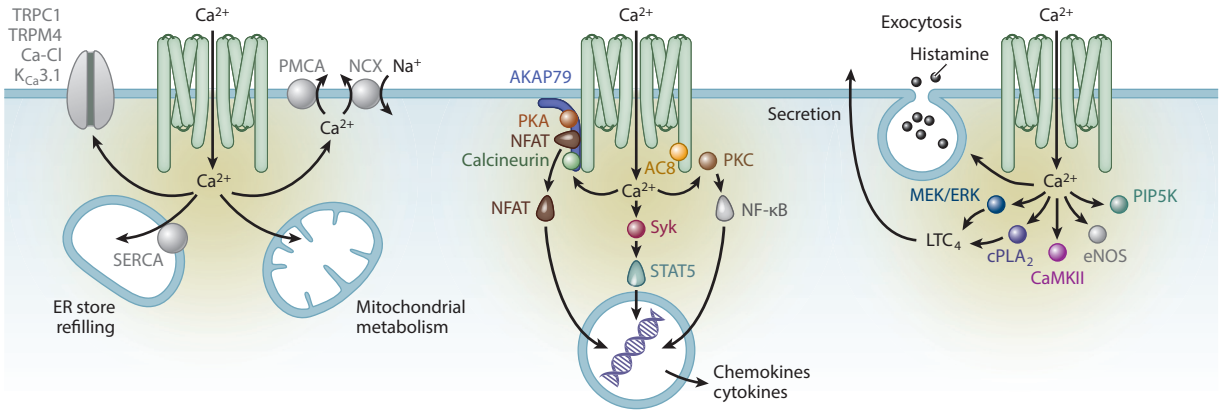

Figure 4

Downstream targets activated by Ca^{2+} entry through CRAC channels. For simplicity, only Orai1 is depicted, and targets have been grouped into ion channels, transporters, and organelles (*left*); transcription factors (*middle*); and enzymes and exocytosis (*right*). See text for more details. Abbreviations: AC8, adenylyl cyclase 8; AKAP79, A-kinase-anchoring protein 79; Ca-Cl, Ca^{2+}-activated chloride channel; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; $cPLA_2$, cytosolic phospholipase A2; CRAC, Ca^{2+} release–activated Ca^{2+}; eNOS, endothelial nitric oxide synthase; $K_{Ca}3.1$, intermediate-conductance Ca^{2+}-activated K^+ channel; LTC_4, leukotriene C_4; MEK/ERK, mitogen-activated protein kinase kinase/extracellular signal–regulated kinase; NCX, Na^+-Ca^{2+} exchanger; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PIP5K, phosphatidylinositol-4-phosphate-5-kinase; PKA, protein kinase A; PKC, protein kinase C; PMCA, plasma membrane Ca^{2+} ATPase; SERCA, sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase; STAT5, signal transducer and activator of transcription 5; Syk, spleen tyrosine kinase/tyrosine-protein kinase.

Many of these responses are activated by local Ca^{2+} signals, or Ca^{2+} nanodomains, that build up rapidly when Ca^{2+} diffuses through open Ca^{2+} channels. Adenylyl cyclase 8, a Ca^{2+}-calmodulin-activated enzyme, binds to an arginine-rich region on the N terminus of Orai1 (68), exposing the enzyme to high local Ca^{2+}. Targets can also be juxtaposed indirectly via a scaffolding protein. AKAP79 binds calcineurin, a Ca^{2+}-activated protein phosphatase that dephosphorylates the transcription nuclear factor of activated T cells (NFAT) (69) to mask a nuclear export and reveal a nuclear import sequence, allowing NFAT to migrate into the nucleus. A portion of the cellular NFAT pool is also associated with AKAP79 (69), which associates with the N terminus of Orai1 after store depletion, placing calcineurin and NFAT in the realm of the Ca^{2+} nanodomain and ensuring robust activation with high fidelity (70). The N terminus of Orai1 also contains an inverted caveolin binding site, mutations in which abolish Orai1 internalization during meiosis (71). Distinct domains of caveolin-1 selectively regulate the ability of Ca^{2+} nanodomains near CRAC channels to activate distinct transcription factors, providing a means to tunnel a local Ca^{2+} signal down a specific pathway (72).

CRAC CHANNELOPATHIES: OF MICE AND MEN

Several inherited null or loss-of-function mutations in STIM1 and ORAI1 that compromise channel gating have been described in patients (**Figure 5**), and the resulting clinical phenotypes have illuminated the role of the channel in humans. Strikingly, loss-of-function mutations in either Orai1 or STIM1 engender almost identical phenotypes, reinforcing the view that both proteins are necessary and sufficient for functional CRAC channels (73). Loss-of-function mutations in *Orai1* and *STIM1* genes result in a combined immunodeficiency with susceptibility to recurrent

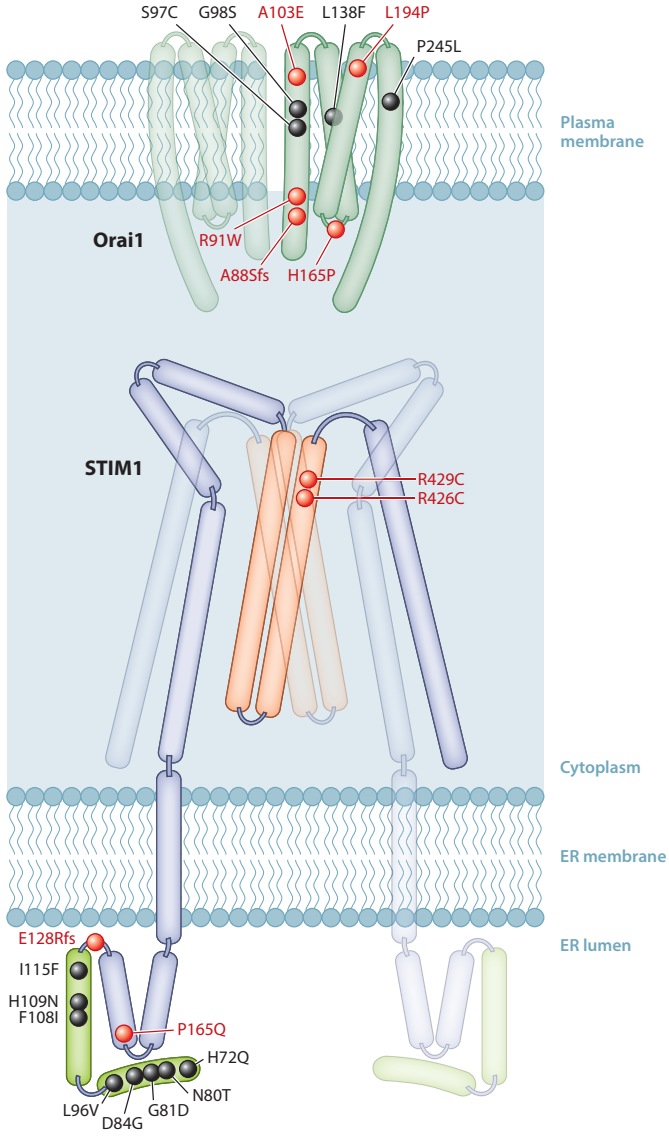

Figure 5

Human disease–associated mutations in STIM1/Orai1. Loss-of-function mutations are shown in red and gain-of-function mutations in black. Abbreviation: fs, frame shift.

viral, bacterial, and fungal infections; autoimmunity; a generalized muscle weakness due to hypotonia and muscle fiber atrophy; anhidrosis; and tooth enamel defects.

Excessive Ca^{2+} influx arising from a small number of gain-of-function mutations in *STIM1* and *Orai1* (**Figure 5**) cause tubular aggregate myopathy and Stormorken syndrome (66). Both disorders involve muscle weakness and can variably present with thrombocytopenia, miosis, hyposplenism, dry and scaly skin, and dyslexia. While it is possible to envisage how prolonged Ca^{2+} entry in immune cells and smooth muscle cells accounts for many of these clinical characteristics, the association with dyslexia is unclear. Of the 13 patients reported with Stormorken syndrome,

Table 2 Indications where CRAC channel blockers may be of therapeutic benefit

Indication (references)	Strength of rationale	CRAC channel blocker studies	Human clinical trials
Acute pancreatitis (88–90)	+++	+++	Phase II (NCT03401190)
Asthma (94, 96–98)	+++	+++	Phase I (NCT02958982, terminated)
Cancer (83, 84)	++	+++	Phase I/Ib (NCT03119467) Phase III (NCT00003869)
Rheumatoid arthritis (102–105)	+++	+++	NA
Atopic dermatitis[a]	+++	++	NA
Psoriasis[a]	+++	++	Phase I
Inflammatory bowel disease (99, 100)	+++	++	NA
Traumatic brain injury/stroke[a]	++	++	NA
Thrombosis[a]	++	+	NA
Huntington's disease[a]	+	+	NA
Renal ischemia reperfusion injury[a]	+	+	NA
Multiple sclerosis[a]	+	+	NA
Chronic pain[a]	+	+	NA
Alzheimer's disease[a]	+	NA	NA
Duchenne muscular dystrophy[a]	+	NA	NA

Plus signs are used above to denote the strength of rationale and the results of CRAC channel blocker studies, with more plus signs indicating a better rationale/results and fewer plus signs indicating a poorer rationale/results.
[a]See the **Supplemental Appendix** for additional information.
Abbreviation: NA, not applicable.

dyslexia was noted in three (74). In the 10 remaining patients, it is unknown whether they had dyslexia. Interestingly, six patients had learning difficulty (unknown for the remaining seven). The mechanism linking gain-of-function mutations in *STIM/Orai* with dyslexia is unknown.

Supplemental Material >

Studies of mice with genetic ablations in *Orai* and *STIM* genes have revealed a much more severe impact than in humans, with broader effects in multiple organ systems (75), eyelid irritation, and sporadic hair loss (76). Potential explanations for the striking differences in phenotype between humans and mice are discussed in the **Supplemental Appendix** (see the section titled Squaring the Circle: Reconciling Mouse and Human Phenotypes in the **Supplemental Appendix**).

INTERVENTIONS TARGETING CRAC CHANNELS: CLINICAL TARGETS

CRAC channels are implicated in a variety of diseases (**Table 2**), particularly in those associated with the immune system. Because of space constraints, we describe below indications based on in vitro experiments, animal models, and effects of CRAC channel blockers in animals and human tissue. For other indications, evidence is presented in the **Supplemental Appendix**.

Cancer

Although Orai channels are unlikely to be drivers of transformation, altered Ca^{2+} entry or Orai expression could enhance cancer growth and invasiveness. Mutations in Orai1 that result in

constitutive Ca^{2+} entry and activation of NFAT have been found in patients with colorectal, stomach, and uterine cancer (64). The profile of Orai expression seems to vary in a cancer-specific context (77). Orai1 levels are increased in liver (78), renal (79), and stomach (80) cancers, whereas Orai3 is elevated in lung cancer (81) and breast cancer (57). By contrast, Orai1 is downregulated in castration-resistant prostate cancer, perhaps reflecting a reduced sensitivity to apoptosis (82).

A role for Orai channels in proliferation and metastasis has come from studies of breast cancer. Orai1 expression increases in basal molecular breast cancer where there is no overexpression of estrogen and progesterone receptors or human epidermal growth factor receptor 2 (77). By contrast, Orai3 expression increases in estrogen receptor–positive breast cancer cells (57). Knockdown of Orai1 in MCF-7 breast cancer cells inhibited proliferation, prevented Ras activation, and reduced tumor generation in nude mice (83), and lung metastasis of MDA-MB-231 breast cancer cells in immunodeficient mice was suppressed by knockdown of STIM1 or Orai1 and by the Ca^{2+} channel blocker SKF96365 (84).

An important finding by Hoth and colleagues (85) is that efficient killing of cancer cells by cytotoxic T cells is enhanced by limiting a cytosolic Ca^{2+} rise through a partial block of endogenous Orai1. Targeting CRAC channels should reduce cancer cell growth and metastasis and simultaneously enable more effective killing by cytotoxic T cells.

Acute Pancreatitis

An important role for CRAC channels has been identified in acute pancreatitis, an inflammation of the pancreas brought about by alcohol abuse or complications from biliary disease. Acute pancreatitis is caused by the activation of proenzymes stored within zymogen granules in the apical pole of pancreatic acinar cells, which then autodigest the pancreas, resulting in necrosis (86). Agents that trigger acute pancreatitis such as fatty acid ethyl esters induce a sustained elevation of cytosolic Ca^{2+} due to Ca^{2+} entry, which leads to the activation of trypsin within endocytic vacuoles (87). The Ca^{2+} entry pathway was identified as the CRAC channel based on its electrophysiological features and sensitivity to the CRAC channel inhibitor GSK-7975A (88). GSK-7975A prevented Ca^{2+} overload, reduced intracellular protease activation, and decreased necrosis induced by palmitoleic acid ethyl ester. In three different mouse models of acute pancreatitis, two distinct CRAC channel blockers (GSK-7975A and CM_128/CM4620) each suppressed local and systemic pathophysiology (89). CM4620 reduced the severity of acute pancreatitis in a rat model but did so not only by targeting the acinar and stellate cells in the pancreas but also by reducing the neutrophil oxidative burst and the release of inflammatory mediators from invading immune cells (90). Therefore, a CRAC channel blocker targets multiple cell types associated with the acute pancreatitis. A clinical trial addressing the impact of a CRAC channel blocker on acute pancreatitis is currently ongoing (**https://clinicaltrials.gov/ct2/show/NCT04195347**).

Asthma

Asthma is a heterogenous disease of the respiratory system, a syndrome of different airway pathophysiologies that cause shortness of breath, cough, chest tightness, and a reduction in expiratory airflow. Patients typically present with airway hyperresponsiveness, often with underlying chronic airway inflammation and remodeling (91).

Since many phenotypes have been described and different disease etiologies suggested, the clinical management of these subtypes can be challenging. Asthma endotypes can represent quite different disease mechanisms, which has led to interest in subtype-specific therapies, especially for the refractory cases of severe disease that account for most asthma morbidity and mortality.

CRAC channels are an attractive potential target; apart from their role in airway immune cell function (whose inhibition modulates the chronic inflammation that characterizes most asthma cases), they are also expressed in other cell types implicated in the disease. In lung epithelial cells, store-operated Ca^{2+} channels couple to the NFAT- and c-fos-dependent transcriptional responses (92) that are critical for the sentinel role of these cells in monitoring the lung environment (91). Aberrant, exaggerated epithelium responses can contribute to airway hyperresponsiveness and remodeling. Release of proinflammatory mediators from human mast and T cells is suppressed by the CRAC channel blockers GSK-5498A and GSK-7975A (93). Store-operated Ca^{2+} channels may play an especially prominent role in allergic asthma because they are activated by the allergens from house dust mites via a protease stimulation of protease-activated receptors (PARs) (94, 95).

Several CRAC channel inhibitors have been tested in preclinical models of asthma. BTP2 inhibited eosinophil infiltration and reduced cytokine release in rat and guinea pig models (96), and Synta66 suppressed CD2/3/28-stimulated cytokine release from lymphocytes that were isolated by bronchoalveolar lavage of healthy and asthmatic human donors and inhibited NFAT activation and cytokine production to antigen challenge in rat lung (97). The pyrazole RP3128 is likewise a bronchodilator and reduces airway sensitivity to histamine and acetylcholine in the guinea pig. It too was shown to reduce cytokine release (IL-4, IL-5, IL-13, and TNF-α) and eosinophil and mast cell infiltration following antigen challenge of sensitized animals (98).

Inflammatory Bowel Disease

Di Sabatino et al. (99) investigated whether the inhibition of CRAC channels suppressed intestinal inflammation, which is characteristic of inflammatory bowel disease (IBD). They demonstrated that treating anti-CD3/anti-CD28-stimulated lamina propria mononuclear cells and biopsy specimens from 51 patients with IBD with CRAC channel inhibitors resulted in altered inflammatory profiles. These changes included reduced T-bet expression and decreased production of IFN-γ, IL-2, and IL-17, identifying CRAC channels as a potential therapeutic strategy for IBD via suppressing the exaggerated T cell response that is characteristic of the inflamed gut seen in these patients. T cells from homozygous Orai1 knock-in mice expressing the inactive mouse R93W Orai1 (human R91W Orai1) failed to induce colitis in an adoptive transfer model of IBD (100). CalciMedica reported efficacy of their CRAC channel inhibitors in a rat model of IBD, in which animals were treated for 7 days following the induction of distal colitis by intracolonic instillation of dinitrobenzene sulfonic acid (101).

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease that usually initiates in the synovium and eventually leads to progressive destruction of cartilage and bone.

The ORAi1 single-nucleotide polymorphism (SNP) rs7135617 correlates with susceptibility to RA in the Taiwanese population (102). This SNP is intronic, and a bioinformatics approach identified it as a potential binding site for the splicing factor SR SC35, which helps influence the selection of the splicing site. How this affects Orai1 expression or function is unclear. Naïve $CD4^+$ T cells of RA patients show increased Orai1 expression, exaggerated Ca^{2+} influx, and aberrant cytokine release (103). Moreover, intra-articular delivery of short hairpin RNA to Orai1 reduced inflammatory cytokines and disease score in a mouse model of collagen-induced arthritis (104). Pharmacological inhibition of CRAC channels with BTP2 or treatment with an anti-Orai1 antibody was also effective against inflammation and bone and cartilage erosion in a human NOD/SCID xenograft RA model (105).

THE QUEST FOR A CRAC CHANNEL BLOCKER

Different screening strategies have been employed in the pursuit of CRAC channel blockers. Assays of Ca^{2+} influx using Ca^{2+}-sensitive fluorescent dyes are especially adaptable to high-throughput automation but are also prone to artifacts. A major determinant of Ca^{2+} flux through the CRAC channel is the prevailing electrical driving force, and I_{CRAC} increases nonlinearly as the membrane potential hyperpolarizes; depolarization reduces flux through the channels. Since various ion channels and electrogenic transporters determine the membrane potential, inhibition of any of these pathways will affect the driving force for Ca^{2+} entry and therefore flux through CRAC channels. Inhibition of a K^+ channel, for example, would depolarize the membrane potential and reduce store-operated Ca^{2+} entry, which might erroneously be interpreted as a CRAC channel block. Careful follow-up is essential to validate screening hits as true CRAC channel blockers. Direct electrophysiological measurement provides the only unambiguous interrogation of CRAC channel activity, but measuring native picoamp currents is not conducive to high-throughput screening. One solution has been to transiently overexpress STIM1 and Orai1 to generate large currents that can be measured in an automated patch clamp system. However, these Ca^{2+} currents cannot be adequately buffered by Ca^{2+} chelators in the intracellular recording solution, which results in strong Ca^{2+}-dependent inactivation that can reduce peak amplitude and preclude testing of more than one concentration of an inhibitor. Moreover, the pharmacological sensitivity to certain compounds changes in cells overexpressing STIM1 and Orai1. 2-Aminoethoxydiphenyl borate (2-APB) inhibits STIM1 puncta formation in cells that overexpress STIM1 alone but fails to do so when Orai1 is coexpressed (106). The ability of the molecule AncoA4 to suppress store-operated Ca^{2+} entry was reduced following STIM1 overexpression (107).

PHARMACOLOGY OF CRAC CHANNELS

Supplemental Material >

Various small-molecule inhibitors have been reported to act as CRAC channel inhibitors (**Figure 6**; **Supplemental Appendix**, table 1).

The Dark and Middle Ages

Early CRAC channel blockers included the lanthanides, imidazole antimycotics, diethylstilbestrol, carboxyamidotriazole, and 2-APB. Many of these compounds are still routinely used as evidence for the involvement of CRAC channels in either contributing to Ca^{2+} signals or driving a response. However, none of these compounds are specific and they act on multiple targets.

Lanthanides. Like other Ca^{2+} channels, CRAC channels are inhibited by low concentrations of lanthanides, including lanthanum and gadolinium (Gd^{3+}) (108), and the block of Ca^{2+} influx by micromolar Gd^{3+} is often considered to be evidence for store-operated Ca^{2+} entry. However, low concentrations of lanthanides affect many other ion channels (see the **Supplemental Appendix**), including voltage-gated Ca^{2+} channels and TRPV2 channels. Gd^{3+} activates TRPV1 and TRPC5 channels and is a potent agonist of the Gq/phospholipase C-coupled Ca^{2+}-sensing receptor. Therefore, Gd^{3+} should not be considered to be a specific inhibitor of CRAC channels, even at submicromolar concentrations.

Imidazole antimycotics. SK&F 96365 was the first small-molecule inhibitor of Ca^{2+} influx in nonexcitable cells (109) (**Figure 6**) and was subsequently shown to inhibit store-operated Ca^{2+} entry through an extracellular site of action (see the **Supplemental Appendix**). However, patch

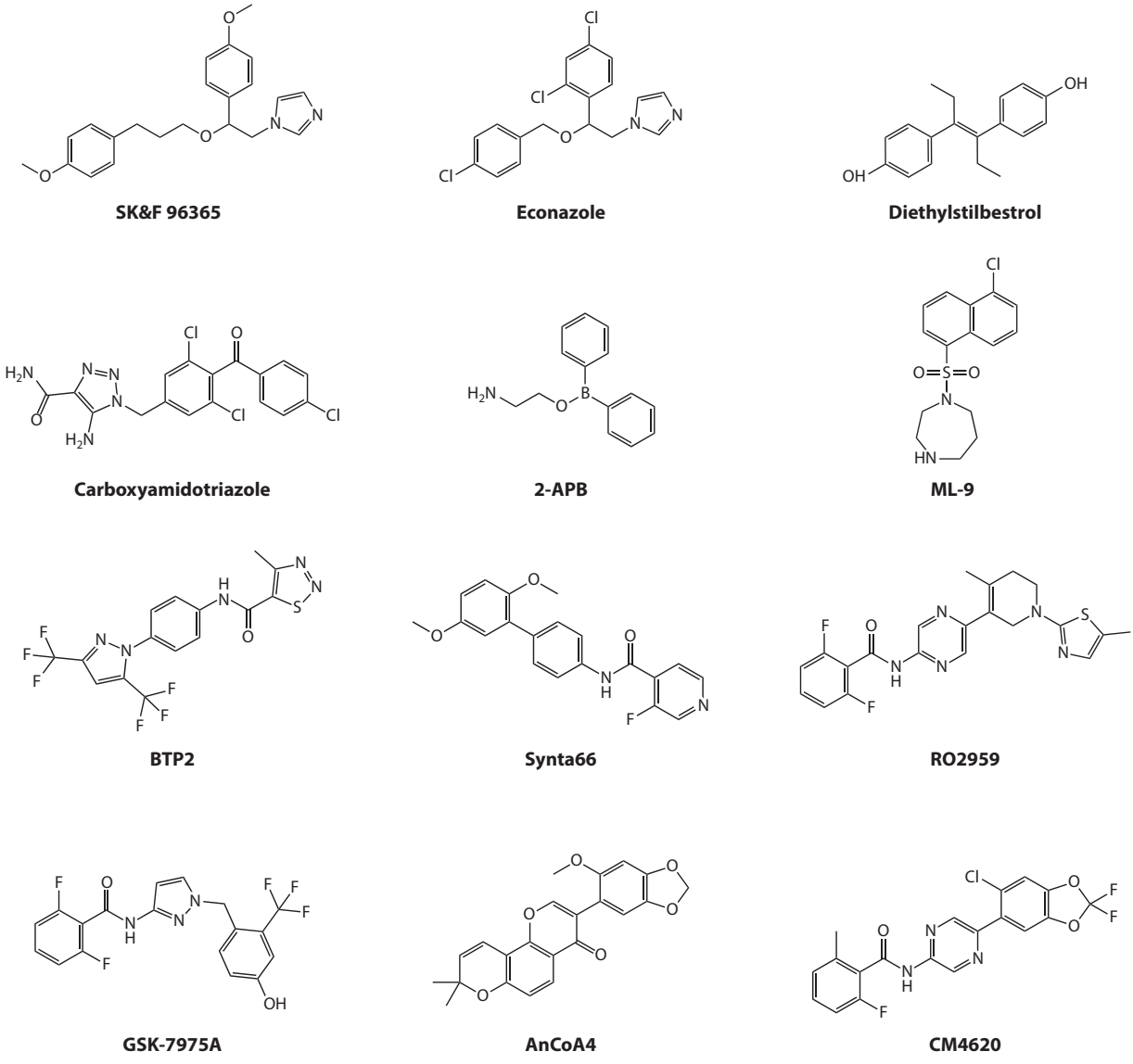

Figure 6

Structures of CRAC channel blockers. Inhibitors include the imidazole antimycotics (econazole and SK&F 96365); the synthetic estrogen agonist diethylstilbestrol; the anticancer agent carboxyamidotriazole; the diphenylborinate 2-APB; the myosin light-chain kinase inhibitor ML-9; the pyrazole derivatives BTP2, GSK-7975A, and RO2959; the dimethoxybenzene ring-containing Synta66; the isoflavone AnCoA4; and the CalciMedica compound CM4620.

clamp experiments revealed that SK&F 96365 also blocked chloride- and second messenger–activated nonselective cation channels over a similar concentration range (110). SK&F 96365 inhibits voltage-gated Ca^{2+} channels and cytochrome P450, and at higher concentrations it activates a Ca^{2+}-permeable cation channel in HL60 cells (see the **Supplemental Appendix**). These off-target effects limit its use as a CRAC channel blocker.

Supplemental Material >

Diethylstilbestrol. The synthetic estrogen agonist diethylstilbestrol inhibits store-operated Ca^{2+} entry in rat basophilic leukemia (RBL)-1 cells, platelets, and aortic smooth muscle (111, 112). It also inhibits other ion channels at similar concentrations, including minK protein-induced K^+ currents, voltage-gated Ca^{2+} channels, Ca^{2+}-permeable nonselective cation channels, and Gd^{3+}-activated TRPC5 channels (see the **Supplemental Appendix**). Diethylstilbestrol also stimulates mitochondrial Ca^{2+} uptake in MM5 cells (see the **Supplemental Appendix**). Poor channel selectivity and estrogenic activity significantly limit the use of diethylstilbestrol as a CRAC channel blocker, especially in vivo.

Carboxyamidotriazole. Carboxyamidotriazole (CAI) inhibits the invasion and metastasis of several human cancer cell lines (113) as well as Ca^{2+} influx and subsequent Ca^{2+}-dependent gene expression evoked by thapsigargin stimulation (114). CAI shows modest efficacy against solid tumors (115), although whether this is due to CRAC channel inhibition is unclear. The impact of CAI on other ion channels has not been reported. However, CAI depolarizes mitochondria and reduces mitochondrial Ca^{2+} uptake as effectively as protonophores (116), which limits its clinical utility. Mitochondrial depolarization impairs cytosolic Ca^{2+} buffering, which enhances Ca^{2+}-dependent slow inactivation of CRAC channels (14, 15), a possible mechanism of channel inhibition by CAI.

2-APB. Inhibition of store-operated Ca^{2+} entry by the small-molecule inhibitor 2-APB was originally interpreted in light of its known activity as an $InsP_3$ receptor antagonist (117, 118). However, 2-APB is more potent when applied extracellularly (119) and affects I_{CRAC} even in mutant DT40 B cells that lack all three $InsP_3$ receptor isoforms (120). The compound has a profound polypharmacology, enhancing Orai1 activity at low micromolar concentrations and inhibiting it at higher (>40 μM) concentrations (120, 121). 2-APB affects multiple ion transport pathways (see the **Supplemental Appendix**), including mitochondrial Ca^{2+} efflux, SERCA pumps, and many members of the TRP superfamily (e.g., TRPV1, TRPC5). With such broad off-target effects, it is easy to dismiss 2-APB as a tool compound, but it has useful, unexpected properties: At higher concentrations, 2-APB functionally uncouples Orai3 and STIM1, directly activating the channel and dilating the pore (122). The structure-activity relationship of 2-APB has been explored by Mikoshiba and colleagues (123) and others, yielding more potent structural isomers like DPB162-AE.

Supplemental Material >

The Modern Era

New compounds have been developed with better selectivity for the CRAC channel. These are discussed below.

BTP2. The pyrazole BTP2 is the archetype of arguably the most explored inhibitory CRAC channel pharmacophore to date. Identified independently by Abbott Laboratories and Boehringer Ingelheim, BTP2 inhibits CRAC channels at submicromolar concentrations (124), with a measurably higher potency over longer incubation periods. It has reported off-target effects at similar potencies, including TRPM4 agonism (125) and broad inhibitory activity at TRPC channels (126). BTP2 is active in animal models of autoimmune and airway diseases [inhibiting T cell–mediated delayed-type hypersensitivity, antigen-induced eosinophil infiltration, and airway IL-4 and cysteinyl-leukotriene release (96, 127)], but it also shows hepatic and renal toxicity (105).

Anti-Orai1 monoclonal antibodies. One strategy to avoid the historically poor selectivity profile of small-molecule CRAC channel inhibitors has been to raise Orai1-inactivating antibodies. Amgen and Novo Nordisk independently generated monoclonal antibodies (mAbs) against the

second extracellular loop of human Orai1 with nanomolar activity in both in vitro and in vivo models (128, 129). Interestingly, although even the lead Amgen mAb 2C1.1 inhibited Ca^{2+} influx, I_{CRAC}, and NFAT reporter gene expression by at most 40%, it inhibited thapsigargin- and PMA/ionomycin-induced cytokine release in human whole blood by approximately 90%. Unlike cyclosporin A, 2C1.1 did not inhibit a T cell–dependent antibody response (130).

Novo Nordisk attributed at least part of their mAb efficacy to promoting Orai1 internalization (129). As was the case for 2C1.1, inhibition of Ca^{2+} influx in Jurkat cells was partial but yielded antiproliferative and cytokine effects of similar magnitude to those of cyclosporin A. Moreover, the mAb inhibited IL-2 and IFN-γ secretion by anti-CD3/anti-CD28-stimulated synovial fluid cells from patients with RA and attenuated graft-versus-host disease in a humanized mouse model.

Liu et al. (105) raised a neutralizing antibody against human Orai1 that was active in ex vivo T cells from RA patient T cells and that was well tolerated and active in a xenograft human RA mouse model.

Aptamers. Single-stranded oligonucleotides offer another strategy for therapeutic CRAC channel inhibition. Using a systematic evolution of ligands via an exponential enrichment strategy, Sun et al. (131) identified the aptamer Y1 ($K_d = 17.2$ nM), which inhibited antigen-stimulated Ca^{2+} influx into, and beta-hexosaminidase release from, LAD2 mast cells. Unlike the currently known inactivating mAbs, Y1 appears to target the first extracellular domain of Orai.

Synta66. Synta66 inhibits store-operated Ca^{2+} influx in numerous cell types, including RBL-2H3 cells (132), vascular smooth muscle cells (133), and vascular endothelial cells (134). It inhibits native whole-cell I_{CRAC} in RBL-2H3 cells (132) and cytokine production by lamina propria mononuclear cells from patients with IBD (99). Like BTP2, the detailed mechanism of action for Synta66 is unclear but shares similar kinetics of block and lack of effect on STIM1 puncta formation. Its pharmacological selectivity profile is significantly improved over BTP2, with no activity at TRPC1/5/6 or TRPV4 (133) or against a panel of 50 diverse GPCR/transporter/ion channel targets (99). Synta66 is an effective experimental tool to probe CRAC channel function in vitro and in animal models.

RO2959. RO2959 inhibits Ca^{2+} influx and native I_{CRAC} in RBL-2H3 cells and store-operated currents in STIM1/Orai1-expressing CHO cells, where it appears to be modestly selective for Orai1 (135). It also inhibits T cell effector function and proliferation (135). No significant activity was reported against a panel of ion channel targets, including TRPC1 and TRPM4. The in vivo efficacy of RO2959 remains unclear.

GSK-7975A. The pyrazolyl carboxamide GSK-7975A, which also has a pharmacophore related to BTP2, inhibited store-operated currents in wild-type RBL-2H3 and STIM1/Orai1-overexpressing HEK293 cells. The kinetics of block for GSK-7975A were sluggish and its reversibility poor, like BTP2 (136), but there was little off-target activity against TRPC4 or TRPC6. However, block of TRPV6 was reported. Mechanistic FRET studies showed that GSK-7975A impaired neither STIM1 oligomerization nor its gross interaction with Orai1. Interestingly, 2-APB-gated, store-independent Orai3 currents and $Orai1^{E106D}$ currents were less sensitive to GSK-7975A, suggesting that pore geometry influenced GSK-7975A binding (136). This GlaxoSmithKline compound is effective in animal models of acute pancreatitis.

AnCoA4 (jamaicin). Sadaghiani et al. (107) identified the isoflavone AnCoA4 as a CRAC channel inhibitor by using a novel discovery strategy. They generated glutathione-*S*-transferase (GST)

fusions of the STIM1 and Orai1 domains involved in channel gating (107) and used them to probe surface-immobilized small-molecule microarrays, and detected binding using fluorescent anti-GST antibodies. AnCoA4 was confirmed as an inhibitor of CRAC channels and of NFAT signaling. Mechanism-of-action studies suggested that AnCoA4 competed with and perturbed Orai1/STIM1 interaction. The selectivity and in vivo efficacy of AnCoA4 remain unclear.

RP3128 and RP4010. Rhizen Pharmaceuticals developed RP3128, an inhibitor of I_{CRAC}, IgE-induced RBL-2H3 cell degranulation (98), and IL-4 and IL-5 release from human whole blood. RP3128 is active in allergic asthma and chronic airway inflammation models, but a phase I clinical trial for asthma was terminated at the recruitment stage. RP4010 inhibits I_{CRAC} and nuclear factor κB translocation and suppresses tumor growth in xenografted mice (137). Although it is active in preclinical models of gastrointestinal cancers, a phase I/Ib trial for lymphoma was terminated in 2019 following a review of the compound's pharmacokinetics and safety.

CM2489, CM3457, and CM4620. CM2489, CM3457, and CM4620 are CRAC channel blockers from CalciMedica that potently inhibit I_{CRAC}, T cell release of proinflammatory cytokines IL-2 and IL-17, and neutrophil activation, and they look to be promising candidates for clinical use. CM2489 was the first CRAC channel inhibitor to reach phase I clinical trials, for psoriasis, and CM4620 is effective in experimentally induced acute pancreatitis (89, 90). Patent literature suggests that these molecules are well tolerated in humans, and a phase I/II trial is currently recruiting (**https://clinicaltrials.gov/ct2/show/NCT04195347**).

CONCLUDING REMARKS

In recent years, spectacular progress has been made in our understanding of the structure, gating, and function of CRAC channels. However, potent and selective channel blockers with good efficacy in vivo have been less forthcoming. There has been a reluctance in the pharmaceutical industry to pursue CRAC channels for two reasons. First, the broad tissue distribution of CRAC channels is seen as a potential for pleiotropy. Second, loss-of-function Orai1 mutations cause SCID, which might mean that CRAC channel blockers are immunosuppressive and increase the risk of opportunistic infection. However, several considerations suggest these properties should not be major concerns. First, CRAC channel blockers, including Synta66 and the Rhizen and CalciMedica compounds, have been dosed successfully for weeks in animal models. Second, CalciMedica completed phase I clinical trials with their lead candidates and disclosed no safety concerns. These data are consistent with the human Orai1 loss-of-function mutant R91W; although homozygous R91W individuals present with SCID, heterozygotes have no overt phenotype (48). Loss-of-function mutations result in the complete loss of functional CRAC channels, but complete inhibition is unlikely to be necessary for CRAC channel blockers to be clinically effective, especially if the goal is only to dampen exaggerated Ca^{2+} entry to normal levels. Finally, it is now known that CRAC channel function in the immune system is nuanced and that different effector T cell populations, for example, are differentially affected by partial CRAC channel block. Th1 and Th17 cells are strongly dependent on CRAC channels, and partial channel inhibition can reduce function (138). By contrast, effector regulatory T cells function even after partial channel block (138, 139). Cytotoxic T cell activity is increased by partial CRAC channel inhibition, enhancing tumor elimination (85). Therefore, targeting CRAC channels could impact Th1/Th17-dependent autoimmune disease or enhance tumor killing while leaving other immune responses intact. Therapeutic windows in other cell types could permit CRAC channel targeting for the management of specific diseases.

DISCLOSURE STATEMENT

D.B., F.M., and A.B.P. worked together at Calcico Therapeutics (2012–2019), where A.B.P. was Scientific Founder, F.M. was Head of Biology, and D.B. was Senior Scientist.

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LITERATURE CITED

1. Putney JWJ. 1986. A model for receptor-regulated calcium entry. *Cell Calcium* 7:1–12
2. Putney JWJ. 1990. Capacitative calcium entry revisited. *Cell Calcium* 11:611–24
3. Thastrup O, Dawson AP, Scharff O, Foder B, Cullen PJ, et al. 1989. Thapsigargin, a novel molecular probe for studying intracellular calcium release and storage. *Agents Actions* 27:17–23
4. Takemura H, Hughes AR, Thastrup O, Putney JWJ. 1989. Activation of calcium entry by the tumour promoter thapsigargin in parotid acinar cells. Evidence that an intracellular calcium pool and not an inositol phosphate regulates calcium fluxes at the plasma membrane. *J. Biol. Chem.* 264:12266–71
5. Hoth M, Penner R. 1992. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* 355:353–56
6. Hofer A, Fasolato C, Pozzan T. 1998. Capacitative Ca^{2+} entry is closely linked to the filling state of internal Ca^{2+} stores: a study using simultaneous measurements of I_{CRAC} and intraluminal [Ca^{2+}]. *J. Cell Biol.* 140:325–34
7. Parekh AB, Penner R. 1997. Store-operated calcium influx. *Physiol. Rev.* 77:901–30
8. Prakriya M, Lewis RS. 2015. Store-operated calcium channels. *Physiol. Rev.* 95:1383–436
9. Prakriya M, Lewis RS. 2002. Separation and characterization of currents through store-operated CRAC channels and Mg^{2+}-inhibited cation (MIC) channels. *J. Gen. Physiol.* 119:487–508
10. Zweifach A, Lewis RS. 1995. Rapid inactivation of depletion-activated calcium current (ICRAC) due to local calcium feedback. *J. Gen. Physiol.* 105:209–26
11. Fierro L, Parekh AB. 1999. Fast calcium-dependent inactivation of calcium release-activated calcium current (CRAC) in RBL-1 cells. *J. Membr. Biol.* 168:9–17
12. Zweifach A, Lewis RS. 1995. Slow calcium-dependent inactivation of depletion-activated calcium current. *J. Biol. Chem.* 270:14445–51
13. Parekh AB. 1998. Slow feedback inhibition of calcium release-activated calcium current by calcium entry. *J. Biol. Chem.* 273:14925–32
14. Hoth M, Button D, Lewis RS. 2000. Mitochondrial control of calcium channel gating: a mechanism for sustained signaling and transcriptional activation in T lymphocytes. *PNAS* 97:10607–12
15. Gilabert J-A, Parekh AB. 2000. Respiring mitochondria determine the pattern of activation and inactivation of the store-operated Ca^{2+} current I_{CRAC}. *EMBO J.* 19:6401–7
16. Palty R, Raveh A, Kaminsky I, Meller R, Reuveny E. 2012. SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling. *Cell* 149:425–38
17. Parekh AB, Putney JWJ. 2005. Store-operated calcium channels. *Physiol. Rev.* 85:757–810
18. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, et al. 2005. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J. Cell Biol.* 169:435–45
19. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, et al. 2005. STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. *Curr. Biol.* 15:1235–41
20. Soboloff J, Rothberg BS, Madesh M, Gill DL. 2012. STIM proteins: dynamic calcium signal transducers. *Nat. Rev. Mol. Cell Biol.* 13:549–65
21. Covington ED, Wu MM, Lewis RS. 2010. Essential role for the CRAC activation domain in store-dependent oligomerization of STIM1. *Mol. Biol. Cell* 21:1897–907
22. Muik M, Fahrner M, Derler I, Schindl R, Bergsmann J, et al. 2009. A cytosolic homomerization and a modulatory domain within STIM1 C terminus determine coupling to ORAI1 channels. *J. Biol. Chem.* 284:8421–26

23. Zhou Y, Srinivasan P, Razavi S, Seymour S, Meraner P, et al. 2010. Initial activation of STIM1, the regulator of store-operated calcium entry. *Nat. Struct. Mol. Biol.* 20:973–81
24. Yang X, Jin H, Cai X, Li S, Shen Y. 2012. Structural and mechanistic insights into the activation of Stromal interaction molecule 1 (STIM1). *PNAS* 109:5657–62
25. Stathopulos PB, Li GY, Plevin MJ, Ames JB, Ikura M. 2006. Stored Ca^{2+} depletion-induced oligomerization of stromal interaction molecule 1 (STIM1) via the EF-SAM region: an initiation mechanism for capacitive Ca^{2+} entry. *J. Biol. Chem.* 281:35855–62
26. Liou J, Fivaz M, Inoue T, Meyer T. 2007. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after calcium store depletion. *PNAS* 104:9301–6
27. Muik M, Frischauf I, Derler I, Fahrner M, Bergsmann J, et al. 2008. Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation. *J. Biol. Chem.* 283:8014–22
28. Li Z, Lu J, Xu P, Xie X, Chen L, Xu T. 2007. Mapping the interacting domains of STIM1 and Orai1 in Ca^{2+} release-activated Ca^{2+} channel activation. *J. Biol. Chem.* 282:29448–56
29. Korzeniowski MK, Manjarrés IM, Varnai P, Balla T. 2010. Activation of STIM1-Orai1 involves an intramolecular switching mechanism. *Sci. Signal.* 3:ra82
30. Muik M, Fahrner M, Schindl R, Stathopulos P, Frischauf I, et al. 2011. STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation. *EMBO J.* 30:1678–89
31. Wu MM, Buchanan J, Luik RM, Lewis RS. 2006. Ca store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J. Cell Biol.* 174:803–13
32. Lur G, Haynes LP, Prior IA, Gerasimenko OV, Feske S, et al. 2009. Ribosome-free terminals of rough ER allow formation of STIM1 puncta and segregation of STIM1 from IP_3 receptors. *Curr. Biol.* 19:1648–53
33. Zheng L, Stathopulos PB, Li GY, Ikura M. 2008. Biophysical characterization of the EF-hand and SAM domain containing Ca^{2+} sensory region of STIM1 and STIM2. *Biochem. Biophys. Res. Commun.* 369:240–46
34. Brandman O, Liou J, Park WS, Meyer T. 2007. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum calcium levels. *Cell* 131:1327–39
35. Alonso MT, Barrero MJ, Carnicero E, Montero M, Garcia-Sancho J, Alvarez J. 1998. Functional measurements of $[Ca^{2+}]$ in the endoplasmic reticulum using a herpes virus to deliver targeted aequorin. *Cell Calcium* 24:87–96
36. Hofer AM, Schulz I. 1998. Quantification of intraluminal free [Ca] in the agonist-sensitive internal calcium store using compartmentalized fluorescent indicators: some considerations. *Cell Calcium* 20:235–42
37. Luik RM, Wang B, Prakriya M, Wu MM, Lewis RS. 2008. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. *Nature* 454:538–42
38. Parekh AB, Fleig A, Penner R. 1997. The store-operated calcium current I_{CRAC}: nonlinear activation by $InsP_3$ and dissociation from calcium release. *Cell* 89:973–80
39. Fierro L, Parekh AB. 2000. Substantial depletion of the intracellular Ca^{2+} stores is required for macroscopic activation of the Ca^{2+} release-activated Ca^{2+} current in rat basophilic leukaemia cells. *J. Physiol.* 522:247–57
40. Oh-hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, et al. 2008. Dual functions for the endoplasmic reticulum calcium sensors for STIM1 and STIM2 in T cell activation and tolerance. *Nat. Immunol.* 9:432–42
41. Bird GS, Hwang SY, Smyth JT, Fukushima M, Boyles RR, Putney JWJ. 2009. STIM1 is a calcium sensor specialised for digital signalling. *Curr. Biol.* 19:1724–29
42. Kar P, Bakowski D, Di Capite J, Nelson C, Parekh AB. 2012. Different agonists recruit different stromal interaction molecule proteins to support cytoplasmic Ca^{2+} oscillations and gene expression. *PNAS* 109:6969–74
43. Ong HL, Brito de Souza L, Zheng C, Cheng KT, Liu X, et al. 2015. STIM2 enhances receptor-stimulated Ca^{2+} signaling by promoting recruitment of STIM1 to the endoplasmic reticulum–plasma membrane junctions. *Sci. Signal.* 8:ra3

44. Williams RT, Manji SS, Parker NJ, Hancock MS, Van Stekelenburg L, et al. 2001. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem. J.* 357:673–85
45. Darbellay B, Arnaudeau S, Bader CR, Konig S, Bernheim L. 2011. STIM1L is a new actin-binding splice variant involved in fast repetitive Ca^{2+} release. *J. Cell Biol.* 194:335–46
46. Rana A, Yen M, Sadaghiani AM, Malmersjö S, Park CY, et al. 2015. Alternative splicing converts STIM2 from an activator to an inhibitor of store-operated calcium channels. *J. Cell Biol.* 209:653–66
47. Miederer AM, Alansary D, Schwär G, Lee PH, Jung M, et al. 2015. A STIM2 splice variant negatively regulates store-operated calcium entry. *Nat. Commun.* 6:6899
48. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel S-H, et al. 2006. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* 441:179–85
49. Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, et al. 2006. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* 312:1220–23
50. Zhang SL, Yeromin AV, Zhang XH-F, Yu Y, Safrina O, et al. 2006. Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. *PNAS* 103:9357–62
51. Peinelt C, Vig M, Koomoa DL, Beck A, Nadler MJS, et al. 2006. Amplification of CRAC current by STIM1 and CRACM1 (Orai1). *Nat. Cell Biol.* 8:771–73
52. Vig M, Beck A, Billingsley JM, Lis A, Parvez S, et al. 2006. CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr. Biol.* 16:2073–79
53. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. 2006. Orai1 is an essential pore subunit of the CRAC channel. *Nature* 443:230–33
54. Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. 2006. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* 443:226–29
55. McNally BA, Somasundaram S, Yamashita M, Prakriya M. 2012. Gated regulation of CRAC channel ion selectivity by STIM1. *Nature* 482:241–45
56. Gwack Y, Srikanth S, Feske S, Cruz-Guilloty F, Oh-hora M, et al. 2007. Biochemical and functional characterization of Orai proteins. *J. Biol. Chem.* 282:16232–43
57. Motiani RK, Abdullaev IF, Trebak M. 2010. A 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. *J. Biol. Chem.* 285:19173–83
58. Lis A, Peinelt C, Beck A, Parvez S, Monteilh-Zoller M, et al. 2007. CRACM1, CRACM2 and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties. *Curr. Biol.* 17:794–800
59. McNally BA, Yamashita M, Engh A, Prakriya M. 2009. Structural determinants of ion permeation in CRAC channels. *PNAS* 106:22516–21
60. Zhou Y, Ramachandran S, Oh-hora M, Rao A, Hogan PG. 2010. Pore architecture of the ORAI1 store-operated calcium channel. *PNAS* 107:4896–901
61. Hou X, Pedi L, Diver MM, Long SB. 2012. Crystal structure of the calcium release-activated calcium channel Orai. *Science* 338:1308–13
62. Lewis RS, Prakriya M. 2015. Store-operated calcium channels. *Physiol. Rev.* 95:1383–436
63. Hou X, Burstein SR, Long SB. 2018. Structures reveal opening of the store-operated calcium channel Orai. *eLife* 7:e36758
64. Frischauf I, Litvinukova M, Schober R, Zayats V, Svobodova B, et al. 2017. Transmembrane helix connectivity in Orai1 controls two gates for calcium-dependent transcription. *Sci. Signal.* 10:eaao0358
65. Liu X, Wu G, Yu Y, Chen X, Ji R, et al. 2019. Molecular understanding of calcium permeation through the open Orai channel. *PLOS Biol.* 17:e3000096
66. Bohm J, Laporte J. 2018. Gain-of-function mutations in STIM1 and ORAI1 causing tubular aggregate myopathy and Stormorken syndrome. *Cell Calcium* 76:1–9
67. Derler I, Plenk P, Fahrner M, Muik M, Jardin I, et al. 2013. The extended transmembrane Orai1 N-terminal (ETON) region combines binding interface and gate for Orai1 activation by STIM1. *J. Biol. Chem.* 288:29025–34
68. Willoughby D, Everett KL, Halls ML, Pacheco J, Skroblin P, et al. 2012. Direct binding between Orai1 and AC8 mediates dynamic interplay between Ca^{2+} and cAMP signaling. *Sci. Signal.* 5:ra29

69. Li H, Pink MD, Murphy JG, Stein A, Dell'Acqua ML, Hogan PG. 2012. Balanced interactions of calcineurin with AKAP79 regulate Ca^{2+}-calcineurin-NFAT signaling. *Nat. Struct. Mol. Biol.* 19:337–45
70. Kar P, Samanta K, Kramer H, Morris O, Bakowski D, Parekh AB. 2014. Dynamic assembly of a membrane signaling complex enables selective activation of NFAT by Orai1. *Curr. Biol.* 24:1361–68
71. Yu F, Sun L, Machaca K. 2010. Constitutive recycling of the store-operated Ca^{2+} channel Orai1 and its internalization during meiosis. *J. Cell Biol.* 191:523–35
72. Yeh Y-C, Parekh AB. 2015. Distinct structural domains of caveolin-1 independently regulate Ca^{2+} release-activated Ca^{2+} channels and Ca^{2+} microdomain-dependent gene expression. *Mol. Cell. Biol.* 35:1341–49
73. Lacruz RS, Feske S. 2015. Diseases caused by mutations in *ORAI1* and *STIM1*. *Ann. N. Y. Acad. Sci.* 1356:45–79
74. Morin G, Bruechle NO, Singh AR, Knopp C, Jedraszak G, et al. 2014. Gain-of-function mutation in STIM1 (P.R304W) is associated with Stormorken syndrome. *Hum. Mutat.* 35:1221–32
75. Feske S. 2019. CRAC channels and disease—from human CRAC channelopathies and animal models to novel drugs. *Cell Calcium* 80:112–16
76. Gwack Y, Srikanth S, Oh-hora M, Hogan PG, Lamperti E, et al. 2008. Hair loss and defective T- and B-cell function in mice lacking ORAI1. *Mol. Cell. Biol.* 28:5209–22
77. Chalmers SB, Monteith GR. 2018. ORAI channels and cancer. *Cell Calcium* 74:160–67
78. Tang BD, Xia X, Lv XF, Yu BX, Yuan J-N, et al. 2017. Inhibition of Orai1-mediated Ca^{2+} entry enhances chemosensitivity of HepG2 heptacarcinoma cells to 5-fluorouracil. *J. Cell Mol. Med.* 21:904–15
79. Kim JH, Lkhagvadorj S, Lee MR, Hwang KH, Chung HC, et al. 2014. Orai1 and STIM1 are critical for cell migration and proliferation of clear cell renal cell carcinoma. *Biochem. Biophys. Res. Commun.* 448:76–82
80. Xia J, Wang H, Huang H, Sun L, Dong S, et al. 2016. Elevated Orai1 and STIM1 expressions upregulate MACC1 expression to promote tumor cell proliferation, metabolism, migration, and invasion in human gastric cancer. *Cancer Lett.* 381:31–40
81. Benzerdjeb N, Sevestre H, Ahidouch A, Ouadid-Ahidouch H. 2016. Orai3 is a predictive marker of metastasis and survival in resectable lung adenocarcinoma. *Oncotarget* 7:81588–97
82. Perrouin-Verbe M-A, Bruyere F, Rozet F, Vandier C, Fromont G. 2016. Expression of store-operated channel components in prostate cancer: the prognostic paradox. *Hum. Pathol.* 49:77–82
83. Feng M, Grice DM, Faddy HM, Nguyen N, Leitch S, et al. 2010. Store-independent activation of Orai1 by SPCA2 in mammary tumors. *Cell* 143:84–98
84. Yang S, Zhang JJ, Huang XY. 2009. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. *Cancer Cell* 15:124–34
85. Zhou X, Friedmann KS, Lyrmann H, Zhou Y, Schoppmeyer R, et al. 2018. A calcium optimum for cytotoxic T lymphocyte and natural killer cell cytotoxicity. *J. Physiol.* 596:2681–98
86. Gerasimenko JV, Gerasimenko OV, Petersen OH. 2014. The role of Ca^{2+} in the pathophysiology of pancreatitis. *J. Physiol.* 592:269–80
87. Raraty M, Ward J, Erdemli G, Vaillant C, Neoptolemos JP, et al. 2000. Calcium-dependent enzyme activation and vacuole formation in the apical granular region of pancreatic acinar cells. *PNAS* 97:13126–31
88. Gerasimenko JV, Gryshchenko O, Ferdek PE, Stapleton E, Hébert TO, et al. 2013. Ca^{2+} release-activated Ca^{2+} channel blockade as a potential tool in antipancreatitis therapy. *PNAS* 110:13186–91
89. Wen L, Voronina S, Javed MA, Awais M, Szatmary P, et al. 2015. Inhibitors of ORAI1 prevent cytosolic calcium-associated injury of human pancreatic acinar cells and acute pancreatitis in 3 mouse models. *Gastroenterology* 149:481–92
90. Waldron RT, Chen Y, Pham H, Go A, Su HY, et al. 2019. The Orai Ca^{2+} channel inhibitor CM4620 targets both parenchymal and immune cells to reduce inflammation in experimental acute pancreatitis. *J. Physiol.* 597:3085–105
91. Holgate ST. 2011. The sentinel role of the airway epithelium in asthma pathogenesis. *Immunol. Rev.* 242:205–19
92. Samanta K, Bakowski D, Parekh AB. 2014. Key role for store-operated Ca^{2+} channels in activating gene expression in human airway bronchial epithelial cells. *PLOS ONE* 9(8):e105586

93. Rice LV, Bax HJ, Russell LJ, Barrett VJ, Walton SE, et al. 2013. Characterization of selective calcium-release activated calcium channel blockers in mast cells and T-cells from human, rat, mouse and guinea-pig preparations. *Eur. J. Pharmacol.* 704:49–57
94. Lin YP, Nelson C, Kramer H, Parekh AB. 2018. The allergen Der p3 from house dust mite stimulates store-operated Ca^{2+} channels and mast cell migration through PAR4 receptors. *Mol. Cell* 70:228–41
95. Jairaman A, Yamashita M, Schleimer RP, Prakriya M. 2015. Store-operated Ca^{2+} release-activated Ca^{2+} channels regulate PAR2-activated Ca^{2+} signaling and cytokine production in airway epithelial cells. *J. Immunol.* 195:2122–33
96. Yoshino T, Ishikawa J, Ohga K, Morokata T, Takezawa R, et al. 2007. YM-58483, a selective CRAC channel inhibitor, prevents antigen-induced airway eosinophilia and late phase asthmatic responses via Th2 cytokine inhibition in animal models. *Eur. J. Pharmacol.* 560:225–33
97. Kaur M, Birrell MA, Dekkak B, Reynolds S, Wong S, et al. 2015. The role of CRAC channel in asthma. *Pulm. Pharmacol. Ther.* 35:67–74
98. Sutovska M, Kocmalova M, Franova S, Vakkalanka S, Viswanadha S. 2016. Pharmacodynamic evaluation of RP3128, a novel and potent CRAC channel inhibitor in guinea pig models of allergic asthma. *Eur. J. Pharmacol.* 772:62–70
99. Di Sabatino A, Rovedatti L, Kaur R, Spencer JP, Brown JT, et al. 2009. Targeting gut T cell Ca^{2+} release-activated Ca^{2+} channels inhibits T cell cytokine production and T-box transcription factor T-bet in inflammatory bowel disease. *J. Immunol.* 183:3454–62
100. McCarl C-A, Khalil S, Ma J, Oh-hora M, Yamashita M, et al. 2010. Store-operated Ca^{2+} entry through ORAI1 is critical for T cell-mediated autoimmunity and allograft rejection. *J. Immunol.* 185:5845–58
101. Whitten JP. 2010. *Inhibitors of store operated calcium release*. US Patent Appl. 2010/0152241A1
102. Yen JH, Chang CM, Hsu YW, Lee CH, Wu MS, et al. 2014. A polymorphism of *ORAI1* rs7135617, is associated with susceptibility to rheumatoid arthritis. *Mediators Inflamm*. 2014:834831
103. Liu S, Watanabe S, Shudou M, Kuno M, Miura H, Maeyama K. 2014. Upregulation of store-operated Ca^{2+} entry in the naïve $CD4^{+}$ T cells with aberrant cytokine releasing in active rheumatoid arthritis. *Immunol. Cell Biol.* 92:752–60
104. Liu S, Kiyoi T, Takemasa E, Maeyama K. 2017. Intra-articular lentivirus-mediated gene therapy targeting CRACM1 for the treatment of collagen-induced arthritis. *J. Pharmacol. Sci.* 133:130–38
105. Liu S, Hasegawa H, Takemasa E, Suzuki Y, Oka K, et al. 2017. Efficiency and safety of CRAC inhibitors in human rheumatoid arthritis xenograft models. *J. Immunol.* 199:1584–95
106. DeHaven WI, Smyth JT, Boyles RR, Bird GS, Putney JW. 2008. Complex actions of 2-aminoethyldiphenyl borate (2-APB) on store-operated calcium entry. *J. Biol. Chem.* 283:19265–73
107. Sadaghiani AM, Lee SM, Odegaard JI, Leveson-Gower DB, McPherson OM, et al. 2014. Identification of Orai1 channel inhibitors by using minimal functional domains to screen small molecule microarrays. *Chem. Biol.* 21:1278–92
108. Putney JW. 2001. Pharmacology of capacitative calcium entry. *Mol. Interv.* 1:84–94
109. Merritt JE, Armstrong WP, Benham CD, Hallam TJ, Jacob R, et al. 1990. SK&F 96365, a novel inhibitor of receptor-mediated calcium entry. *Biochem. J.* 271:515–22
110. Franzius D, Hoth M, Penner R. 1994. Non-specific effects of calcium entry antagonists in mast cells. *Pflugers Arch.* 428:433–38
111. Zakharov SI, Smani T, Dobrydneva Y, Monje F, Fichandler C, et al. 2004. Diethylstilbestrol is a potent inhibitor of store-operated channels and capacitative Ca^{2+} influx. *Mol. Pharmacol.* 66:702–7
112. Brueggemann LI, Markun DR, Henderson KK, Cribbs LL, Byron KL. 2006. Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca^{2+} entry in vascular smooth muscle cells. *J. Pharmacol. Exp. Ther.* 317:488–99
113. Kohn EC, Sandeen MA, Liotta LA. 1992. In vivo efficacy of a novel inhibitor of selected signal transduction pathways including calcium, arachidonate, and inositol phosphates. *Cancer Res.* 52:3208–12
114. Rodland KD, Wersto RP, Hobson S, Kohn EC. 1997. Thapsigargin-induced gene expression in nonexcitable cells is dependent on calcium influx. *Mol. Endocrinol.* 11:281–91
115. Hussain MM, Kotz H, Minasian L, Premkumar A, Sarosy G, et al. 2003. Phase II trial of carboxyamidotriazole in patients with relapsed epithelial ovarian cancer. *J. Clin. Oncol.* 21:4356–63

116. Mignen O, Brink C, Enfissi A, Nadkarni A, Shuttleworth TJ, et al. 2005. Carboxyamidotriazole-induced inhibition of mitochondrial calcium import blocks capacitative calcium entry and cell proliferation in HEK-293 cells. *J. Cell Sci.* 118:5615–23
117. Maruyama T, Kanaji T, Nakade S, Mikoshiba K. 1997. 2APB, 2-aminoethoxydeiphenyl borate, a membrane-permeable modulator of Ins(1,4,5)P_3-induced Ca^{2+} release. *J. Biochem.* 122:498–505
118. Ma HT, Patterson RL, Van Rossum DB, Birnbaumer L, Mikoshiba K, Gill DL. 2000. Requirement of the inositol trisphosphate receptor for activation of store-operated Ca^{2+} channels. *Science* 287:1647–51
119. Bakowski D, Glitsch MD, Parekh AB. 2001. An examination of the secretion-like coupling model for the activation of the Ca^{2+} release-activated Ca^{2+} current I_{CRAC} in RBL-1 cells. *J. Physiol.* 532:55–71
120. Prakriya M, Lewis RS. 2001. 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.* 536:3–19
121. Mercer JC, DeHaven W, Smyth JT, Wedel B, Boyles RB, et al. 2006. Large store-operated calcium-selective currents due to co-expression of Orai1 with the intracellular calcium sensor, STIM1. *J. Biol. Chem.* 281:24979–90
122. Schindl R, Bergsmann J, Frischauf I, Derler I, Fahrner M, et al. 2008. 2-Aminoethoxydiphenyl borate alters selectivity of Orai3 channels by increasing their pore size. *J. Biol. Chem.* 283:20261–67
123. Goto J, Suzuki AZ, Ozaki S, Matsumoto N, Nakamura T, et al. 2010. Two novel 2-aminoethyl diphenylborinate (2-APB) analogues differentially activate and inhibit store-operated Ca^{2+} entry via STIM proteins. *Cell Calcium* 47:1–10
124. Djuric SW, BaMaung NY, Basha A, Liu H, Luly JR, et al. 2000. 3,5-Bis(trifluoromethyl)pyrazoles: a novel class of NFAT transcription factor regulator. *J. Med. Chem.* 43:2975–81
125. Takezawa R, Cheng H, Beck A, Ishikawa J, Launay P, et al. 2006. A pyrazole derivative potently inhibits lymphocyte Ca^{2+} influx and cytokine production by facilitating transient receptor potential melastatin 4 channel activity. *Mol. Pharmacol.* 69:1413–20
126. He LP, Hewavitharana T, Soboloff J, Spassova MA, Gill DL. 2005. Functional link between store-operated and TRPC channels revealed by the 3,5-bis(trifluoromethyl)pyrazole derivative, BTP2. *J. Biol. Chem.* 280:10997–1006
127. Ishikawa J, Ohga K, Yoshino T, Takezawa R, Ichikawa A, et al. 2003. A pyrazole derivative, YM-58483, potently inhibits store-operated sustained calcium influx and Il-2 production in T lymphocytes. *J. Immunol.* 170:4441–49
128. Lin FF, Elliott R, Colombero A, Gaida K, Kelley L, et al. 2013. Generation and characterization of fully human monoclonal antibodies against human Orai1 for autoimmune disease. *J. Pharmacol. Exp. Ther.* 345:225–38
129. Cox JH, Hussell S, Søndergaard H, Roepstorff K, Bui JV, et al. 2013. Antibody-mediated targeting of the Orai1 calcium channel inhibits T cell function. *PLOS ONE* 8:e82944
130. Gaida K, Salimi-Moosavi H, Subramanian R, Almon V, Knize A, et al. 2017. Inhibition of CRAC with a human anti-ORAI1 monoclonal antibody inhibits T-cell-derived cytokine production but fails to inhibit a T-cell-dependent antibody response in the cynomolgus monkey. *J. Immunotoxicol.* 12:164–73
131. Sun R, Yang Y, Ran X, Yang TT. 2016. Calcium influx of mast cells is inhibited by aptamers targeting the first extracellular domain of Orai1. *PLOS ONE* 11:e0158223
132. Ng S-W, DiCapite JL, Singaravelu K, Parekh AB. 2008. Sustained activation of the tyrosine kinase Syk by antigen in mast cells requires local Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} channels. *J. Biol. Chem.* 283:31348–55
133. Li J, McKeown L, Ojelabi O, Stacey M, Foster R, et al. 2011. Nanomolar potency and selectivity of a Ca^{2+} release-activated Ca^{2+} channel inhibitor against store-operated Ca^{2+} entry and migration of vascular smooth muscle cells. *Br. J. Pharmacol.* 164:382–93
134. Li J, Cubbon RM, Wilson LA, Amer MS, McKeown L, et al. 2011. Orai1 and CRAC channel dependence of VEGF-activated Ca^{2+} entry and endothelial tube formation. *Circ. Res.* 108:1190–98
135. Chen G, Panicker S, Lau KY, Apparsundaram S, Patel VA, et al. 2013. Characterization of a novel CRAC inhibitor that potently blocks human T cell activation and effector functions. *Mol. Immunol.* 54:355–67
136. Derler I, Schindl R, Fritsch R, Heftberger P, Riedl MC, et al. 2013. The action of selective CRAC channel blockers is affected by the Orai pore geometry. *Cell Calcium* 53:139–51

137. Cui C, Chang Y, Zhang X, Choi S, Tran H, et al. 2018. Targeting Orai1-mediated store-operated calcium entry by RP4010 for anti-tumor activity in esophagus squamous cell carcinoma. *Cancer Lett.* 432:169–79
138. Vaeth M, Maus M, Klein-Hessling S, Freinkman E, Yang J, et al. 2017. Store-operated Ca^{2+} entry controls clonal expansion of T cells through metabolic reprogramming. *Immunity* 47:664–79.e6
139. Kaufmann U, Shaw PJ, Kozhaya L, Subramanian R, Gaida K, et al. 2016. Selective Orai1 inhibition ameliorates autoimmune central nervous system inflammation by suppressing effector but not regulatory T cell function. *J. Immunol.* 196:573–85
140. Jacob R. 1990. Agonist-stimulated divalent cation entry into single cultured human umbilical vein endothelial cells. *J. Physiol.* 421:55–77
141. Parekh AB, Foguet M, Luebbert H, Stuehmer W. 1993. Ca^{2+} oscillations and Ca^{2+} influx in *Xenopus* oocytes expressing a novel 5-hydroxytryptamine receptor. *J. Physiol.* 469:653–71
142. Bautista DM, Lewis RS. 2004. Modulation of plasma membrane calcium-ATPase activity by local calcium microdomains near CRAC channels in human T cells. *J. Physiol.* 556:805–17
143. Hogan PG, Chen L, Nardone J, Rao A. 2003. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev.* 17:2205–32
144. Machaca K. 2003. Ca^{2+}-calmodulin dependent protein kinase II potentiates store-operated Ca^{2+} current. *J. Biol. Chem.* 278:33730–37
145. Chang WC, Parekh AB. 2004. Close functional coupling between CRAC channels, arachidonic acid release and leukotriene secretion. *J. Biol. Chem.* 279:29994–99
146. Chang W-C, Nelson C, Parekh AB. 2006. Ca^{2+} influx through CRAC channels activates cytosolic phospholipase A2, leukotriene C_4 secretion and expression of c-fos through ERK-dependent and independent pathways in mast cells. *FASEB J.* 20:1681–93
147. Lefkimmiatis K, Srikanthan M, Maiellaro I, Moyer MP, Curci S, Hofer AM. 2009. Store-operated cyclic AMP signalling mediated by STIM1. *Nat. Cell Biol.* 11:433–42
148. Alswied A, Parekh AB. 2015. Ca^{2+} influx through store-operated calcium channels replenishes the functional phosphatidylinositol 4,5-bisphosphate pool used by cysteinyl leukotriene type I receptors. *J. Biol. Chem.* 290:29555–66
149. Lin S, Fagan KA, Li K-X, Shaul WPW, Cooper DMF, Rodman DM. 2000. Sustained endothelial nitric oxide-synthase activation requires capacitative Ca^{2+} entry. *J. Biol. Chem.* 275:17979–85
150. Feldman B, Fedida-Metula S, Nita J, Sekler I, Fishman D. 2010. Coupling of mitochondria to store-operated Ca^{2+} signaling sustains constitutive activation of protein kinase B/Akt and augments survival of malignant melanoma cells. *Cell Calcium* 47:525–37
151. Kaufmann U, Kahlfuss S, Yang J, Ivanova E, Koralov SB, Feske S. 2017. Calcium signaling controls pathogenic Th17 cell-mediated inflammation by regulating mitochondrial function. *Cell Metab.* 29:1104–18
152. Steinckwich N, Frippiat JP, Stasia MJ, Erard M, Boxio R, et al. 2007. Potent inhibition of store-operated Ca^{2+} influx and superoxide production in HL60 cells and polymorphonuclear neutrophils by the pyrazole derivative BTP2. *J. Leukoc. Biol.* 81:1054–64
153. Vig M, DeHaven WI, Bird GS, Billingsley JM, Wang HJ, et al. 2008. Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels. *Nat. Immunol.* 9:89–96
154. Maul-Pavicic A, Chiang SCC, Rensing-Ehl A, Jessen B, Fauriat C, et al. 2011. ORAI1-mediated calcium influx is required for human cytotoxic lymphocyte degranulation and target cell lysis. *PNAS* 108:3324–29
155. DiCapite JL, Shirley A, Nelson C, Bates G, Parekh AB. 2009. Intercellular calcium wave propagation involving positive feedback between CRAC channels and cysteinyl leukotrienes. *FASEB J.* 23:894–905
156. Wilson SR, The L, Batia LM, Beattie K, Katibah GE, et al. 2013. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. *Cell* 155:285–95
157. Toth AB, Hori H, Novakovic MM, Bernstein NG, Lambot L, Prakriya M. 2019. CRAC channels regulate astrocyte Ca^{2+} signaling and gliotransmitter release to modulate hippocampal GABAergic transmission. *Sci. Signal.* 12:eaaw5450
158. Steinckwich N, Myers P, Janardhan KS, Flagler ND, King D, et al. 2015. Role of the store-operated calcium entry protein, STIM1, in neutrophil chemotaxis and infiltration into a murine model of psoriasis-inflamed skin. *FASEB J.* 29:3003–13

159. Cheng KT, Liu X, Ong HL, Swaim W, Ambudkar IS. 2011. Local Ca^{2+} entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca^{2+} signals required for specific functions. *PLOS Biol.* 9:e10011025
160. Lemonnier L, Prevarskaya N, Shuba Y, Vanden-Abeele F, Nilius B, et al. 2002. Ca^{2+} modulation of volume-regulated anion channels: evidence for colocalization with store-operated channels. *FASEB J.* 16:222–24
161. Zholos A, Beck B, Sydorenko V, Lemonnier L, Bordat P, et al. 2005. Ca^{2+}- and volume-sensitive chloride currents are differentially regulated by agonists and store-operated Ca^{2+} entry. *J. Gen. Physiol.* 125:197–211
162. Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet JP. 2004. TRPM4 regulates calcium oscillations after T cell activation. *Science* 306:1374–77
163. Pulina MV, Zulian A, Baryshnikov SG, Linde CI, Krashima I, et al. 2013. Cross-talk between plasma membrane Na^{+}/Ca^{2+} exchanger-1 and TRPC/Orai containing channels: key players in arterial hypertension. In *Sodium Calcium Exchange: A Growing Spectrum of Pathophysiological Implications*, ed. L Annunziato, pp. 365–74. New York: Springer
164. Duffy SM, Ashmole I, Smallwood DT, Leyland ML, Bradding P. 2015. Orai/CRACM1 and $K_{Ca}3.1$ ion channels interact in the human lung mast cell plasma membrane. *Cell Commun. Signal.* 13:32
165. Dolmetsch RE, Xu K, Lewis RS. 1998. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* 392:933–36
166. Di Capite J, Ng S-W, Parekh AB. 2009. Decoding of cytoplasmic Ca^{2+} oscillations through the spatial signature drives gene expression. *Curr. Biol.* 19:853–58
167. Ng S-W, Nelson C, Parekh AB. 2009. Coupling of Ca^{2+} microdomains to spatially and temporally distinct cellular responses by the tyrosine kinase Syk. *J. Biol. Chem.* 284:24767–72
168. Davis FM, Janoshazi A, Janardhan KS, Steinckwich N, D'Agostin DM, et al. 2015. Essential role of Orai1 store-operated calcium channels in lactation. *PNAS* 112:5827–32