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Orai channels: key players in Ca^{2+} homeostasis

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Maintaining a precise calcium (Ca^{2+}) balance is vital for cellular survival. The most prominent pathway to shuttle Ca^{2+} into cells is the Ca^{2+} release activated Ca^{2+} (CRAC) channel. Orai proteins are indispensable players in this central mechanism of Ca^{2+} entry. This short review traces the latest articles published in the field of CRAC channel signalling with a focus on the structure of the pore-forming Orai proteins, the propagation of the binding signal from STIM1 through the channel to the central pore and their role in human health and disease.

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History

In 2006, RNA interference (RNAi) approaches together with human genetic linkage analysis identified a 33 kDa (301 amino acids) cell surface protein genetically encoded on chromosome 12 [1–3]. First described as CRACM1 and later named after the keepers of the gates of heaven in Greek mythology – the *Horae* – this protein was then referred to as Orai1 and proven as the pore forming unit of one of the most prominent Ca^{2+} entry pathway – the Ca^{2+} release activated Ca^{2+} (CRAC) channel. The activation process of CRAC channels is highly unusual as it involves proteins residing within different cellular compartments: STIM1 acting as the Ca^{2+} sensor in the ER (for a detailed review on STIM proteins see Fahrner *et al.* within this issue) and Orai1 as the pore-forming subunit in the plasma membrane.

Importance

Ca^{2+} signalling through CRAC channels is a complex network of intertwining pathways and tight regulation of Ca^{2+} is vital for cellular survival. Ca^{2+}-dysregulation has been shown to be involved in several pathophysiological cellular malfunctions. Serious clinical human phenotypes result from loss-of-function or gain-of-function (LOF and GOF, respectively) mutations [4]. Initially discovered in patients with severe combined immunodeficiency (SCID; R91W mutation leading to lack of CRAC channel function in T-cells [1]), Orai proteins have now been associated with various diseases [5]. Several mutations within transmembrane (TM) domains of Orai1 are the cause for tubular aggregate myopathy (TAM) (TM1: S97C, G98S, V107M TM2: L138F TM3: T184M TM4: P245L; Table 1) [6–9]. A single nucleotide polymorphism in Orai1 (S218G [10•]) is associated with atopic dermatitis and alterations in store-operated Ca^{2+} entry (SOCE) via CRAC channels are related to end-stage human failing myocardium [11]. It has also been shown that Orai1–3 are upregulated in pregnancy and type 1 diabetes [12]. Accumulating evidence suggests that altered Ca^{2+} influx due to oncogenic remodelling of Orai proteins plays a critical role in cancer hallmarks like unrestricted proliferation, resistance to cell death, metastasis as well as tumour vascularization and antitumor immunity (reviewed in Refs. [13,14]). Orai1 and Orai3 proteins have been related to tumorigenesis of breast cancer as well as lung adenocarcinoma and play a role in cell migration and metastatic invasiveness [14–21]. In the context of breast cancer as well as prostate cancer cells, altered expression levels of Orai channels remodels the Ca^{2+} signalling pathways to avoid cell death [14]. Overexpression of Orai channels in prostate cancer has been linked with a decreased risk of recurrence after prostatectomy [22]. Angiogenesis, crucial for tumour development, has been shown to be dependent on calcium signalling [23]. SOCE is also responsible for the secretion of vascular endothelial growth factor (VEGF) [15,24]. Pharmacological inhibition of Orai can diminish the growth of colorectal, breast, liver, melanoma and clear cell renal cancer cells [15]. Understanding the molecular architecture and choreography of CRAC channels, therefore, has significant potential for therapeutic applications.

Orai proteins

There are three highly conserved homologous proteins of Orai1 known (Orai 1–3). They are located and distributed uniformly in the plasma membrane and consist of four, highly conserved transmembrane domains (Orai1: TM1–4; aa92–106, aa118–140, aa174–197 and aa236–258) sharing ~81–87% sequence similarity within TM2-4 and complete identity in the functionally critical TM1 helix. Both N-terminal and C-terminal strands reside in the cytoplasm (Figure 1a). The cytoplasmic strands exhibit considerable sequence homology within the segments concerned with direct STIM-Orai interaction. TM-domains are connected via two extra- (loop1, loop3) as well as one intracellular loop (loop2), showing isoform

Table 1

The effect of identified Orai1 mutations on channel function

Residue/mutation[reference]	Location	Channel behaviour	Associated disease
K85E [61]	N-terminus	Inactive	
R91W [1]	TM1	Inactive	SCID
S97C [9]		Constitutive	TAM
G98C/D/P [38,62]		Constitutive	
G98R [63]		Inactive	CID; autoimmunity
G98S [8]		Constitutive	TAM
F99C/G/M/S/T/Y/W [38]		Constitutive	
V102A/C/G/S/T [64]		Constitutive	
V102I/L/M/V [64]		Store-operated	
E106Q [65]		Inactive	
V107M [8]		Constitutive	TAM
H134S/A/C/T/V/Q/E/M [37,60•]	TM2	Constitutive	
H134K/W [60•]		Inactive	
A137V [37]		Constitutive	Colorectal tumor
L138F [6]		Constitutive	TAM
L174D [49]	TM3	Inactive	
W176C [66]		Constitutive	
G183A [66]		Inactive	
G183D [37]		Inactive	Glioblastoma
T184M [7,8]		Store-operated	TAM
E190C [60•]		Constitutive	
S218G [10•]	Loop3	Store operated	Atopic dermatitis
N223S [10•]		Store operated	
P245L [67]	TM4	Constitutive	Stormorken like syndrome
L261A V262N H264G K265A (ANSGA) [49]	C-terminus	Constitutive	
L273S [68]		Inactive	
L273D [69]		Inactive	
L276D [70]		Inactive	

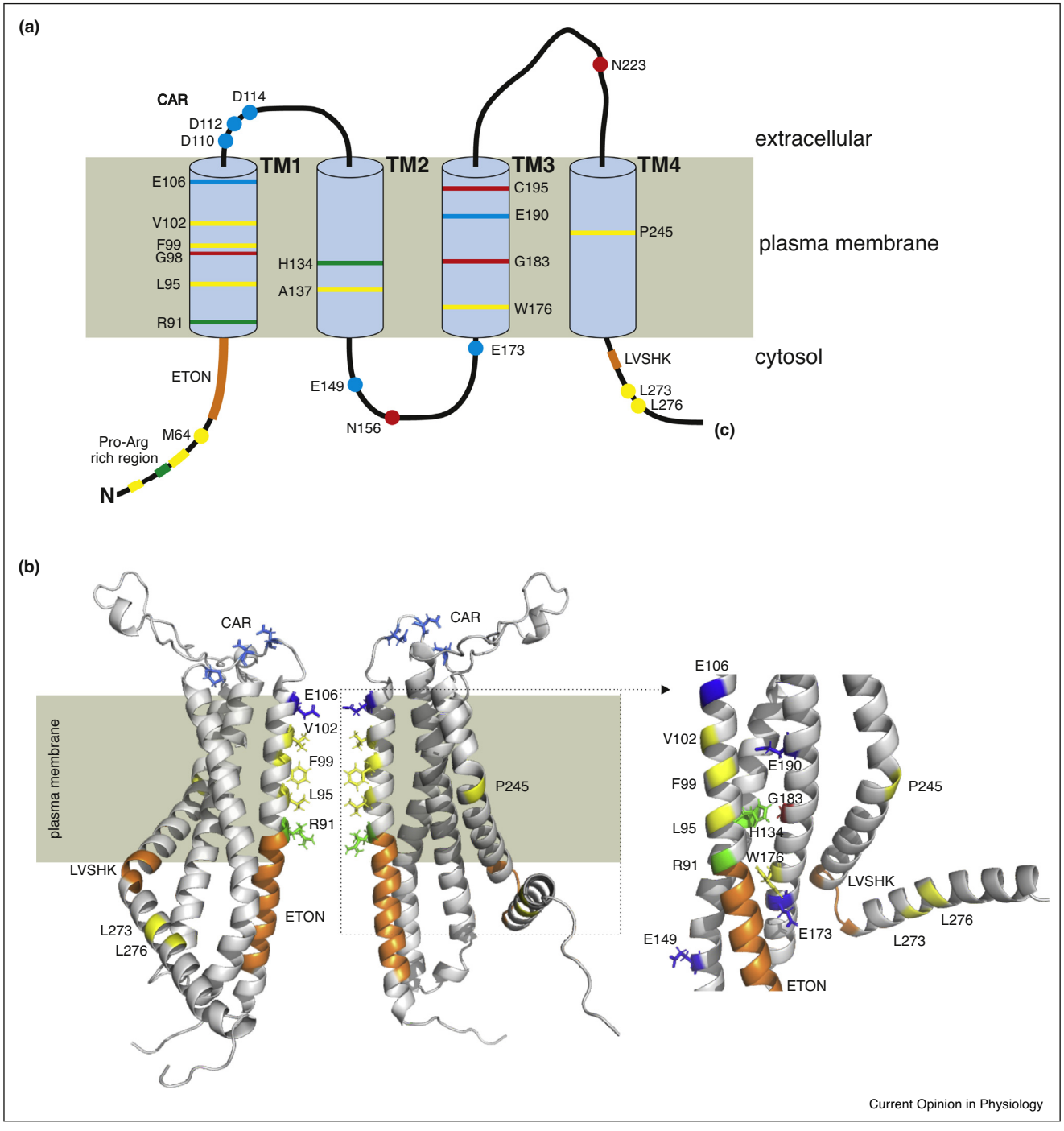
Most important currently identified mutations within Orai1 mentioned within this review, their location, resulting channel behaviour and associated diseases are indicated. (SCID: severe combined immunodeficiency; TAM: tubular aggregate myopathy; CID: combined immunodeficiency).

specific characteristics and sequence deviations especially within the loop3 (Figure 1a). Much less is known on the function of Orai2 and Orai3 compared to Orai1, therefore this review mainly focuses on the most prominent isoform, Orai1 (for a detailed review on Orai2 and Orai3 see Ref. [25]). In humans the Orai1 protein is expressed in a long (Orai1α) and a short (Orai1β) form originating from alternative translation initiation at Met_{64} [26,27].

Orai1 structure

In 2012, against all expectations and countering the prevailing evidence for a tetramer, the crystal structure of the *Drosophila melanogaster* Orai (dOrai) showed that the channel is hexameric [28]. The closed dOrai channel structure has revealed that six monomers are arranged around a central axis, in which TM1 of each subunit forms the inner surface of the pore, thereby confirming earlier studies that identified pore-lining residues [29] (Figure 1a,b). TM2-4 are positioned in concentric rings around this ion conducting pore [28]. dOrai proteins exhibit close similarity (~73% in TM regions) to human Orai1. This suggests and electrophysiological data strongly implies that also human Orai1 proteins assemble as hexamers [30,31]. Nonetheless, one should keep in mind that the dOrai crystal has been achieved by using various deletions and mutations, facilitating protein purification (reviewed in Ref. [32]). Store-dependent activation of CRAC channels involves direct binding of STIM1 to Orai1 as has been demonstrated by several groups [29,32]. Although a binding of STIM1 to the Orai1 N-terminus is still under debate, it has been shown via progressive N-terminal deletions that this region is essential for STIM1 coupling and that isolated fragments are able to bind to the STIM1 CAD domain [29,33,34]. Pre-binding of STIM1 to the Orai1 C-terminus followed by coupling to the Orai1 N-terminus in a stepwise manner has been suggested by Niu *et al.* [35•]. A 17 residues long conserved sequence, termed 'extended transmembrane Orai1 N-terminal' (ETON, aa73–90) region is suggested to be involved during STIM1/Orai1 activation [34]. Amino acid residues located within the ETON region and at the beginning of TM1 (K85, S89, S90, R91) were suggested to play important roles during STIM-Orai coupling [1,33,34] (Figure 1a,b). Yet, a full picture of the binding/activation mechanism between Orai1 N-terminus and STIM1 is still missing. The about 6 Å narrow, ion-conducting pore of the Orai channel is formed by

Figure 1



Schematic and structural representation of important domains in Orai1 proteins.
(a) Schematic representation of one hOrai1 monomer with important amino acids and regions highlighted. N-termini and C-termini are located inside the cytosol and range from aa1–91 and aa259–301, respectively. The ETON region (aa73–90) is highlighted in orange. The four TM-domains (TM1: aa92–106; TM2: aa118–140; TM3: aa174–197 and TM4: aa236–258) are connected via two extracellular loops (loop1 and loop3) and one intracellular loop (loop2). Important hOrai1 residues and domains highlighted in yellow represent unpolar, green basic, blue acidic and red neutral/polar side chains.
(b) Cross section through a 3D model of a hexameric hOrai1 channel, displaying two hOrai1 monomers facing each other with TM2-TM4 stabilizing the pore-forming TM1-helices. Important amino acid residues are highlighted as in (a). Additionally, side chains of amino acid residues facing the ion-conducting pore (left) and side chains of residues within TM2 and TM3 (right) crucial for stability of TM domains are depicted. (PDB number: 4HKR with adapted hOrai1 sequence described in Frischauf *et al.* [39]).

TM1 helices according to the dOrai crystal structure [28]. The channels entrance is surrounded by 6 highly conserved glutamate residues (E106 - selectivity filter) resulting in a hydrophobic surrounding and concentrated negative charges attracting Ca^{2+} ions [36] (Figure 1b). Residues within TM1 that are discussed to have specific functions in channel gating are V102 (hydrophobic gate), G98 (gating hinge), L95 as well as F99 (hydrophobic gate) [32,36] (Figure 1a,b). Additionally, R91, together with the formation of a water layer, may act as gate of the channel [37]. V102 and F99 have been proposed to work in concert forming a hydrophobic gate, as mutation to more polar amino acids leads to leaky gates [38]. Within the extracellular loop1 Frischauf *et al.* described a Ca^{2+} accumulating region (CAR) that enhances permeation at physiologically low Ca^{2+} levels via three negatively charged aspartates (D110, D112, D114) [29,39]. TM2-4 seem to stabilize the channel by forming a second layer around the central pore-forming TM1 helix [28]. Frischauf *et al.* [37] also identified several mutations (e.g. H134A, A137V) in TM2 affecting Orai channel gating. Especially the powerful H134A gain-of-function mutation developed constitutively active currents, completely independent of STIM1 activation. Using combined approaches including molecular dynamics simulations (MD-simulations), electrophysiology and cysteine-crosslinking, H134A proteins were shown to create hydrogen bonds between amino acid side chains facing the channels pore (S90 and R91). Additionally, mutating H134 to alanine decreases hydrophobic gating barriers by creating a chain of water molecules through the channels pore.

The role of the intracellular loop2 in humans is not fully clarified yet. It has been shown in *Caenorhabditis elegans* that STIM1 binds to loop2, pointing to a different activation mechanism than in humans [40]. In addition, loop2 seems to be responsible for the different, isoform-specific behaviour of human Orai1 and Orai3 proteins shown with various N-truncated mutants that interact between residues Y80 in the N-terminus and N156 in loop2 [41]. Mutation of W176 to cysteine (W176C) and G183 to alanine (G183A) within TM3 has dramatic effects on gating and selectivity of the channel [34]. MD-simulations have predicted that also residue E190 contributes to selectivity and gating by reducing the number of water molecules in this region when mutated to glutamine [42]. The E190 residue was also shown to be responsible for the external pH sensitivity if Ca^{2+} is not present [43]. Niemeyer *et al.* discovered redox-dependent regulation of Orai1 proteins which they attributed to the cysteine 195 residue (C195) close to the extracellular loop3 [44]. There is no corresponding C195 residue within Orai3, which makes Orai3 isoforms insensitive to H_2O_2-induced inhibition [44,45]. The extracellular loop3 exhibits the lowest sequence-conservation among Orai isoforms. Molecular modelling together with MD-simulations identified loop3 as highly flexible region, electrostatically interacting with amino acids in the loop1 CAR, thereby modulating Ca^{2+} permeation [10•,39]. In loop3, only Orai1 proteins contain an *N*-glycosylation site at position 223 (N223). Different glycosylation states of Orai1 might manipulate SOCE-mediated Ca^{2+} signalling, thereby playing a crucial role in pathophysiological processes involved in diseases and cancer related aberrations [46]. The outer shell of a hexameric Orai1 channel is formed by TM4 [28]. Quite recently the Long lab crystallized the open dOrai channel by taking advantage of the H134A Orai1 gain-of-function mutation [37] (H206A in dOrai) [47••]. The open crystal suggests conformational changes and straightening of the TM4 and the extended TM4 region (M4ext) upon channel opening. Additionally, it has been shown that the open pore is dramatically dilated, being ~10 Å apart on the cytosolic end. A release of cytosolic latches between P245 (P288 in dOrai) and the C-terminally located SHK motif (aa263, 264 and 265 in hOrai1) leading to a straightened TM4 helix, seems to expose cytosolic docking sites for STIM1. The TM4 helix bends at position P245 which enables cytosolic M4ext to point in opposite directions and interact through coiled-coils. The M4ext segment is widely accepted to be the major binding site for STIM1 and seems to be crucial in stabilizing the closed state of the channel [33]. The C-terminus is attached to TM4 via a flexible linker region that is required for coupling between Orai1 C-terminus and STIM1 [48]. Within this linker region one can find a five amino acid long sequence (aa 261–265, LVSHK) termed 'nexus' (Figure 1a). Mutation of these amino acids from LVSHK to ANSGA led to a constitutively active channel. Hydrophobic attachment of TM4 residues (L261, V262) to TM3 residue L174 is proposed by Zhou *et al.* [49]. The cytosolic C-terminal strand of Orai is predicted to arrange in paired structures by forming coiled-coil interactions with other Orai1 subunits bending in opposite directions. Two hydrophobic residues (L273, L276) were shown to play significant roles during coiled-coil formation, STIM-Orai binding as well as channel activation [32,50,51] (Figure 1a,b).

STIM1/Orai1 binding

Two models have been proposed to describe STIM1 binding to the cytosolic Orai1 C-terminus. The dimeric model postulates binding of a STIM1 dimer to a pair of M4ext, based on an NMR solution structure where the CC2 domains of CAD/SOAR fold as a binding pocket [52]. This binding model is not easily reconciled with the open dOrai structure. In the monomeric binding model, a STIM1 dimer engages only one C-terminus of Orai1, based on findings were a binding-deficient F394H STIM1 mutant within a STIM1 dimer is still able to activate Orai1 to its full extent [53]. With this model, one could possibly explain how the free CAD/SOAR of the dimer can crosslink Orai1 channels into clusters thereby slowing their diffusion [54]. Still, both models can represent different stages in the CRAC activation process. In

earlier studies it has been hypothesized that the Orai1 C-termini straighten, which breaks their coiled-coil interaction allowing for STIM1 binding [28]. Which conformational rearrangements take place within Orai upon STIM binding, are only partially resolved. It is highly likely that STIM1 binding to one or both Orai1 termini induces signal propagation by exerting a force on the four TM regions. By now, there are several mutations identified that constitutively activate Orai1 — most of them located within TM2 to TM4 (H134A, P245L, W176C, G183A, ANSGA; Table 1) [7,37,49]. It is the prevailing view that the closed channel is stabilized by multiple TM-interactions which are released upon the signal from STIM1 to open the gate allowing for Ca^{2+} influx.

Mechanisms of Ca^{2+} permeation

One essential characteristic of the CRAC channel is its very high selectivity for Ca^{2+} over Na^{+} and its small unitary current: features that point to an energetic barrier for ion passage through the pore — either by repulsive forces or purely by the narrow pore diameter (~039 nm) [29,36,55]. Therefore, conformational changes need to take place within the pore to allow for Ca^{2+} influx. The group of Prakriya has proposed a pore rotation model where torsion of the TM1 helix is linked with conformational changes in the Orai1 C-terminus upon STIM1 binding and channel opening [56]. Hydrophobic side chains of pore-lining residues V102 and F99 create a barrier for ion permeation in the closed state, which are rotated outwards (~20°) upon STIM1 binding [38]. A recent study by Dong *et al.* [57•] supports this 'twist-to-open' gating mechanism. By the use of MD-simulations based on the dOrai structure they show that coupling of TM1 to TM3 (R83-E149 and K85-E173) is crucial for graded activation of Orai channels and that a series of motions lead to channel opening without relaxing structural integrity. In contrast, Frischauf *et al.* have shown a small local widening of the pore (~1−2 Å) occurring during channel opening, proposing two gates in both the hydrophobic and basic region of the pore [37]. In line, the open dOrai crystal reveals a widening of the basic region in the channels pore although the limited resolution may have prevented the identification of a slight rotation of the pore helix [47••]. A recent study combining crystallization and cryo-electron microscopy compared the closed and open state of the dOrai channel by use of the constitutively open dOrai P288L (P245L in hOrai1) mutant that mimics the action of STIM1 binding [58••]. They propose a model for the conformational transduction pathway from the peripheral TM4 to the pore lining TM1 helix: Latched TM4 helices close the pore from the cytosolic side in closed channel configuration and Ca^{2+} flux is blocked by positive charge repulsion and anion plugs. Upon channel opening, the basic section of TM4 helices moves outwards, facilitating Ca^{2+} permeation (anion recruitment model). Lui *et al.* were not able to observe pore helix rotation comparing closed and open states of Orai similar to the results of Hou *et al.* [47••], although both groups used two different open Orai mutants (P245L and H134A, respectively). Nowadays, MD-simulations are widely used to gain mechanistic insights into Orai channels, an approach that is in detail reviewed in [59•]. Merging the two different gating models is therefore tricky as the basis used for MD-simulations differ (dOrai in Refs. [38,57•] and a modelled hOrai structure in Refs. [37,39]). Ultimately, an atomic-resolution structure is needed for resolving the native behaviour of human Orai1.

Perspectives/open questions

Undoubtedly, Orai1 TM domains are critically involved in transmitting the STIM1 signal to the ion-conducting pore [60•]. Discovering the exact, physiological mechanism of the allosteric conformational switch necessary to convey the signal from STIM1 binding to Orai1 channel opening is an exciting focus for further research. Constitutively active mutants within Orai1 can help to understand the transition from the closed to the open channel may takes place. The recent open dOrai crystal structure was achieved by introducing the H206A mutation (corresponding to H134A in hOrai1) [47••]. Considering this structure one has to keep two things in mind: (1) the relatively low sequence homology between dOrai and hOrai1 of about 73% and evolutionary divergence can lead to differences in structural and functional characteristics as already shown for the *C. elegans* Orai channel [40] and (2) STIM1 as physiological activator of Orai1 is missing in the structure. A crystal structure of the entire STIM1/Orai1 complex is required to clarify all remaining open questions. Such a resolution of the CRAC channel complex would also unravel the unique pore opening mechanism of Orai1. This will resolve if either a rotation of the TM1 helix is linked with conformational changes upon STIM1 binding and channel opening as proposed by Prakriya *et al.* [56] or if channel opening is achieved via small local widening of the pore as described by Frischauf *et al.* [37].

Additional proteins modulating STIM1/Orai1 function even enhance the complexity of the CRAC channel system and also have to be taken into account to gain a thorough understanding of the native STIM1/Orai1 system and its regulatory role in downstream signalling pathways involved in human health and disease.

Conflict of interest statement

Nothing declared.

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CRediT authorship contribution statement

Matthias Sallinger: Conceptualization, Writing - original draft. **Sascha Berlansky:** Conceptualization, Writing - original draft. **Irene Frischauf:** Funding acquisition,

Conceptualization, Resources, Project administration, Supervision, Writing - review & editing.

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- •• of outstanding interest

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