JGP Journal of General Physiology

REVIEW

Ion Channels in Health and Disease

The many faces of Orai1 dysfunction: Molecular mechanisms and clinical manifestations

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Orai1, the pore-forming subunit of the calcium (Ca^{2+}) release-activated Ca^{2+} (CRAC) channel, plays a central role in store-operated Ca^{2+} entry (SOCE) in animal cells and thereby serves as a key regulator of intracellular Ca^{2+} homeostasis. Disruption of this tightly controlled process is associated with a wide spectrum of human diseases. Dysfunction can result from a multitude of remodeling mechanisms, including altered protein expression (up- or downregulation), assembly remodeling, or mutations. We focus in particular on Orai1 mutations, which have been linked to severe combined immunodeficiency (SCID) as a result of channel loss-of-function (LoF), as well as to disorders like tubular aggregate myopathy (TAM) and Stormorken syndrome (STRMK) arising from gain-of-function (GoF) alterations. These mutation-induced functional defects can be attributed to a wide variety of disruptions in the complex activation cascade of the Orai1 channel. Under physiological conditions, Orai1 activation involves all four transmembrane (TM) domains and follows a sophisticated interaction mechanism that ensures accurate signal transmission from the protein periphery toward its central Ca^{2+}-conducting pore. In this Review, we compile all currently known disease-associated Orai1 mutations, delineate the mechanisms by which they interfere with the activation cascade, and discuss their pathological relevance. Their widespread distribution across all the domains of this Ca^{2+} channel highlights that malfunctions at virtually any point along the Orai1 TM domain interfaces can profoundly impair its activation mechanism, ultimately leading to severe diseases.

Introduction

Calcium (Ca^{2+}) ions are among the most versatile intracellular second messengers in animal cells, regulating a broad range of processes including proliferation, muscle contraction, fertilization, secretion, apoptosis, and cellular development (Berridge et al., 2000; Nijenhuis et al., 2005; Clapham, 2007; Berridge, 2012). In resting cells, cytosolic Ca^{2+} concentrations are maintained at remarkably low concentrations of ~100 nM (Clapham, 2007), and even small fluctuations in the Ca^{2+} level are involved in modulating cellular downstream signaling. To maintain this precise balance, multiple channels, pumps, exchangers, and Ca^{2+}-binding proteins are required to work together in a tightly coordinated way. Among them, the SOCE machinery represents a principal pathway to replenish ER Ca^{2+} stores and sustain cytosolic Ca^{2+} signals, most prominently via the Ca^{2+} release-activated Ca^{2+} (CRAC) channel (Parekh and Putney, 2005; Putney, 2011; Hogan and Rao, 2015; Ambudkar et al., 2017; Prakriya and Lewis, 2015).

Putney first proposed the concept of SOCE in 1986 (Putney, 1986). In the 1990s, patients with severe combined immunodeficiency (SCID) were found to have defective CRAC channels, laying the foundation for the pathway's importance in human physiology and disease (Partiseti et al., 1994; Feske et al., 2001; Le Deist et al., 1995). Nevertheless, the molecular identity of store-operated Ca^{2+} channels—and the defect underlying SCID—remained unresolved for nearly a decade. In 2005, the stromal interaction molecules STIM1 and STIM2 were identified as key components of the CRAC channel, functioning as the ER membrane–resident Ca^{2+} sensors (Zhang et al., 2005; Roos et al., 2005; Liou et al., 2005). One year later, sequencing studies of two SCID patients and their unaffected relatives pinpointed a homozygous R91W mutation in a protein that was not yet characterized, establishing the genetic basis of the disease (Yeromin et al., 2006; Prakriya et al., 2006; Vig et al., 2006; Feske et al., 2006). This discovery led to the identification of Orai1 and its isoforms Orai2 and Orai3 as the pore-forming subunits of the CRAC channel. During CRAC channel activation, STIM detects ER Ca^{2+} store depletion via its luminal Ca^{2+}-sensing domain, undergoes conformational rearrangements,

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This work is part of a special issue on Emerging Research on Ion Channels in Health and Disease.

and relocates to ER–plasma membrane (PM) junctions, where it directly couples to and gates Orai, thereby mediating Ca^{2+} influx from the extracellular space into the cytosol. Together, these discoveries established the molecular framework of CRAC channels and directly linked Ca^{2+} dysregulation caused by Orai dysfunction to human disease. Moreover, the identification of Orai and STIM further emphasized the importance of tightly controlled Ca^{2+} homeostasis, essential for physiological cellular function (Butorac et al., 2020; Parekh and Putney, 2005; Sallinger et al., 2024; Tiffner et al., 2021b; Feske et al., 2006; Prakriya and Lewis, 2015).

Importantly, while CRAC channels can be fully reconstituted by STIM1 and Orai1, physiological CRAC channels are not limited to STIM1 and Orai1. Depending on the tissue context, they may also include the isoforms STIM2, Orai2, and Orai3, thereby expanding the functional repertoire of CRAC channels (Grabmayr et al., 2021; Lis et al., 2007; Kraft, 2015; Mercer et al., 2006; Emrich et al., 2022). This ensures, on the one hand, tissue-specific roles of CRAC channels, while, on the other hand, remodeling of isoform compositions in CRAC channel assembly or up- or downregulation of Orai isoforms can promote pathological phenotypes. While we provide a brief overview of these kinds of dysregulation, in this Review, we focus on disease-associated mutations in Orai channels, with particular emphasis on Orai1, as the majority of pathogenic variants identified to date affect the Orai1 channel complex (Vaeth and Feske, 2018; Feske, 2019; Lacruz and Feske, 2015; Emrich et al., 2022). A possible explanation for the latter might be that Orai1 represents the main isoform in CRAC channel formation, which may compensate for dysregulation of Orai2 and Orai3 when mutated. Apart from the already mentioned SCID, which arises from CRAC channel loss-of-function (LoF), there are also several diseases associated with Orai1 gain-of-function (GoF)—most prominently, Stormorken syndrome (STRMK) and tubular aggregate myopathy (TAM). Notably, mutations within STIM1, which represents the Orai activator, can give rise to similar diseases, mainly STRMK, TAM, and immune deficiencies, as mutations within Orai1 do. Several publications, including our recent one (Atzgerstorfer et al., 2026), have so far comprehensively reviewed the effect of STIM1 mutations on the CRAC channel system and the resulting phenotypes (Berna-Erro et al., 2023; Böhm et al., 2014; Böhm and Laporte, 2018; Lacruz and Feske, 2015; Markello et al., 2015; Morin et al., 2020). In the scope of this Review, only Orai1 and its isoforms will be discussed.

Orai structure and isoforms

As briefly introduced above, the Orai family comprises three isoforms, Orai1, Orai2, and Orai3 (Yoast et al., 2020; Zhang et al., 2020; Prakriya and Lewis, 2015). First, we present the current understanding of the channel structure of Orai1. After that, we will highlight the differences between the three isoforms.

Understanding how Orai1 mediates Ca^{2+} influx at the molecular level has been a central focus of structural and functional biophysical studies over the past two decades. A cryo-EM structure of human Orai1 (Orai1) has been resolved (Zhang et al., 2026) recently, which revealed a hexameric stoichiometry in accord with structural resolution obtained from *Drosophila melanogaster* Orai (dOrai) (Hou et al., 2012; Hou et al., 2018; Hou et al., 2020; Liu et al., 2019). However, as its low resolution does not allow for mechanistic insights, a fundamental understanding of CRAC channel activation dynamics has been derived from the dOrai structures. dOrai shares high sequence conservation with its human counterpart. X-ray crystallographic analyses captured the hexameric structure of the WT dOrai channel in the closed conformation together with its pore organization (Hou et al., 2012). Furthermore, structural characterization of the mutant dOrai K163W—corresponding to the human LoF variant Orai1 R91W—provided important insights into the disease-relevant closed state of the pore (Hou et al., 2012). In contrast, X-ray and cryo-EM structures of the constitutively active mutants including dOrai H206A (analogous to human Orai1 H134A) and dOrai P288L (corresponding to human Orai1 P245L) (Nesin et al., 2014; Noyer et al., 2025), revealed open-state features associated with channel activation (Hou et al., 2018; Hou et al., 2020; Liu et al., 2019). Notably, Orai1 P245L has been linked to STRMK-like syndrome and TAM (Nesin et al., 2014; Noyer et al., 2025).

All reported dOrai structures revealed that the PM-resident protein Orai1 consists of four transmembrane (TM) helices (TM1–TM4), with both N and C termini located in the cytosol, schematically depicted in Fig. 1, A and B. TM1 and TM2 as well as TM3 and TM4 are connected by extracellular loops, while an intracellular loop links TM2 and TM3. Functional channel formation requires the assembly of six Orai1 subunits arranged such that the TM1 helices form a central ring constituting the ion-conducting pore of the channel (Hou et al., 2012). The TM2 and TM3 create a tightly packed intermediate structure surrounding the TM1 domains, while the outermost concentric layer is formed by the TM4 domains of each subunit (Hou et al., 2012; Yeung et al., 2018). In Fig. 1 C, a top view of the channel highlights this concentric organization of the TM helices. The Orai1 pore comprises four key functional regions that together establish a highly Ca^{2+} selective, yet low unitary conductance environment: an extracellular Ca^{2+}-accumulating region, the E106 selectivity filter, a central hydrophobic gate mainly formed by V102 and F99, and an intracellular basic region, also denoted as the extended TM Orai1 N-terminal region (Najjar et al., 2026; Kraft, 2015; Prakriya et al., 2006; McNally et al., 2009; Hou et al., 2012; Derler et al., 2013; Li et al., 2007; Frischauf et al., 2017), as depicted in Fig. 1 D.

Structural and functional studies indicate that pore opening entails a concerted conformational rearrangement of the entire Orai1 hexamer. Upon STIM1 engagement at the Orai1 C terminus—the principal coupling site (Muik et al., 2008; Zhou et al., 2010; Park et al., 2009; Palty et al., 2015; McNally et al., 2013; Li et al., 2007)—the activation signal propagates from the channel periphery through all TM helices toward the pore forming TM1, culminating into pore dilation (Yeung et al., 2018; Yeung et al., 2020b; Tiffner et al., 2021c; Yeung et al., 2018; Tiffner et al., 2021a). Beyond these global structural rearrangements, recent findings point to early events following STIM1 binding that involve dilation of the peripheral TM interfaces

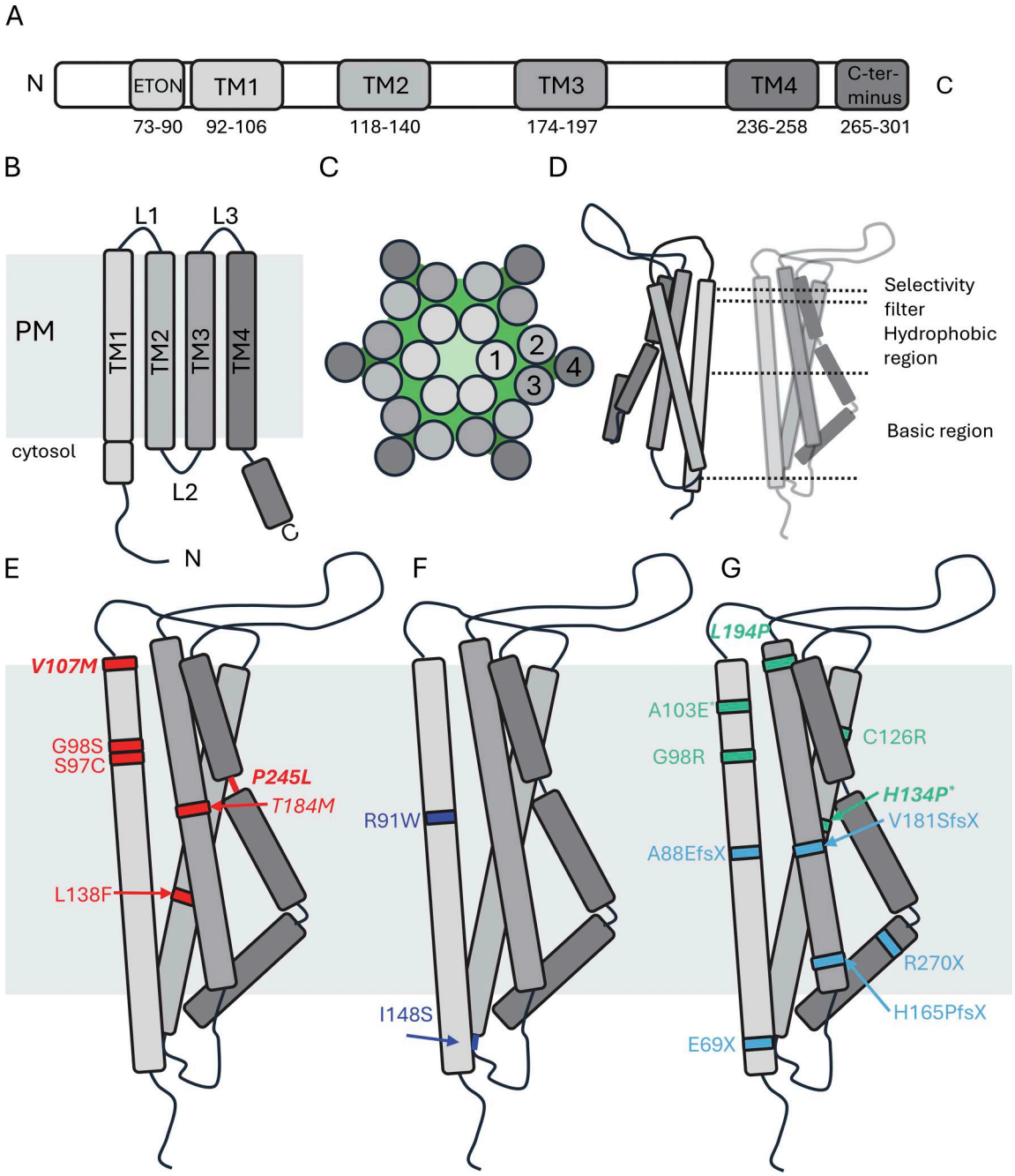

Figure 1. **Schematic insight into the structure of Orai1 and location of disease-causing mutants. (A)** Domain structure of Orai1. **(B)** Schematic representation of an Orai1 subunit. Each subunit has 4 TM helices TM1–TM4, cytosolic N and C termini, an intracellular loop, and two extracellular loops. **(C)** Top view showing the arrangement of the Orai1 hexameric channel. The six TM1 helices form the pore of the channel (light green). The TM1 helices together with the TM2 and TM3 helices constitute the inner interface (middle green). The TM2/3 helices together with TM4 form the outer interface (dark green). **(D)** Side view of two Orai1 subunits. The pore of the channel can be divided into three functional regions: the selectivity filter at the pore entrance, followed by a hydrophobic region and a basic region toward the cytosolic side. **(E)** Disease-causing GoF mutations found in Orai1 (red). **(F)** Disease-causing LoF Orai1 mutants that leave channel expression and trafficking unaffected (blue). **(G)** Disease-causing Orai1 LoF variants resulting from premature translation termination, leading to impaired expression or trafficking, are shown in light blue. Orai1 variants that cause LoF due to reduced channel stability or trafficking defects are shown in turquoise. Mutations marked with * occur in patients only in combination with other mutants, and bold and cursive written mutations differ from a simple GoF or LoF phenotype.

(TM4–TM3) (Hopl et al., 2024; Najjar et al., 2025; Najjar et al., 2026; Söllner et al., 2026). This peripheral rearrangement is proposed to initiate pore opening via a rotation of the pore-lining TM1 accompanied by dilation of the pore region (Yamashita et al., 2017; Yeung et al., 2020b; Huang et al., 2010; Palty et al., 2017; Navarro-Borelly et al., 2008).

Having outlined the structure of Orai1, we want to briefly highlight the main differences from Orai2 and Orai3, whereas a

more detailed and comprehensive comparison of the three isoforms has been provided by Tiffner and Derler (Tiffner and Derler, 2021). While the general channel structure, comprised of 4 TM domains, a cytosolically located N and C terminus, two extracellular and one intracellular loop, is conserved between all three isoforms, their sequence identity only amounts to 50–60%. The TM1 domains are conserved throughout the isoforms, and TM2–TM4 share a sequence identity of over 80%. Notably, the N terminus is longest in Orai1; in the loop 2 region, four residues in Orai2 and eight residues in Orai3 are not conserved, and the C termini of Orai2/3 possess a stronger coiled-coil probability than Orai1. These sequence differences, together with isoform-specific properties along TM3 and the nexus region connecting TM4 with the C terminus, lead to differences in functional properties of the isoforms, likely due to structural alterations that are still pending to be resolved. Under physiological conditions, Orai proteins are thought to assemble into both homo- and heteromeric channel complexes, and interactions among all three isoforms have been demonstrated by Gwack et al. (2007) and most recently by Zhang et al. (2026). While Orai1 is essential for canonical CRAC channel function, Orai2 and Orai3 are generally considered modulatory subunits that fine-tune SOCE activity (Yoast et al., 2020; Zhang et al., 2020; Korshunov and Prakriya, 2025).

While all Orai isoforms are widely expressed, their relative abundance varies considerably between tissues, cell types, and differentiation states (Yoast et al., 2020; Zhang et al., 2020; Vaeth et al., 2017; Chin-Smith et al., 2014; Prakriya and Lewis, 2015; Chalmers and Monteith, 2018; Feske, 2010; Čendula et al., 2021). Orai1 is mostly found in immune cells, cardiomyocytes, vascular smooth muscle cells, melanocytes, and airways (Vig et al., 2008; Feske, 2009; Feske, 2010; Tiffner and Derler, 2021; Berna-Erro et al., 2012). This specific isoform controls a wide range of immune system functions. In this context, Orai2 and Orai3 are thought to assume a critical role in the fine-tuning of immune cell responses, cardiac as well as muscle function. In comparison, Orai2 expression is mainly restricted to the brain (Tiffner and Derler, 2021). Additionally, lower expression levels are found in the spleen, the lung, and the small intestine. Lastly, Orai3 is expressed predominantly in the brain, the heart, the lung, the kidney, the skeletal muscle, as well as other organs (Feske, 2009; Feske, 2010; Vig et al., 2008; Tiffner and Derler, 2021; Berna-Erro et al., 2012).

The modulatory role of the Orai isoform is demonstrated, for example, by the fact that Orai2 negatively affects CRAC channel activity in T cells (Vaeth et al., 2017; Yoast et al., 2020). In neurons, evidence suggests that the classical STIM1–Orai1 pathway is substituted by STIM2–Orai2-mediated SOCE (Berna-Erro et al., 2009; Chauvet et al., 2016; Yoast et al., 2020). Beyond the nervous system, Orai2 has been reported as the predominant Orai isoform in the pregnant human myometrium (Chin-Smith et al., 2014). Orai3 likewise exhibits cell type–specific functions. It is found in various cell types of the nervous system (Korshunov and Prakriya, 2025), in differentiated human T helper lymphocytes (Bogeski et al., 2010), and forms heteromeric complexes with Orai1 in vascular smooth muscle cells (González-Cobos et al., 2013).

While variations in these isoform expression levels across tissues ensure specificity to different biological processes, the remodeling of CRAC channels through changes in isoform expression levels can be the driving force behind severe pathologies, which will be discussed in detail in the following chapter.

Orai channelopathies

The discovery of Orai1 as a core component of the CRAC channel was accompanied by the recognition that genetic mutations in this protein can disrupt the tightly regulated SOCE signaling pathway, giving rise to a group of rare disorders collectively referred to as Orai1 channelopathies. These conditions are primarily caused by point mutations, which can be broadly classified into two categories: LoF or GoF variants (Böhm et al., 2017; Feske, 2019; Lacruz and Feske, 2015).

Orai1 GoF mutations are autosomal dominant, result in constitutive channel activity, and have been identified in patients presenting with TAM and STRMK-like phenotypes. These disorders share a characteristic clinical spectrum involving myalgia, muscle cramps and progressive muscle weakness, often accompanied with thrombocytopenia, miosis, ichthyosis, hypocalcemia, elevated creatine kinase (CK), short stature, and dyslexia (Böhm et al., 2013; Böhm et al., 2017; Nesin et al., 2014; Endo et al., 2015; Markello et al., 2015; Morin et al., 2020). Notably, all GoF mutations connected to these diseases (as identified to date) are located in the highly conserved TM domains of Orai1 (Nesin et al., 2014; Böhm et al., 2017; Garibaldi et al., 2017). Their spatial distribution within the channel is depicted in Fig. 1 E.

In contrast, Orai1 LoF mutations are autosomal recessive and are among the genetic defects responsible for a rare form of SCID. In affected individuals, CRAC channel activity is either completely absent or severely reduced (Lacruz and Feske, 2015). Clinically, patients with Orai1-induced SCID exhibit muscular hypotonia, anhidrosis, chronic infections, ectodermal dysplasia, mydriasis, and autoimmunity (Picard et al., 2009; Feske et al., 2006; Lacruz and Feske, 2015). The first Orai1 mutation linked to SCID, Orai1 R91W, results in a nonfunctional channel despite normal expression levels (Feske et al., 2006). This discovery was followed by the identification of additional variants, which alter channel function through defective channel gating mechanisms (McCarl et al., 2009; Lian et al., 2018) or through altered expression or trafficking of the protein. All disease-associated Orai1 LoF mutations and their positions are summarized in Fig. 1, F and G.

A comprehensive overview of all currently known disease-associated Orai1 mutations is provided in Table 1. Although these variants are classified as either GoF or LoF for practical reasons, the functional consequences of several mutations are more complex than this binary distinction. Accordingly, variants that cannot be assigned unambiguously to either category are written bold and cursive in Fig. 1, E and G. Mutations such as H134P/L194P, T184M, V107M, and P245L are not fully represented by this simple distinction since they present a mixed phenotype, rely on STIM1 for a GoF phenotype, are still partially impacted by STIM1, and show altered CDI, respectively. The mechanistic basis of these mutations is discussed individually in the corresponding sections of this review.

Table 1. **This table contains all currently known disease-associated Orai1 mutations**

Mutation	Reference	Functional effect	Disease	Location
E69X	Baskar et al. (2024)	LoF	SCID	N terminus
A88SfsX25	McCarl et al. (2009)	LoF	SCID	TM1
R91W	Feske et al. (2006)	LoF	SCID	
S97C	Garibaldi et al. (2017)	GoF	TAM	
G98R	Lian et al. (2018)	LoF	SCID	
G98S	Böhm et al. (2017)	GoF	TAM and STRMK spectrum	
A103E	McCarl et al. (2009)	LoF	SCID	
V107M	Böhm et al. (2017)	GoF (facilitated by STIM1 activation)	TAM and STRMK spectrum	
C126R	Yu et al. (2021)	LoF	SCID	TM2
L138F	Böhm et al. (2017)	GoF	TAM	
I148S	Klemann et al. (2017)	LoF	SCID and HLH	Loop 2
H165PfsX1	Chou et al. (2015) and Lacruz and Feske (2015)	LoF	SCID	
V181SfsX8	Lian et al. (2018)	LoF	SCID	TM3
T184M	Böhm et al. (2017)	GoF (only with STIM1)	TAM	
L194P	Lian et al. (2018)	LoF	SCID	
H134P/L194P	Noyer et al. (2025)	GoF/LoF	SCID and HLH	TM2 + TM3
P245L	Nesin et al. (2014) and Palty et al. (2015)	GoF (altered CDI)	TAM and STRMK spectrum	TM4
R270X	Badran et al. (2016)	LoF	SCID	C terminus

For each mutation, the first report is listed, as well as its functional impact on the protein. Further, the associated diseases are matched to the mutations. Lastly, the location of the mutations in the Orai1 domains is described.

As established in the previous chapter, the tissue-specific expression patterns of Orai isoforms are important determinants of CRAC channel function and should therefore also be considered in the context of Orai channelopathies. Consequently, the functional consequences of pathogenic Orai1 variants are likely influenced by the local molecular composition of CRAC channel complexes and the capacity of alternative isoforms to compensate for impaired Orai1 function. Such differences may contribute to the marked phenotypic heterogeneity observed in affected individuals, including immunological, muscular, hematological, and ectodermal manifestations.

Importantly, most functional studies, as is the case for the ones presented in this review, of disease-associated Orai1 variants have been conducted in heterologous expression systems (typically the HEK 293 cell line) that primarily examine Orai1 in combination with STIM1. While these models provide valuable mechanistic insights, they may not fully represent the diversity of native CRAC channel assemblies.

An additional challenge in understanding Orai1 channelopathies is the interpretation of genotype–phenotype relationships. Although this review summarizes the currently reported disease-associated Orai1 variants, not all mutations have been functionally characterized. For several variants, particularly those identified only in individual case reports, the molecular, biophysical, and cellular consequences remain unknown. Consequently, the observed clinical manifestations may arise not only from altered channel gating or activation but also from changes in Orai1 expression, protein stability, trafficking, or overall SOCE. Furthermore, as in many inherited disorders, phenotypic variability is likely influenced by modifier genes, epigenetic regulation, environmental factors, and other individual-specific determinants. Therefore, genotype–phenotype relationships discussed throughout this review should be interpreted with appropriate caution, particularly for variants lacking comprehensive functional validation.

Beyond diseases directly attributed to specific GoF or LoF mutations in Orai1, dysregulated Orai activity or overexpression of the protein has been implicated in a broader range of pathological conditions (Prakriya and Lewis, 2015). These include cardiorespiratory and cardiovascular diseases (Ruhle and Trebak, 2013; Humer et al., 2022a; Mammadova-Bach et al., 2019; Johnson and Trebak, 2019; Shawer et al., 2021) as well as neurological (Wegierski and Kuznicki, 2018; Mei et al., 2018) and neurodegenerative disorders (Collins et al., 2022) as detailed in Emrich et al. (2022). Furthermore, inflammatory disorders such as pulmonary arterial hypertension (Masson et al., 2022), asthma (Zou et al., 2011; Spinelli et al., 2012), and dermatitis (Chang et al., 2012) can be caused by Orai channel remodeling. While studies referenced in this paragraph provide a comprehensive overview of the identified roles of Orai isoforms in cardiovascular disease, neuronal disorders, or others, below we highlight a few of the most important examples of Orai remodeling in disease.

For instance, pathophysiological remodeling of the heart and vessels is associated with greatly enhanced Orai1 expression in cardiomyocytes and smooth muscle cells (Johnson and Trebak, 2019). Similarly, in hypotensive rats, Orai1 has been found to be

upregulated in vascular smooth muscle cells (Johnson et al., 2020), whereas elevated Orai1 expression has also been observed in atherosclerotic lesions (Shawer et al., 2021). Conversely, decreased Orai1 expression has been reported in failing myocardium (Jeong et al., 2021), illustrating that Orai1 remodeling is disease-specific rather than uniformly increased.

Other isoforms also contribute to cardiovascular pathology. Orai3 appears to play both a homeostatic role under physiological conditions and in pathological cardiac remodeling. Notably, increased Orai3 expression has been observed in multiple disease models, whereas Orai3 knockdown has been shown to attenuate pathological remodeling (Zhang et al., 2015; Saliba et al., 2015; González-Cobos et al., 2013). Furthermore, excessive activation of cardiac fibroblasts, a characteristic of many cardiac pathological conditions, has been linked to an increase in the expression of the Orai2 isoform and a change in the Orai1/Orai3 ratio (Čendula et al., 2021).

Within the nervous system, several conflicting reports indicate the expression and contribution of specific Orai isoforms to Ca^{2+} signals in various neuron subsets. In this context, a potential role of Orai2 has been identified in certain subtypes of neuronal cells (Berna-Erro et al., 2009; Stegner et al., 2019; Chen-Engerer et al., 2019; Hartmann et al., 2014); however, the exact role requires further investigation.

Additionally, Orai channels have emerged as important regulators of cancer-associated Ca^{2+} signaling, with growing evidence indicating that altered Orai expression and activity contribute to multiple hallmarks of cancer as detailed in Chalmers and Monteith, 2018; Hoth, 2016; Tiffner et al., 2022; Fiorio Pla et al., 2016. Although activating Orai1 mutations have been identified in several cancer types, including colorectal, gastric, and uterine cancers (Frischauf et al., 2017; Chalmers and Monteith, 2018), these alterations are not considered primary drivers of oncogenic transformation. Instead, remodeling of Orai-mediated Ca^{2+} influx appears to support disease progression by promoting cellular processes such as proliferation, migration, invasion, metastasis, and resistance to cell death (Chalmers and Monteith, 2018; Hoth, 2016; Tiffner et al., 2022). In particular, altered expression levels of STIM1/STIM2, Orai1, and Orai3 seem to be a common effect appearing in various types of cancer, such as prostate cancer, gastric cancer, breast cancer, oral cancer, and glioblastoma (Fiorio Pla et al., 2016; Zhang et al., 2024). A detailed overview of altered gene expression in cancerous versus healthy tissue is provided in other reviews (Fiorio Pla et al., 2016; Tiffner et al., 2022).

Among the three Orai isoforms, Orai1 is the most extensively studied and is upregulated in many cancers, like liver, esophageal, renal, and stomach cancer, where the elevated expression is often associated with poor clinical outcomes (Chalmers and Monteith, 2018; Hoth, 2016; Xia et al., 2016; Zhang et al., 2024). Other studies on prostate cancer reported that Orai1 expression is reduced while alternative Ca^{2+} channels such as the transient receptor potential vanilloid (TRPV6) channel are upregulated (Hoth, 2016), highlighting the diversity of Ca^{2+} signaling remodeling mechanisms in different cancers.

Orai3 has attracted particular attention due to its elevated expression in breast cancer, although findings from clinical samples remain inconsistent. Nevertheless, studies in cancer cell lines generally support a tumor-promoting function for Orai3 (Chalmers and Monteith, 2018; Hoth, 2016). Orai3 is typically upregulated in estrogen receptor-positive breast cancers (Motiani et al., 2010; Motiani et al., 2013; Nieto-Felipe et al., 2025) and in lung cancer, whereas in prostate cancer a shift in the Orai1:Orai3 ratio due to an upregulation of Orai3 leading to the formation of Orai1/3 heteromeric assemblies has been reported (Yoast et al., 2020).

Limited evidence suggests that Orai2 may contribute to cellular proliferation, as its silencing reduces growth in leukemia cells and increased expression has been observed in parathyroid tumors (Chalmers and Monteith, 2018; Hoth, 2016). The current lack of comprehensive investigations into Orai2 and Orai3 across different malignancies represents a significant gap in the field.

Notably, altered expression of Orai and other CRAC channel components does not necessarily imply a direct role in driving cancer progression. In some cases, changes in channel expression may serve primarily as biomarkers of aggressive disease or specific molecular subtypes (Hoth, 2016).

Importantly, the contribution of Orai channels extends beyond cancer cells themselves and includes cells within the tumor microenvironment. Ca^{2+} signaling is essential not only for cancer cell survival and progression but also for the activation and cytotoxic function of immune cells responsible for tumor surveillance and elimination. Consequently, Orai-dependent Ca^{2+} entry must be considered within the broader context of tumor–immune interactions rather than as a cancer cell–specific mechanism. This complexity presents a challenge for therapeutic targeting, as inhibition of Orai channels may suppress tumor-promoting pathways while simultaneously impairing antitumor immune responses (Chalmers and Monteith, 2018; Hoth, 2016).

Overall, current evidence supports the view that Orai channels are not independent oncogenic drivers but rather integral components of a broader Ca^{2+} signaling network whose remodeling facilitates tumor progression in a highly context-dependent manner. Future studies addressing the roles of Orai2 and Orai3, together with investigations into tumor–immune interactions and therapy resistance, will be essential for determining whether Orai channels can serve as reliable biomarkers or therapeutic targets in cancer.

Expression or trafficking defective mutants

For Orai1 to assemble a functional pore in the PM, several steps must proceed correctly: accurate transcription and translation to produce the correct mRNA and protein, respectively, correct folding and assembly, and efficient trafficking from the ER to the PM. Disruptions in any of these steps can prevent Orai1 from reaching the PM, resulting in CRAC channel LoF, and subsequently leading to diseases.

Truncations due to frameshift or premature stop codons

To date, five disease-relevant Orai1 mutations, depicted in Fig. 1 G, arising from frameshifts or preliminary stop codons have been identified: E69X (Baskar et al., 2024), A88fsX25

(McCarl et al., 2009), H165PfsX1 (Chou et al., 2015), V181SfsX8 (Lian et al., 2018), and R270X (Badran et al., 2016). Because these mutations cause a termination at the beginning of Orai1 translation, they yield a nonfunctional or absent (due to nonsense-mediated decay) protein, resulting in LoF.

Consistent with CRAC channel LoF, patients carrying these Orai1 mutations typically present with a SCID-like phenotype, characterized by severe, recurrent infections. Unlike many forms of classical SCID, lymphocyte counts are often relatively preserved, yet lymphocyte function is profoundly impaired. Especially, SOCE is typically absent, and T cells fail to proliferate upon activation. The clinical spectrum arising from these mutations comprises early-onset immune failure alongside congenital muscle involvement, with muscular hypotonia or myopathy being frequent non-immunological features. The range of the syndromic phenotype of patients varies with the specific variant, which also influences the prominence of ectodermal, neurological, or hematologic features. It therefore would be beneficial to examine each mutation individually (McCarl et al., 2009; Chou et al., 2015; Badran et al., 2016; Lian et al., 2018; Baskar et al., 2024; Lacruz and Feske, 2015).

One intriguing variant is the truncation variant Orai1 E69X, located in the Orai1 N terminus. Orai1 E69X causes a predominantly myopathic phenotype accompanied by slowly progressive proximal weakness, ophthalmoparesis, and biopsy abnormalities (Baskar et al., 2024). Notably, this patient did not experience recurrent infections, indicating that Orai1 truncations do not necessarily manifest as SCID. Unexpectedly, the patient reported by Baskar et al. (2024) carried a homozygous mutation and yet presented a purely muscular phenotype.

By contrast, A88fsX25, a frameshift mutation leading to a premature stop codon and located at the very beginning of TM1, shows absent mRNA, consistent with nonsense-mediated decay (McCarl et al., 2009). Notably, apart from typical symptoms for Orai1 deficiency, like recurring infections, developmental delay, and congenital muscular hypotonia, the patient carrying this mutation presented with autoimmunity (Picard et al., 2009). The authors speculate that autoimmunity may be underrecognized because most of the patients with Orai1 deficiencies either passed away only a few months after birth or received a stem cell transplantation, leaving insufficient time for autoimmune manifestation to emerge (McCarl et al., 2009).

Although H165PfsX1, located in the loop2 region, was not assessed at the mRNA level, the mutant was not detectable upon heterologous expression of an N-terminally tagged construct, and endogenous Orai1 was absent in patient fibroblasts by C-terminal immunoblotting. Notably, patient fibroblasts retained residual SOCE, consistent with measurable contributions from paralogue channels when Orai1 was absent. Individuals homozygous for this frameshift presented with features typical for Orai1 deficiency, whereas heterozygous relatives remained unaffected (Chou et al., 2015).

V181SfsX8, a frameshift mutation in TM3 of Orai1 that leads to a premature stop codon, abolishes SOCE in patient fibroblasts similar to A88fsX25. Unlike A88fsX25, however, Orai1 V181SfsX8 transcript levels are not markedly reduced, while surface Orai1 is undetectable, pointing to a posttranscriptional instability or trafficking issue. Clinically, the patient exhibited severe infections in infancy as well as muscular hypotonia and tooth defects among other symptoms consistent with CRAC channel LoF (Lian et al., 2018).

R270X is distinct from the other truncation variants in that it truncates the Orai1 C terminus, the main interaction site for STIM1. Because the stop codon occurs late, the mutant protein is detectable by western blot experiments; however, CRAC currents are abolished (Badran et al., 2016). Studies using C-terminally truncated Orai1 fragments support the notion that loss of the C terminus disrupts STIM1 coupling and consequently channel function (Muik et al., 2008; Li et al., 2007; Palty et al., 2015). Among these, Orai1 $\Delta C_{273-301}$ and Orai1 $\Delta C_{276-301}$, artificial C-terminal truncations, prevented co-localization with STIM1 CAD (CRAC activation domain, the minimal C-terminal STIM1 region that elicits constitutive CRAC currents) and abolished CRAC currents. However, introduction of the constitutively active pore mutant V102A into Orai1 $\Delta C_{273-301}$ or Orai1 $\Delta C_{276-301}$ restored Ca^{2+} permeation, indicating that LoF occurs due to defective STIM1 coupling (Palty et al., 2015). This is consistent with the finding that positions L273 and L276 on the Orai1 C terminus are required for STIM1 binding (Muik et al., 2008; Frischauf et al., 2009; Navarro-Borelly et al., 2008). The patient carrying the Orai1 R270X mutation described by Badran et al. (2016) presented with hypotonia as well as recurrent respiratory tract infections, among other symptoms leading to a SCID diagnosis.

Overall, Orai1 premature stop codons or frameshift variants converge in a LoF phenotype but do so through distinct mechanistic bottlenecks. Truncations close to the N terminus predominantly cause nonsense-mediated decay as the primary cause of LoF, while other variants impair intact protein expression. Thus, WT Orai1 must be produced, correctly folded, and stably delivered to the PM to support channel activity. Finally, stop codon mutants around the C terminus highlight the domains' critical role in STIM1 coupling and CRAC channel activation.

Insufficient channel stability and trafficking defects

Orai1 LoF is not restricted to prematurely truncated variants. Missense mutations can also create LoF phenotypes by destabilizing Orai1 at the protein level, as identified for A103E in the compound A103E/L194P genotype (McCarl et al., 2009), and likely G98R (Lian et al., 2018). Moreover, even when transcripts are preserved and the protein is synthesized, defective trafficking to the PM can abolish CRAC activity, as reported for L194P (Lian et al., 2018) and C126R (Yu et al., 2021). Notably, the L194P variant also occurs in a patient with a combined L194P/H134P (Noyer et al., 2025) genotype resulting in a mixed LoF/GoF phenotype discussed later in this review. The location of these mutants in the protein is shown in Fig. 1 G.

Clinically, patients with these variants exhibit combined immunodeficiency, congenital muscle involvement, and ectodermal features, most prominently anhidrosis and enamel defects. Differences arise mainly from the type of immunological features emphasized and whether or not immune dysregulation is noted (McCarl et al., 2009; Lian et al., 2018; Yu et al., 2021;

Noyer et al., 2025). In particular, G98R leads to additional immune dysregulation, namely autoimmune hemolytic anemia (Lian et al., 2018).

In patient primary cells carrying Orai1 G98R, mRNA levels are not significantly reduced, yet surface Orai1 is undetectable by flow cytometry using an antibody against an extracellular Orai1 epitope (Lian et al., 2018). G98R lies in TM1 at a pore-lining position and replaces a small glycine with a large, positively charged arginine. This substitution is likely incompatible with the tightly packed, largely hydrophobic pore helix and thus prone to disrupt folding and/or pore architecture. However, because subcellular localization was not assessed, a trafficking defect cannot be excluded.

The A103E mutant, present in the biallelic mutation A103E/L194P genotype, is located in the TM1 helix of Orai1 near the selectivity filter (E106), while L194P resides in the TM3. Although mRNA levels are preserved, protein expression of Orai1 is undetectable in patient fibroblasts via flow cytometry. Moreover, Ca^{2+} influx is impaired across multiple patient cell types. To dissect the contributions of the two mutations, they were expressed separately in HEK293 cells. Immunoblotting showed that both mutants independently abolish stable expression. Mechanistically, the A103E mutation introduces an acidic side chain near the selectivity filter, which could destabilize TM1 and/or perturb pore architecture via unfavorable electrostatics, thereby compromising protein stability and expression (McCarl et al., 2009).

In contrast, L194P, located at the end of TM3, and C126R, in TM2, are best characterized as trafficking or surface expression defects. Although Orai1 mRNA is preserved, patient cells show no detectable Orai1 in the PM and abolish SOCE. Heterologous expression investigated by fluorescence microscopy revealed that Orai1 L194P predominantly localizes intracellularly, with only a minority of cells displaying detectable surface Orai1. Based on the human Orai1 structural model Lian et al. (2018) proposed that introducing the helix-breaking proline at position 194 destabilizes Orai1 monomers, impairs proper Orai1 hexamer assembly, and thus promotes protein degradation (Yu et al., 2021; Lian et al., 2018). Notably, C126R was the first TM2 mutation associated with Orai1 LoF. While overall protein expression was not impaired, the mutated protein failed to traffic properly. Further investigation of the TM2 interface showed that introducing positive side chains in this region leads to misfolding, defective bilayer insertion, and disrupted trafficking, providing a mechanistic explanation for the C126R phenotype (Yu et al., 2021).

To summarize, impaired SOCE due to these mutants can be explained by reduced or absent protein expression or trafficking defects toward the PM. While TM1 substitutions like A103E and G98R likely act through protein destabilization, L194P and C126R abolish SOCE due to abolished PM expression.

Mechanisms of disease-associated Orai mutants

Nevertheless, successfully overcoming the hurdles of correct protein assembly, transport to the PM, and expression does not necessarily entail proper function of the Orai1 protein. From now on, the focus will shift to disease-associated Orai1 mutants that impair the activation mechanism of the protein, while PM integration remains intact. To understand how these variants cause channelopathies, it is necessary to outline the current model of WT Orai1 activation, comprehensively reviewed by Najjar et al. (2026). Here, Orai1 is discussed in terms of its major functional regions: its termini and loops, the periphery and outer interface, the inner interface, and the pore. Each section also covers the relevant disease-associated variants discussed alongside the molecular mechanisms underlying their dysfunction.

The termini and loop regions

The cytosolic termini and loop2 region of Orai1 play important roles in STIM1–Orai1 coupling, channel gating, and modulation. While the C terminus represents the main coupling site for STIM1 and is essential for channel activation (Muik et al., 2008; Frischauf et al., 2009; Fahrner et al., 2009; Tirado-Lee et al., 2015; Palty and Isacoff, 2016; Park et al., 2009; Yuan et al., 2009; Zhou et al., 2010; Palty et al., 2015; McNally et al., 2013; Li et al., 2007). The intracellular loop2 is assumed to directly support STIM1-induced gating (Butorac et al., 2020; Humer et al., 2022b; Srikanth et al., 2010; Fahrner et al., 2018; McNally et al., 2013; Tiffner et al., 2021a). Notably, a study conducted by Kim et al. (Kim et al., 2018) showed that in *Caenorhabditis elegans,* contrary to mammals, the channel gating mechanism is facilitated by STIM binding to loop2 of Orai. The N terminus contributes to STIM1-dependent modulation of channel function, though it remains unknown whether it directly couples to STIM1 (Derler et al., 2013; McNally et al., 2013). All three cytosolic regions have been implicated in fine tuning STIM1-mediated Orai1 gating (Fahrner et al., 2018; Tiffner et al., 2021a; Humer et al., 2022b; McNally et al., 2013), selectivity, and Ca^{2+}-dependent inactivation (CDI) (Lee et al., 2009; Parekh, 2017; Srikanth et al., 2010; Krizova et al., 2019; Frischauf et al., 2011; Mullins et al., 2009; Mullins et al., 2016). While the exact molecular activation and gating mechanism involving the N terminus and loop2 is not yet fully defined, their communication with each other and with STIM1 is proposed to facilitate transmission of conformational changes from the channel periphery toward the pore (Fahrner et al., 2009; Fahrner et al., 2018; Tiffner et al., 2021b). Additionally, Orai1 possesses two extracellular loops, loop1 connecting TM1 to TM2 as well as loop3 connecting TM3 to TM4. Both are imperative for channel selectivity and for facilitating Ca^{2+} entry to the pore (Frischauf et al., 2015; Tiffner et al., 2021b; Najjar et al., 2026).

I148S

Orai1 I148S is, to date, the only disease-associated variant in the cytosolic segments and is located in the cytosolic loop2 between TM2 and TM3. The homozygous Orai1 I148S variant causes a severe LoF CRAC channelopathy characterized by SCID and hyperinflammation (HLH), notably without ectodermal dysplasia or anhidrosis (Klemann et al., 2017). In Ca^{2+} imaging studies carried out on patient cells, SOCE was absent compared with healthy control cells (Klemann et al., 2017). As loop2

mediates communication between cytosolic channel regions and the pore (Fahrner et al., 2018), this substitution possibly interferes with local interactions necessary for efficient signal transmission during channel activation. Consistent with this finding, substitutions of neighboring residues, such as P146 and E149, also impair channel function. As part of an alanine screening of loop2 to demonstrate its involvement in fast CDI, substitution of these residues leads to significantly reduced maximal intracellular Ca^{2+} concentrations after store depletion compared with the WT control (Srikanth et al., 2010; Klemann et al., 2017). In particular, it has been reported that E149 forms salt-bridge interactions with K85 and R83 (Dong et al., 2019; Tiffner et al., 2021c; Najjar et al., 2026). Disruption of these interactions impairs proper channel function, likely due to erroneous long-range effects in the transmission of the activation signal. Furthermore, the loop2 residue E166 in Orai1 is essential for STIM1-mediated propagation of the gating signal, either through direct contact with STIM1 or via indirect coupling mechanisms (Butorac et al., 2019). This supports the idea that the region around I148 is critical for coupling cytosolic rearrangements to pore opening.

The periphery and outer interface

After STIM1 coupling to the C terminus of Orai1, the activation signal propagates through the outer interface consisting of TM4, TM3, and TM2 toward the pore. Fig. 2, A and B further illustrate the location of the outer interface. An early key step in this process involves the highly conserved nexus region, particularly the $_{261}$LVSHK$_{265}$ motif, which connects the C-terminal extension to TM4 and couples STIM1 binding to channel opening (Zhou et al., 2016; Palty et al., 2015). The signal then proceeds along TM4, where the central P245 kink, depicted in Fig. 2 C, acts as a structural checkpoint for Orai1 gating (Palty et al., 2015). It stabilizes the helix in a bent conformation assumed to contribute to the maintenance of the closed state, while upon opening, a straightening has been proposed (Hou et al., 2012; Hou et al., 2018; Liu et al., 2019). Notably, P245 is a residue highly conserved from flies to humans (Liu et al., 2019). From there, conformational changes are transmitted through contacts between TM4, TM3, and TM2 toward the inner interface and ultimately the pore (Najjar et al., 2026). Recent work further suggests that dilation of both the nexus-TM3 region and the TM3/TM4 interface contribute to this process (Söllner et al., 2026; Najjar et al., 2026; Tiffner et al., 2021a), while increased hydration within this peripheral channel region may facilitate progression toward the open state, as derived from the GoF mutant Orai1 V181K (Hopl et al., 2024).

P245L

Within the peripheral, outer interface region, Orai1 P245L is the best-characterized disease-associated GoF mutation. Clinically, the mutation is associated with a STRMK-like phenotype and TAM (Nesin et al., 2014). Functionally, Nesin et al. (Nesin et al., 2014) first characterized Orai1 P245L in HEK293 cells upon co-expression with STIM1 using electrophysiology. They showed that P245L does not alter the activation kinetics or maximal currents compared with WT conditions. However, the mutants exhibited impaired slow CDI, leading to prolonged activation times. Later Ca^{2+} imaging and electrophysiology studies by several independent groups indicated that P245L can confer low-level basal activity with small spontaneous currents and constitutive Ca^{2+} entry (Bulla et al., 2019; Palty et al., 2015). This behavior fits well with the structural role of P245 in TM4. In the closed dOrai structure, TM4 is bent at the residue corresponding to human P245 and this proline kink has been interpreted as a structural feature that stabilizes the resting conformation of Orai1 (Hou et al., 2012; Hou et al., 2018), illustrated in Fig. 2 C. Consistent with this interpretation, systematic mutagenesis at this site shows that only proline is capable of stabilizing the closed channel, whereas all other residues, including P245L, shift the equilibrium toward more activation permissive conformations (Palty et al., 2015; Butorac et al., 2019; Yeung et al., 2018). Importantly, an open-state dOrai structure was resolved with the P288L mutant corresponding to human P245L. In this crystal structure, the TM4 helices in the periphery assume an extended conformation (Liu et al., 2019), as shown in Fig. 2 D. Analogously, the crystal structure of the GoF mutant Orai1 H206A exhibits a fully straightened TM4 C terminus region (Hou et al., 2018). However, apart from the effects of the introduced mutation, it must be considered that this conformation could also arise from potential crystallization artifacts. Corresponding cryo-EM (PDB accession no. 7HR5) structures indicate less drastic structural changes along the TM4 (Hou et al., 2020), indicated in Fig. 2 D. Importantly, however, substantial portions of the cytosolic TM4 extension and the intracellular channel region are not resolved in the cryo-EM density, and the positions of several helices and side chains in these regions were inferred largely from the earlier crystal structure rather than directly visualized. This limitation has to be considered when drawing mechanistic conclusions regarding structural rearrangements of the cytosolic domains (Hou et al., 2012; Hou et al., 2020). Notably, constitutive activity of Orai1 P245L could still be further enhanced by STIM1 expressed in HEK293 cells, indicating additional structural changes by STIM1 (Palty et al., 2015; Palty et al., 2017). We recently performed a screen using photo-cross-linking unnatural amino acids (UAAs) combined with conventional mutagenesis. Our functional analysis in the absence and presence of STIM1 revealed that Orai1 pore opening involves a dilation of the peripheral TM interfaces. Among others, the p-azido-L-phenylalanine (Azi) UAA-containing Orai1 A254Azi mutant exhibited UV-induced activation in the absence of STIM1, which was proposed to be in part associated with a dilation of the TM3/TM4 interface. A combination of P245L with Orai1 A245Azi revealed constitutive activity, which could be further enhanced by UV light application, leading to levels comparable with activation by STIM1 (Maltan et al., 2023). This finding supports the assumption that P245L leads to an intermediate active state. Although it is clear that P245L shifts Orai1 into an active conformation, further investigations are required to understand the extent of structural changes in this peripheral segment of the Orai1 P245L channel complex. Interestingly, immortalized patient lymphocytes showed a slow development of SOCE that persists for longer, which is more consistent with the abrogated slow CDI rather than enhanced amplitudes of

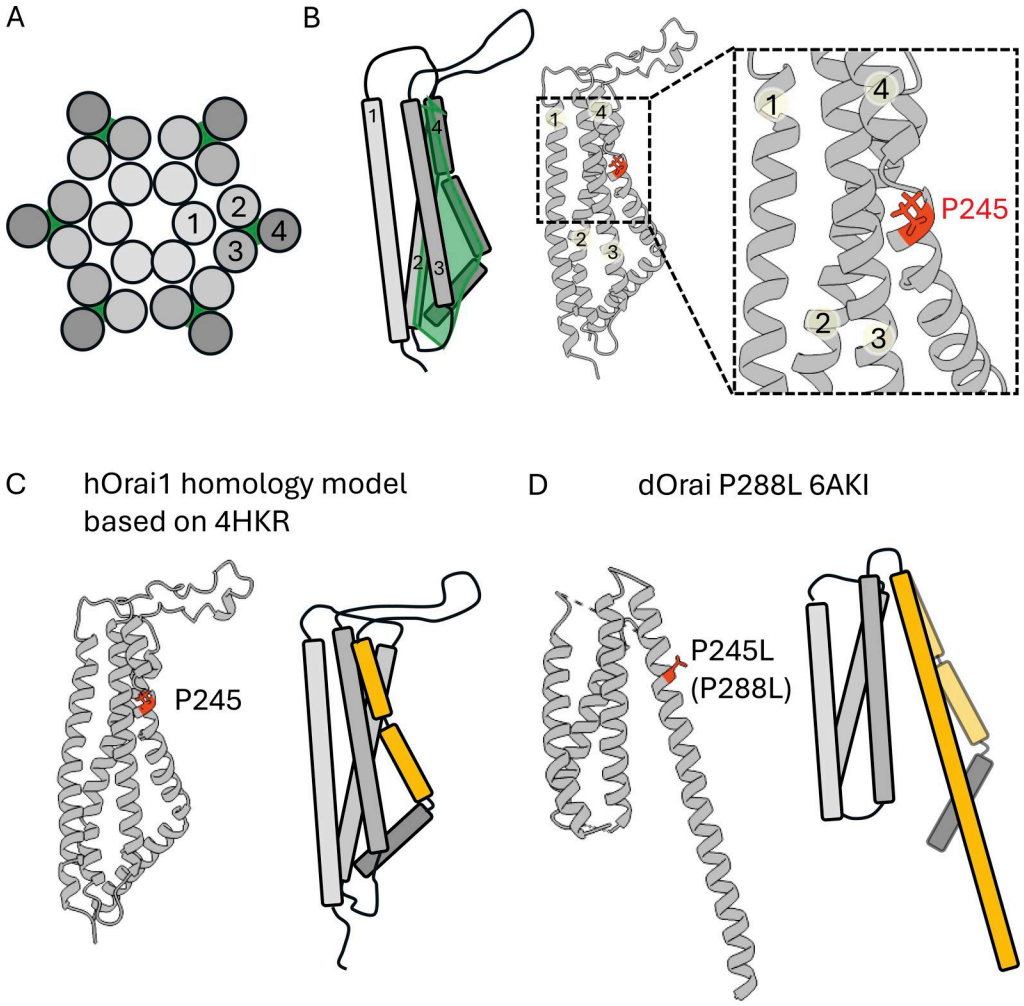

Figure 2. **Potential structural rearrangement of the TM4 due to the P245L mutation in Orai1. (A)** Top view of the Orai1 outer interface between the TM4 and TM2/TM3 unit (green). **(B)** Side view of the outer interface (green), as well as a zoom-in on the disease-relevant residue P245 (red). Circles with numbers indicate the TMs, e.g., 1 corresponding to TM1. **(C)** Schematic depiction of the Orai1 homology model based on PDB accession no. 4HKR. The proline at position 245, marked in red, introduces a kink between the two helices colored in yellow that adds to the stabilization of the resting state. **(D)** This scheme of the resolved dOrai1 P288L (PDB accession no. 6AKI) structure corresponds to the human Orai1 mutation P245L. The mutation leads to an extension of TM4, shown in yellow. While this prominent extension was observed in the resolved structure, additional cryo-EM suggests a less pronounced effect, depicted by the lighter yellow helices.

CRAC currents in Orai1 P245L. In line, patch-clamp recordings of HEK293 cells show significantly reduced slow CDI resulting in sustained currents, thus representing a GoF phenotype (Nesin et al., 2014). In contrast, fast CDI was preserved. Beyond the effects on channel architecture caused by the P245L mutation, which results in GoF, P245 is also associated with proteostatic control. It is proposed to act as a determinant for recognition and cleavage of activated Orai1 by RHBDL2, a rhomboid protease that cleaves membrane proteins. Mutation of the proline could block this recognition, implying that the disease mutation can bias gating and evade a proteostatic brake, thus representing an additional pathway contributing to GoF (Grieve et al., 2021).

Overall, P245 appears to function as a critical regulatory site that couples conformational changes in the peripheral TM helices to the opening of the channel pore. Disease-associated substitutions at this position bias the channel toward constitutive or prolonged activation by altering the gating mechanism. In addition, P245 has been identified as a recognition determinant for RHBDL2-mediated cleavage of activated Orai1. Substitution of this residue may reduce RHBDL2-dependent proteolysis, thereby limiting turnover of activated channels and prolonging their residence at the PM. Thus, P245 substitutions may promote GoF through two complementary mechanisms: altered channel gating and impaired proteostatic regulation.

The inner interface

The TM2/TM3 ring, positioned between TM4 and TM1, is proposed to act as a cohesive unit linking the channel periphery to the channel pore (Yeung et al., 2020b). Together with TM1, it constitutes the inner interface, as depicted in Fig. 3, A and B. Central to this region is a hydrophobic packing network supported by a sulfur-aromatic interaction, which is essential for efficient STIM1-induced activation. Further, a serine-rich

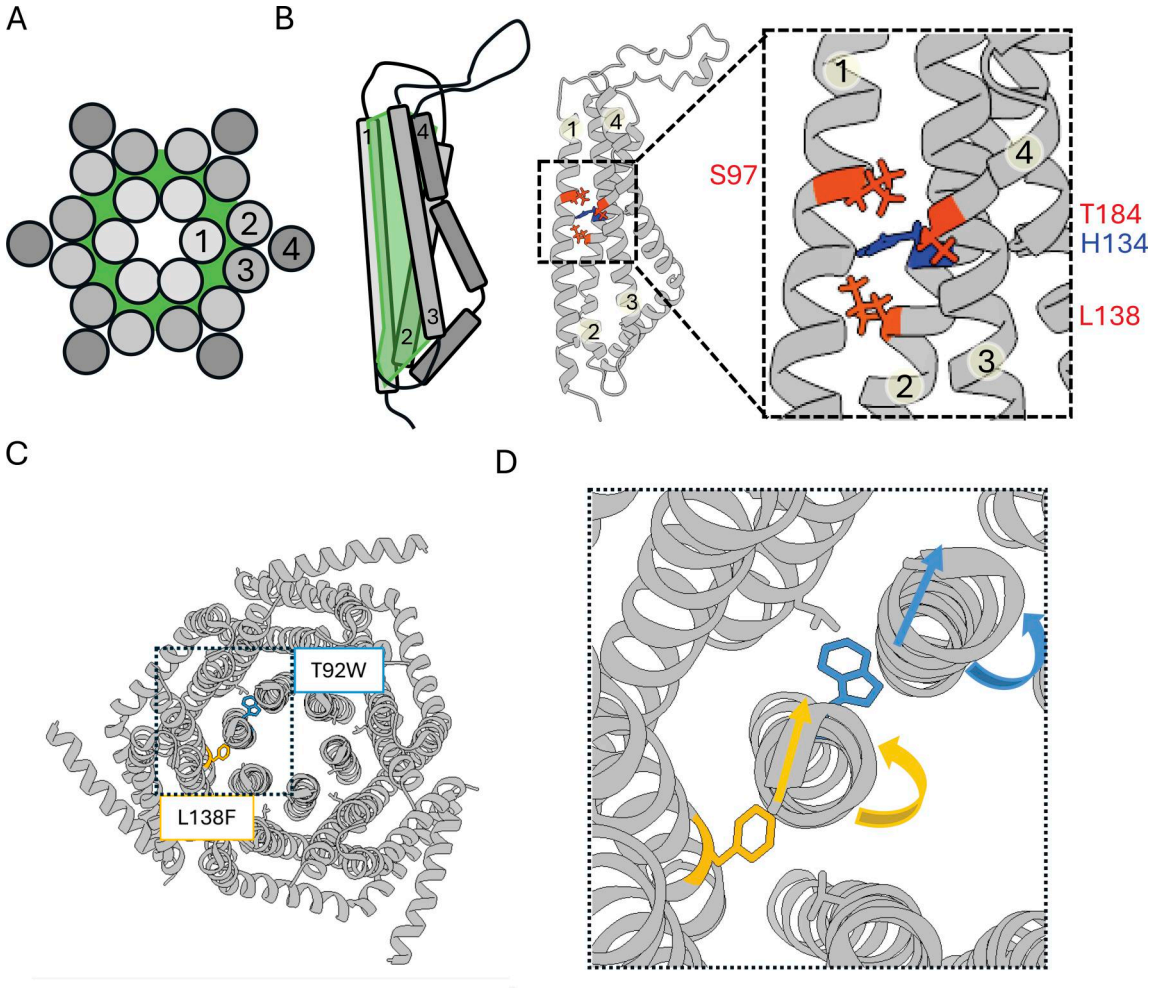

Figure 3. **Rearrangements at the inner interface due to Orai1 disease mutants. (A)** Arrangement of the TM helices in the Orai1 hexamer. The inner interface between TM1 and TM2/3 is shown in green. **(B)** The left subunit shows a side view of an Orai1 subunit and the inner interface (green). The right subunit shows the location of the disease-causing Orai1 mutants. The inset shows a magnified view of the region. GoF mutants are depicted in red, LoF mutants in blue. Circles with numbers indicate the TMs, e.g., 1 corresponding to TM1. **(C)** Sketch of the Orai1 top view and location of the mutants L138F (yellow) and T92W (light blue). **(D)** Potential rearrangements of the TM helices, including rotation and outward movement of TM1 caused by the GoF mutants L138F (yellow) and T92W (light blue), respectively.

region along TM1 forms functionally important contacts with the TM2/3 unit, contributing to the stabilization of the closed state (Yeung et al., 2018; Yeung et al., 2020a; Hou et al., 2018).

In this intramembrane relay, the TM2-resident H134, shown in Fig. 3 B, emerges as a major gating determinant (Frischauf et al., 2017; Yeung et al., 2018). Two complementary mechanistic models have been proposed to understand the function of this position. In one model, H134 stabilizes the closed state by forming hydrogen bonds with the TM1 residues S93 and/or S97, thereby creating interactions that must be disrupted during channel opening (Hou et al., 2020). In contrast, the second model attributes the role of H134 primarily to its steric properties rather than to specific side-chain interactions. It views the bulky histidine side chain as a physical brake that restricts the local conformational rearrangements of the TM1–TM2 interface required for pore opening, whereas substitution with a smaller residue relieves this constraint and facilitates activation (Yeung et al., 2018). Both models support the idea that channel activation is facilitated by releasing the H134-mediated restraint (Najjar et al., 2026). Neighboring TM2 residues, particularly A137 and L138, further support a direct role of the inner interface in pore opening as activating substitutions at these positions have been linked to enhanced pore flexibility, expansion of the hydrophobic pore region and formation of a continuous water chain that may lower the energetic barrier for channel opening (Yeung et al., 2018; Yeung et al., 2023; Frischauf et al., 2017). In addition, several TM3 residues positioned at the inner interface contribute to the coupling between gating and ion permeation. T184 is a potential mediator of the activation signal transfer, whereas other residues are more likely to influence pore selectivity and pore architecture (Bulla et al., 2019; Najjar et al., 2026). Taken together, the inner interface is likely to actively translate peripheral conformational changes into local alterations in packing, hydration, and pore geometry, thereby enabling pore opening of Orai1.

T184M

Another Orai1 mutant, Orai1 T184M, is described as milder, with asymptomatic elevated CK levels and mild generalized weakness, myalgia, and cramps but without prominent extramuscular symptoms (Böhm et al., 2017; Bulla et al., 2019). This mutation is located in the TM3 domain. In transiently transfected human primary myoblasts and HEK293T cells, T184M produces increased Ca^{2+} currents under store-depleted conditions compared with WT. However, when STIM1 is absent, no elevated activity can be observed (Böhm et al., 2017; Bulla et al., 2019), indicating that STIM1 is required for a GoF phenotype. Notably, Orai1 T184M can be gated by the STIM1 F394H mutant, which fails to activate WT Orai1, suggesting that T184M lowers the energetic barrier for STIM1-induced signal propagation (Bulla et al., 2019). Weakening Orai1 C-terminal STIM1 binding by introducing the double mutation L273D/L276D prevents the T184M phenotype, supporting the idea that the GoF phenotype of T184M results from enhancing the coupling efficiency rather than bypassing STIM1 (Bulla et al., 2019). Patch-clamp experiments show larger CRAC currents while fast and slow CDI remain largely conserved, a clear distinction from the effects of P245L (Bulla et al., 2019). Finally, MD simulations are consistent with an allosteric mechanism. Compared with WT Orai1, Orai1 T184M does not show a clear increase in pore hydration or ion penetration in the closed-state model (Bulla et al., 2019). This aligns with a MD-based study of Guardiani et al. (Guardiani et al., 2021), in which TM3 mutations are described as factors that alter the efficiency of signal transduction rather than the pore architecture. It has been further shown that an alanine substitution at position G183 in the immediate vicinity of T184 shifts Orai1 into a more easily activatable state. Orai1 G183A could be activated by the compound 2-APB (100 μM), which normally blocks the CRAC current under WT conditions (Srikanth et al., 2010). Overall, the structural integrity of the T184 region is crucial for Orai1 to remain in an inactive state, yet ready for activation by STIM1, while structural changes in this region can shift the energy barrier for activation.

L138F

A patient with Orai1 L138F showed slowly progressive TAM with muscle weakness and a rigid spine (Böhm et al., 2017). Due to the location of the mutation, it is thought to lead to Orai1 opening by perturbing the inner interface. According to Yeung et al. (Yeung et al., 2023), L138 is one of the strongest contact points between TM2 and TM1. Mutations to aromatic residues at 138 (F or Y) show GoF phenotypes implicating steric shape rather than hydrophobicity as the key factor for the observed effects. They propose that the aromatic residues at position L138 push the TM1 away from the pore, leading to its dilation and opening. Additionally, they identified T92, located on the neighboring TM1 subunit, as interaction partner for L138 and propose a steric clash mechanism between the two residues when a F is introduced at position 138, shown in Fig. 3 C. Introducing a F at position 92 leads to a similar GoF phenotype, while reducing the TM1 side chain volume by introducing a double mutation T92G/L138F reversed this effect and led to a store dependent activation by STIM1. Interestingly, L138F alone produces constitutive CRAC currents; however, STIM1 can further enhance these currents, indicating that STIM1 still modulates the mutant channel (Yeung et al., 2023). Notably, in the Orai1 L138F channels, fast CDI can occur without STIM1, challenging the view that STIM1 is essential for CDI, while STIM1 still tunes the Ca^{2+} sensitivity of inactivation. Consistently, truncation experiments indicate that the Orai1 C terminus contributes to CDI even in constitutively active backgrounds (Yeung et al., 2023). MD simulations by Zhang et al. (Zhang et al., 2021) suggest that the dOrai analog L210F shows increased hydration of the hydrophobic region and a more dilated basic region and selectivity filter, allowing ions to sample deeper pore positions. The authors propose specific local motions, i.e., clockwise rotation of the mutant site, facilitating dilation of the basic region and increased counterclockwise rotation of the phenylalanine that enlarges the hydrophobic gate (Zhang et al., 2021). A schematic representation of this process is depicted in Fig. 3 D. The importance of the L138-adjacent region in controlling pore opening is highlighted by mutations at neighboring residues: substitutions at F136 (Orai1-F136S) or A137 (Orai1-A137V) can confer constitutive activity (Frischauf et al., 2017; Butorac et al., 2020). Complementing these findings, Maltan et al. (Maltan et al., 2023) engineered a light-sensitive Orai1 variant by incorporating the photo–cross-linkable UAA benzoylphenylalanine at A137 (Orai1-A137Bpa), which produced a robust increase in CRAC-like currents upon UV illumination even in the absence of STIM1. However, the molecular underpinning of their activation mechanisms require further investigation. Together, these studies show that the region around L138 is critical for Orai1 gating, where steric perturbations in this part of the inner interface can promote pore opening.

H134P/L194P

The Orai1 mutant H134P appears together with the L194P mutation in a patient with compound heterozygosity. The patient suffered from immunodeficiency associated with immune dysregulation, complicated by serious, ultimately fatal infections. Furthermore, the patient showed muscular hypotonia and ectodermal involvement. In addition, the patient's T cells showed reduced Orai1 levels on the cell surface, indicating that this biallelic mutation disrupts protein expression or trafficking to the PM (Noyer et al., 2025). Further investigation of both mutations separately revealed that H134P alone did not alter Orai1 expression but rather the localization in the PM, which was reduced to some extent. While most of the mutated proteins still localized in the PM, a significant amount was observed intracellularly. For Orai1 L194P, the protein expression was affected, and the localization in the PM was even more diminished than for H134P. Co-expression of Orai1 H134P and Orai1 L194P did not alter the localization compared with Orai1 H134P or Orai1 L194P expressed alone, indicating that the PM-localized channels in patient cells are likely dominated by H134P (Noyer et al., 2025).

Since H134P occurs in the combined form H134P/L194P, the functional interpretation depends on whether the defect in surface expression of L194P can be distinguished from a gating defect caused by H134P. H134P shows modest constitutive activity but fails to activate further upon STIM1 binding. This distinction is important in the interpretation of the functional

phenotype. Whereas L194P primarily compromises channel expression and trafficking, H134P predominantly affects channel gating. H134P exhibits modest constitutive activity but fails to undergo further activation by STIM1, suggesting that the mutation stabilizes the channel in a partially open conformation. Although this conformation permits basal channel activity, it appears to restrict the additional structural rearrangements required for full STIM1-mediated activation (Noyer et al., 2025). Since H134 lies at the inner interface, it controls TM1 pore helix rearrangements. H134 acts as a steric brake stabilizing the closed state, and mutations at the equivalent site in dOrai (H206) show an open pore characterized by increased pore-helix rotation and hydration of the hydrophobic gate (Yamashita et al., 2017; Tiffner et al., 2021c; Najjar et al., 2026; Yeung et al., 2018). The helix-breaking proline at H134 could therefore at the same time weaken the closed packing interface (consistent with constitutive activity) and disrupt the TM2/TM1 mechanical coupling needed for STIM1 to open the channel fully (Yeung et al., 2018).

S97C

The S97C mutation is located at the inner interface on the non-pore-lining side of TM1. Patients carrying this mutation present with mild and late-onset TAM as well as congenital miosis (Garibaldi et al., 2017). This residue forms part of the "serine ridge" (S89, S90, S93, and S97), where hydrophobic and polar side chains alternate and interact with the corresponding polar-hydrophobic residues of the TM2/TM3 ring (Yeung et al., 2018; Najjar et al., 2026). The introduction of hydrophobic residues at position 97 leads to Orai1 opening, the extent of which depends on the specific substitution (Garibaldi et al., 2017; Yeung et al., 2018). It has been suggested that introducing hydrophobic residues in this TM1 region disrupts the interface formed with TM2/TM3 and restricts TM1 movement by decreasing flexibility and leading to Orai1 pore opening (Yeung et al., 2018). Studies on Orai1 S97C, carried out in HEK293 cells and myotubes, showed constitutive Ca^{2+} entry through Orai1, which is possibly explained through a hydrophobic switching mechanism (Yeung et al., 2018).

Collectively, these findings suggest that, on the one hand, mutations in the TM1 and TM2 regions interfere with the interface in terms of flexibility, biasing the pore to increased dilation and therefore leading to channel opening. TM3 mutations, on the other hand, interfere with signal propagation and not with the pore architecture itself.

The pore region

From the inner TM interface, the Orai1 opening signal is transferred toward the ion-conducting pore, shown in Fig. 4 A. Early functional and mutagenesis studies following the identification of Orai1 established many fundamental aspects of pore architecture, including the identification of pore-lining residues, the selectivity filter, and key determinants of channel gating (Feske et al., 2006; Prakriya et al., 2006; Yeromin et al., 2006; McNally et al., 2009; Zhou et al., 2010). Subsequently, Hou et al. (Hou et al., 2012) resolved the first closed-state structure of dOrai, providing a structural framework that confirmed and extended these functional observations. At the extracellular entrance, the E106 glutamate ring forms the selectivity filter and constitutes the narrowest region of the pore. Directly below the selectivity filter, the hydrophobic region, formed by L95, F99, and V102, functions as the principal gate by restricting water and ion permeation. Toward the cytosolic side, the hydrophobic region is followed by a basic region characterized by the charged residues R91, K87, and R83. According to the closed structure, this region likely contributes to the maintenance of the closed state by binding negatively charged ions (Hou et al., 2012). Later structural, computational, and functional work studied transitions to and conformations of open, ion-conducting channels. It was implied that opening of the channel is linked to local rearrangements of the TM1 helices, which increase pore hydration and widening of the pore, especially in the basic region toward the cytosolic side (Yamashita et al., 2019; Hou et al., 2018; Hou et al., 2020). In this context, the highly conserved G98 residue has been proposed to provide the local backbone flexibility required for pore opening involving rotation of G98 and its neighbor F99 (Yamashita et al., 2017). Neighboring residues in the hydrophobic region appear to participate in the rearrangement of packing interactions associated with the transition to the open state (Yamashita et al., 2017; Yeung et al., 2020b). Toward the cytosolic side, the basic region undergoes a pronounced widening in open conformations, which is thought to further support ion conduction. Although the precise mechanism remains under debate, proposed models have included displacement of an anionic plug, rotation of basic side chains, and ion-assisted permeation (Hou et al., 2012; Hou et al., 2018, 2020; Liu et al., 2019). Recent work favors the view that positively charged residues in this region primarily promote hydration of the pore, thereby destabilizing the hydrophobic gate, formed by residues V102 and F99, facilitating channel opening (Yamashita et al., 2019). Taken together, these observations support a model in which Orai1 pore opening involves tightly coupled changes in the geometry of the selectivity filter, hydration of the hydrophobic gate, and dilation of the basic region, allowing the channel to transition from a closed to an open state.

V107M

Several disease-causing GoF as well as LoF mutations have been identified along the Orai1 pore. Patients carrying the V107M mutant present a GoF phenotype with thrombocytopenia, splenomegaly, and reduced body length in addition to muscular symptoms (Böhm et al., 2017). This is corroborated by an in vivo knock-in mouse model carrying the homologous substitution Orai1 $V109M^{+/+}$. These mice exhibit GoF features such as reduced muscle force and elevated resting cytosolic Ca^{2+} in primary myoblasts and TAM. Furthermore, they show thrombocytopenia, splenic abnormalities, and hypocalcemia (Pérez-Guàrdia et al., 2024).

The Orai1 V107M mutant is located in TM1, directly adjacent to the selectivity filter of the channel, as depicted in Fig. 4 B. The PM distribution of the mutant, recorded via TIRF microscopy, is homogeneous in the resting state. After store depletion, the mutant forms clusters, overall exhibiting a WT-like behavior (Böhm et al., 2017). However, Ca^{2+} imaging experiments and patch-clamp recordings show constitutive activity of the mutant

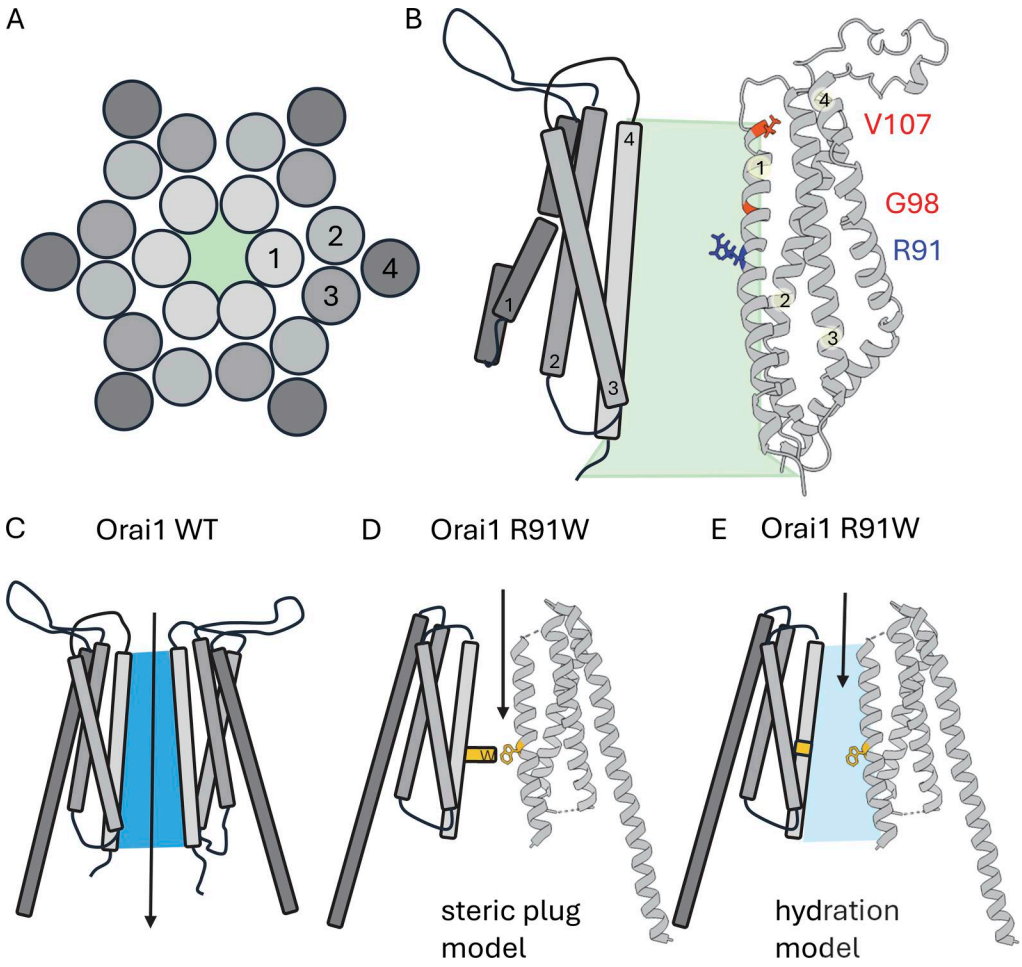

Figure 4. **Orai1 R91W LoF models. (A)** Top view of the pore location in Orai1 (green). **(B)** Side view of the Orai1 pore (green) including the pore-resident disease-associated mutations. Circles with numbers indicate the TMs, e.g., 1 corresponding to TM1. **(C)** Schematic representation of the open Orai1 WT configuration. Dark blue indicates normal pore hydration. **(D)** This model suggests that the W introduced at position 91 leads to a steric plug of the pore, resulting in channel LoF. **(E)** Another model infers that LoF occurs due to diminished hydration of the pore, indicated in light blue, after mutation of R91 to a W.

channel. Notably, the current amplitudes in the presence of STIM1 largely exceed WT currents (Böhm et al., 2017; Bulla et al., 2019). Due to its position near the pore entrance, it is likely that the mutant alters the pore geometry around the selectivity filter, increasing ion permeation not only for Ca^{2+} but also for other ions. In fact, electrophysiological measurements show reduced selectivity for Ca^{2+} and increased Na^{+} permeability, especially in the absence of STIM1. Interestingly, co-expressing STIM1 can partially rescue Ca^{2+} selectivity, which indicates that STIM1-dependent gating can restore the geometry and function of the selectivity filter to some extent (Bulla et al., 2019). Additionally, Ca^{2+} imaging and electrophysiology experiments showed that V107M reduces the pH sensitivity of Orai1, introducing TM1 as a domain relevant for pH sensing, previously associated only with the TM3 region (Beck et al., 2014; Tsujikawa et al., 2015; Bulla et al., 2019). Notably, regulatory mechanisms like fast and slow CDI are preserved in Orai1 V107M. MD simulations of the closed state further suggest that V107M does not change water or ion penetration in the pore, indicating that the mutated channel does not assume an open-like conformation (Bulla et al., 2019). It is, however, possible that differences would become visible in an open-state simulation. Taken all together, the Orai1 V107M mutation increases the permeability of Orai1, decreases its Ca^{2+} selectivity in a manner modulated by STIM1 binding, and reduces the protein's pH sensitivity.

R91W

A prominent example of a LoF pore mutant is Orai1 R91W, causing severe and early-onset SCID with recurrent infections. Affected patients also exhibit congenital muscular hypotonia and ectodermal dysplasia with anhidrosis and enamel defects (Feske et al., 2006). An analogous phenotype is recapitulated in a mouse knock-in model carrying Orai1 R93W (the mouse homolog of human R91W). Since homozygous Orai1 R93W mice are perinatally lethal, Bergmeier et al. (Bergmeier et al., 2009) generated hematopoietic chimeras in which blood cells express Orai1 R93W. In these mice, platelets display markedly reduced SOCE and diminished agonist-induced cytosolic Ca^{2+} elevations, with the defect most evident under weak stimulation. The most prominent functional defect is impaired phosphatidylserine exposure, implying that proper clot formation is impaired. Notably, neither mice nor the human SCID patients exhibit a

spontaneous bleeding tendency. In a different study carried out on chimeric knock-in Orai1 R93W mice, McCarl et al. (2010) showed that T and B cells of these mice presented with severely impaired SOCE as well as CRAC channel function. They also showed that this impairment led to a strongly reduced expression of key cytokines. Notably, they demonstrate that Orai1 is necessary for cell-mediated immune responses in vivo, such as T cell–dependent allograft rejection, hypersensitivity responses, as well as autoimmunity.

While R91W does not abolish STIM1 binding, it prevents channel activation. FRET and co-localization experiments show that STIM1–Orai1 interactions remain intact in Orai1 R91W (Navarro-Borelly et al., 2008). Consistently, R91W abolishes CRAC currents, while preserving store-dependent STIM1–Orai1 coupling, suggesting that increased hydrophobicity and structural constraints at the N-terminal TM1 region underlie a gating defect rather than loss of STIM1 binding (Derler et al., 2009). In a purified, reconstituted system, the STIM1 cytosolic domain robustly gates WT Orai1 but fails to gate Orai1 R91W, and STIM1 does not elicit the pore-opening conformational change observed in WT (Gudlur et al., 2014). Structural findings show that introducing a Trp at this position (K163W in dOrai) forms a tight Trp ring projecting into the pore and is associated with altered ion occupancy consistent with a more occluded, dehydrated conduction pathway (Hou et al., 2012). Recent MD simulations corroborate this finding by showing reduced pore hydration and an increased dehydration barrier in the R91W background (Yamashita et al., 2019). Importantly, weakening the hydrophobic gate (e.g., Orai1 V102A) partially rescues activation, arguing that the R91W defect is tightly coupled to pore hydration and gating energetics rather than arising solely from a steric plug (Yamashita et al., 2019). A comparison of the steric plug and hydration model is illustrated in Fig. 4, C–E.

Conclusion and perspectives

The activation of Orai1 relies on a precisely tuned interplay of its TM helices. Even small conformational changes can determine whether the channel is activatable, closed, or inactive. Individual amino acid substitutions have the potential to substantially influence these molecular motions and shift the conformational ensemble in favor of more closed or more open states, ultimately resulting in LoF or GoF phenotypes. We summarize the current knowledge on disease-inducing variants and their mechanisms: On the one hand, expression, trafficking, and functional consequences are well documented for many disease-causing mutants. On the other hand, only two of them, R91W and P245L, have been structurally resolved in dOrai to date. To extend this understanding and close the gaps between mutation, structure, and functional consequences, a combined structural-dynamic approach will be essential. This includes the elucidation of additional structures across all gating states of Orai (closed, pre-open, and open) and, importantly, in complex with STIM1. High-resolution cryo-EM of human Orai1 with STIM1, supported by state-trapping strategies, may reveal how signals travel from the Orai1 C terminus through the TM4–TM3–TM2–TM1 relay to the pore. Complementary MD simulations with enhanced sampling can map the energy landscape and identify allosteric bottlenecks that control the opening mechanism. Additionally, conformational reporters such as FRET or voltage-clamp fluorometry can directly track early expansions of the peripheral TM interfaces and the subsequent rearrangements of the TM1 pore.

Resolving variant-specific mechanisms could assist in the development of novel therapeutic strategies. For example, GoF effects could be countered via allosteric modulators that shift the TM3–TM4 interface more toward a closed state. Further, stabilizing productive STIM1 coupling to Orai1 could restore LoF dysfunctions. Pharmacological chaperones may rescue folding and trafficking defects, while modulation of proteostasis could diminish ER retention (Wang et al., 2014; Tran et al., 2020; Grasso et al., 2023). In another approach, Orai1 activation might be tuned through interface-selective peptides, mini-proteins, or nanobodies (Baraniak et al., 2021; Ali et al., 2025). For selected variants, genetic approaches such as allele-specific silencing or precise base editing may be appropriate.

Orai1 P245L illustrates how structural and functional insights could provide the basis for a targeted approach to develop modulators of disease mutants. This variant possibly straightens TM4, thereby perturbing the peripheral gating interface. Although it remains unclear to what extent the straightening effect occurs, the hypothesis is that widening of peripheral helices precedes the pore opening. If P245L enhances TM3–TM4 dilation, modulators that reduce this separation could limit TM1 pore widening and Ca^{2+} permeation. Photoswitchable peptides or site-specific incorporation of photosensitive amino acids could first validate the mechanism with state-dependent control, paving the way for small-molecule campaigns.

By incorporating multistate structures, quantitative dynamics, and targeted perturbations, one could identify drug-targetable interfaces and potentially lay the groundwork for rational therapies across the entire spectrum of Orai1 channelopathies. While the mechanistic concepts discussed throughout the review are largely derived from STIM1/Orai1 model systems, an important future challenge will be to determine how these mechanisms are modulated by the tissue-specific composition of native CRAC channel complexes. Future studies should therefore extend these mechanistic insights to physiological systems reflecting variable CRAC channel compositions across different tissues, addressing how molecular defects translate into tissue-specific disease manifestations and therapeutic responses.

Acknowledgments

Christopher J. Lingle served as editor.

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This research was funded by the Open Access Funding by the Austrian Science Fund (FWF) projects https://doi.org/10.55776/P35900, https://doi.org/10.55776/P36202, and https://doi.org/10.55776/PAT1680824.

Author contributions: Magdalena Prantl: conceptualization, visualization, and writing—original draft, review, and editing. Lara Atzgerstorfer: writing—original draft, review, and editing. Tamara Radiskovic: writing—original draft, review, and editing. Marc Fahrner: writing—original draft. Isabella Derler: conceptualization, funding acquisition, project administration, supervision, and writing—original draft, review, and editing.

Disclosures: The authors declare that no competing interests exist.

Submitted: 24 April 2026
Revised: 16 July 2026
Accepted: 11 August 2026

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