Adaptation of STIM1 structure-function relationships for optogenetic control of calcium signaling

Received for publication, March 12, 2024, and in revised form, July 26, 2024 Published, Papers in Press, August 8, 2024,
https://doi.org/10.1016/j.jbc.2024.107636

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Reviewed by members of the JBC Editorial Board. Edited by Roger Colbran

In cellular contexts, the oscillation of calcium ions (Ca^{2+}) is intricately linked to various physiological processes, such as cell proliferation, metabolism, and survival. Stromal interaction molecule 1 (STIM1) proteins form a crucial regulatory component in the store-operated calcium entry process. The structural attributes of STIM1 are vital for its functionality, encompassing distinct domains situated in the endoplasmic reticulum lumen and the cytoplasm. The intraluminal domain enables the timely detection of diminishing Ca^{2+} concentrations, prompting structural modifications that activate the cytoplasmic domain. This activated cytoplasmic domain undergoes conformational alterations and engages with membrane components, opening a channel that facilitates the influx of Ca^{2+} from the extracellular environment. Given its multiple domains and interaction mechanisms, STIM1 plays a foundational role in cellular biology. This review focuses on the design of optogenetic tools inspired by the structure and function of STIM1. These tools offer a groundbreaking approach for studying and manipulating intracellular Ca^{2+} signaling with precise spatiotemporal control. We further explore the practical applications of these tools, spanning fundamental scientific research, clinical studies, and their potential for translational research.

Signal transmission is a crucial and intricate process in biological organisms, occurring within cells and between cells and their microenvironment (1). Calcium ions (Ca^{2+}), serving as a crucial second messenger in signal transduction, play a pivotal role in regulating a multitude of cellular functions (2, 3), including but not limited to the modulation of vital processes, such as cell survival and death signals (4), proliferation (5), differentiation (6), apoptosis (7–9), and gene expression (10, 11). Consequently, disruptions in extracellular and intracellular Ca^{2+} balance can lead to diseases affecting numerous tissues and organs (12, 13). Thus, it is imperative to uphold or regulate the equilibrium of Ca^{2+} concentration within the cytoplasm and various organelles for physiological well-being (14, 15).

Under normal physiological conditions, the cytoplasm maintains a Ca^{2+} concentration of approximately 0.2 μM, while the extracellular Ca^{2+} concentration ranges from 1 to 3 mM, representing a 10,000-fold concentration gradient (16, 17). To precisely maintain these concentration gradients, a multitude of Ca^{2+} channels, ion pumps, and calcium-binding proteins are required within both the cell membrane and intracellular compartments. Among these, Ca^{2+} channels form the structural foundation for Ca^{2+} influx, thereby playing a pivotal role in sustaining the concentration gradient. These Ca^{2+} channels mainly include voltage-operated calcium channels (VOC) (2, 18), receptor-operated calcium channels (ROC) (19, 20), and store-operated calcium (SOC) channels (21) (Fig. 1). Among these, the SOC channel primarily consists of the transmembrane-stromal interaction molecule (STIM) protein located on the endoplasmic reticulum (ER) and the plasma membrane-located calcium release-activated calcium modulator (Orai) protein (10, 22, 23). Recent research has shed light on how this channel is activated and how it controls Ca^{2+} entry into the cell. A key feature of the SOC channel is its unique regulatory mechanism: its opening and closing are solely determined by Ca^{2+} levels within the ER, making it distinct from other channels (24) (Fig. 1).

It is well established that after the concentration of Ca^{2+} within the ER is exhausted, extracellular Ca^{2+} can penetrate the cytoplasm *via* calcium release-activated channels (CRAC) formed by STIM and Orai (25, 26). STIM has been confirmed as an ER transmembrane protein, presenting two mammalian homologs, STIM1 and STIM2. These homologs predominantly consist of the N-terminus located in the ER lumen and the C-terminus situated in the cytoplasm (3, 22, 27) (Fig. 2). In a state of adequate ER Ca^{2+}, STIM remains in a resting state. As Ca^{2+} levels in the ER stores gradually decrease, STIM releases the Ca^{2+} it was holding onto, triggering its own activation (28, 29). Subsequently, activated STIM interacts with Orai

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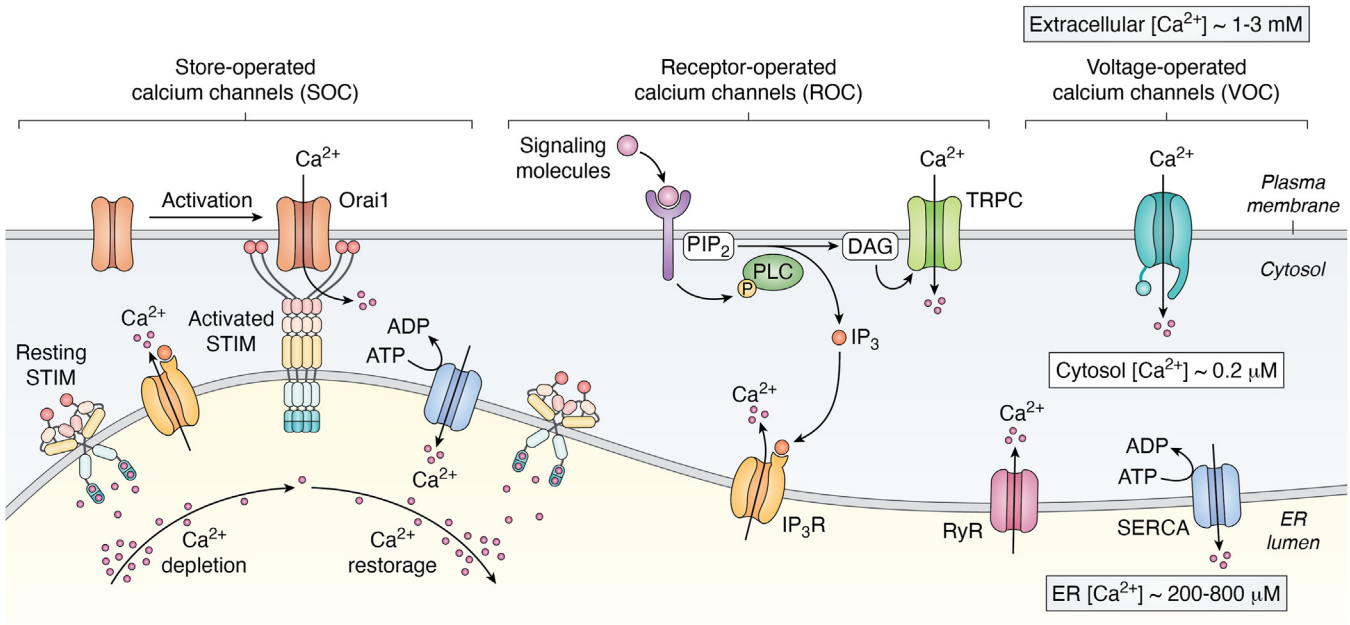

Figure 1. A scheme illustrating the standard mechanism and key players of Ca^{2+} signaling pathway. VOC and ROC channels are influenced by transmembrane voltage and receptor molecules, producing substantial calcium influx rapidly. In contrast, SOC operates differently, responding solely to changes in ER Ca^{2+} concentration and resulting in sustained calcium influx. Additional information is provided in the accompanying text. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca^{2+}, calcium ions; DAG, diacylglycerol; ER, endoplasmic reticulum; IP_3, inositol triphosphate; IP_3R, inositol triphosphate receptor; Orai, calcium release-activated calcium modulator; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; ROC, receptor-operated calcium channels; RyR, ryanodine receptors; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; SOC, stored-operated calcium channels; STIM, stormal interaction molecule; TRPC, transient receptor potential cation channels; VOC, voltage-dependent calcium channels.

proteins on the plasma membrane, opening the CRAC channel and allowing Ca^{2+} to flow into the cell from the extracellular environment (30).

Given the crucial role of the CRAC channel, which is created by the interaction of STIM and Orai, in mediating the influx of Ca^{2+} into the cell, functional mutations in STIM—such as those leading to overactivation or inhibition—have the potential to contribute to diverse diseases (31). For instance, in concurrent studies in 2014, Nesin V (32), Morin G (33), and Misceo D (34) identified the R304W mutation in STIM1, which induces oligomerization in the coiled-coil domain 1 (CC1) region of STIM1—typically observed during STIM1 activation. This mutant exhibits a persistent activation state of STIM1, leading to Stormorken syndrome (35). Previous research findings also suggest that STIM1 mutations may contribute to conditions such as muscle weakness and severe

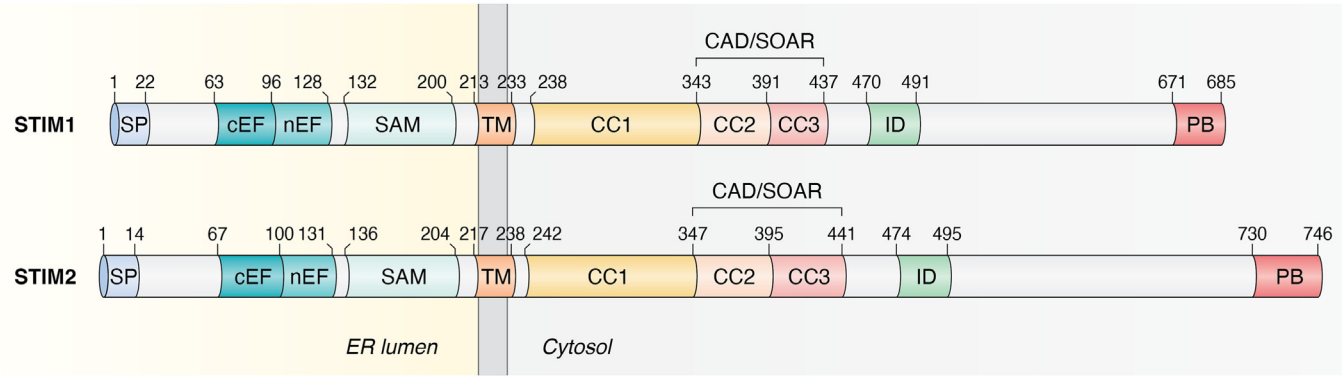

Figure 2. The molecular domains of human STIM1 and STIM2. STIM1 and STIM2 share a 54% sequence homology, each comprising an ER luminal segment and a cytoplasmic segment. The luminal segment includes cEF, nEF, and a SAM domain, crucial for regulating the process of SOCE. Specifically, STIM1 primarily detects changes in ER Ca^{2+} concentration through cEF, while subsequent conformational changes in nEF and SAM form the foundation for STIM1 activation signals. These signals are transmitted through the TM domain to the cytoplasmic segment, facilitating the binding of the CAD/SOAR domain with Orai. The cytoplasmic segment consists of three coiled-coil domains (CC1, CC2, CC3), an ID domain, and a C-terminal PB domain. In the resting state, the three coiled-coil domains adopt a mutually constrained structure. Upon receiving activation signals, CC1 undergoes conformational changes, releasing CC2 and CC3. Subsequently, the CAD/SOAR region formed by CC2 and CC3 binds to Orai, thereby opening the Ca2+ channel. The ID domain primarily functions as a negative feedback inhibitor of the SOCE process, preventing excessive activation. The PB domain, rich in positively charged amino acids, interacts with the negatively charged phospholipids in the plasma membrane, providing the driving force for STIM1 translocation to ER-PM junctions. CAD, calcium release-activated channels (CRAC) activation domain; CC1, coiled-coil domain 1; CC2, coiled-coil domain 2; CC3, coiled-coil domain 3; cEF, canonical EF-hand; ER-PM, endoplasmic reticulum-plasma membrane; ID, inactivation domain; nEF, non-canonical EF-hand; PB domain, polybasic domain; SAM, sterile alpha motif; SOAR, STIM1 Orai1 activating region; SOCE, store-operated calcium entry; SP, signal pepide; TM, transmembrane domain.

immune deficiency (36, 37). Furthermore, investigations in cancer reveal that the STIM1-Orai1 complex also plays a role in promoting the migration and metastasis of breast cancer cells (38).

Calcium signaling within immune cells plays a critical role in regulating T cell function, making it a promising target for novel cancer therapies. In immune cells, modulation of Ca^{2+} signaling activates the nuclear factor of activated T-cells (NFAT), consequently triggering the expression of downstream genes to stimulate T-cell activation (39, 40). Regulated changes in Ca^{2+} concentration within various organelles such as the mitochondria, ER, and Golgi apparatus are crucial for the metabolism, proliferation, and differentiation of immune cells (41). For instance, the three dehydrogenases in the tricarboxylic acid (TCA) cycle, essential for ATP production, are regulated by Ca^{2+} transport between the mitochondria and ER, which is related to T cell activation. Consequently, recent advancements in controlling Ca^{2+} influx have attracted significant attention for their potential clinical applications. Meanwhile, therapeutic intervention in tumor cells involves manipulating Ca^{2+} levels, achieved by elevating the concentration of Ca^{2+}. This process induces damage to subcellular organelles such as mitochondria, leading to structural disruption and oxidative stress. Consequently, tumor cell proliferation, migration, and invasion are inhibited, ultimately resulting in cell death (42, 43). Hence, precise regulation of extracellular Ca^{2+} influx and the maintenance of Ca^{2+} homeostasis is of considerable clinical importance, especially in cancer therapy and managing conditions associated with Ca^{2+} dysregulation.

Optogenetics represents an emerging experimental technique that offers a precise, spatiotemporal, and reversible means of modulating specific biological processes *in vitro* and *in vivo*. By combining optical and genetic approaches, optogenetics enables the creation and expression of fusion proteins integrating photosensitive proteins with target functional proteins. Consequently, these fusion proteins allow for the coordinated modulation of associated functional proteins alongside photosensor proteins upon activation by specific wavelengths of light, thereby facilitating targeted physiological modifications. Given the critical importance of maintaining or regulating Ca^{2+} concentration stability across the cytoplasm and various organelles for Ca^{2+} homeostasis, the utilization of optogenetics presents a promising strategy for optically restoring Ca^{2+} homeostasis (44).

In this review, we will initially introduce the formation and activation of the CRAC channel, with a specific focus on the involvement of the STIM1 protein, which plays a crucial role in developing optogenetic techniques for controlling Ca^{2+} signaling. Following this, we will outline various optogenetic approaches employed in studying Ca^{2+} homeostasis and discuss their potential therapeutic applications.

The identification of STIM proteins as indispensable elements within CRAC channels

Cytoplasmic Ca^{2+} is a crucial second messenger in signal transduction, orchestrating various life processes ranging from cell biogenesis (sperm and ovulation maturation) to programmed cell death (apoptosis and necroptosis) (45, 46). In the exploration of Ca^{2+} signal, Putney suggested that there exists an ER calcium concentration operated Ca^{2+} channel on the cell membrane in 1986 (47). This channel would permit Ca^{2+} entry into the cell following depletion of Ca^{2+} in the ER, a phenomenon termed store-operated calcium entry (SOCE). Unlike channels activated by receptor contact, SOCE activation was solely triggered by signals indicating Ca^{2+} depletion in the ER (48). Subsequent experiments across different cell lines confirmed Putney's hypothesis. The use of Thapsigargin (TG), an inhibitor of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pumps, established a model of ER Ca^{2+} depletion. In 1989, Putney, along with Takemura and colleagues, observed ER Ca^{2+} depletion and extracellular Ca^{2+} entry in cells treated with TG, providing initial validation of the SOCE concept (49). However, it remained unclear whether external stress, such as TG, or the Ca^{2+} depletion itself induced extracellular Ca^{2+} influx. This ambiguity was addressed in 1992 by Hoth and Penner, who systematically applied various non-interfering techniques to selectively diminish Ca^{2+} storage in the ER (50, 51). Their experiments definitively showcased that SOCE was exclusively triggered by the depletion of Ca^{2+} in the ER. This process adeptly responds to the swift decline of Ca^{2+} in the ER, delivering a substantial influx of Ca^{2+} to the cells and playing a pivotal role in maintaining cellular Ca^{2+} homeostasis (14).

SOCE is mediated by the CRAC channel. However, unraveling the composition of CRAC proved to be a protracted process. In 2005, two independent research groups successively unveiled that the STIM1 protein serves as a Ca^{2+} sensor in the ER. Following Ca^{2+} depletion, STIM1 migrates to the ER-plasma membrane (ER-PM) junction, activating the CRAC channel. Initially, Roos *et al.* utilized RNA interference (RNAi) technology, demonstrating the indispensable role of STIM1 rather than its homolog STIM2 in SOCE (52). Nevertheless, subsequent studies revised this understanding, unveiling that STIM2 also contributes to the facilitation of SOCE (53). Ultimately, Zhang *et al.* subsequently confirmed that STIM1, as a Ca^{2+} sensor, serves as the key link between ER Ca^{2+} depletion and extracellular Ca^{2+} influx (54). In 2006, Stefan Feske *et al.* (55, 56) and Monika Vig *et al.* (57) independently employed genetic and expression cloning methods to ascertain that Orai1 is the pore-forming subunit of the CRAC channel. However, subsequent studies demonstrated that the CRAC channel remains inactive unless the cytoplasmic domain of STIM1 binds to the cytoplasmic domain of Orai1. Thus, it was established that both STIM1 and Orai1 are indispensable for calcium influx mediated by SOCE (58).

The distinct domains of STIM proteins and their respective roles

STIM proteins represent a class of transmembrane proteins situated on the ER. This protein family primarily comprises two mammalian homologs, namely, STIM1 and STIM2, sharing a sequence homology of 54% and exhibiting significant

structural similarity (59, 60) (Fig. 2). Each member features an N-terminal ER luminal domain, a single transmembrane domain, and a C-terminal cytoplasmic domain (61). Following the depletion of Ca^{2+} stores in the ER, these proteins undergo conformational changes, the cytoplasmic domain of STIM proteins translocate to a plasma membrane-adjacent ER compartment, and subsequently activate the CRAC channel, facilitating the entry of Ca^{2+} into the cell (62, 63).

The distinctions between STIM1 and STIM2 are primarily evident in the following aspects:

- Calcium sensing capability: The baseline concentration of free luminal Ca^{2+} in the ER ranges from 400 to 600 μM in most cell lines (64, 65). Both STIM1 and STIM2 proteins can activate CRAC channels in response to a decrease in ER Ca^{2+} concentration. However, STIM2 is more sensitive to smaller decreases in ER Ca^{2+} levels, typically being activated at an EC50 of 406 μM, compared to 210 μM for STIM1 (65, 66). With a slight decrease in ER Ca^{2+} concentration, STIM2 discerns the alteration, gradually activating the CRAC and resulting in a modest Ca^{2+} influx. In contrast, STIM1 rapidly triggers the CRAC channel once ER Ca^{2+} is depleted, causing a substantial influx of Ca^{2+} (67). It is evident that STIM2 exhibits greater sensitivity to changes in ER Ca^{2+} levels but produces a comparatively weaker response.
- Translocation and aggregation capacity: Following the depletion of Ca^{2+} in the ER, the entire STIM1 rapidly translocates to the ER-PM junction, forming conspicuous aggregates (68). In contrast, STIM2 translocates at a slower pace in response to the decrease in Ca^{2+} concentration, resulting in smaller and less easily detectable aggregates (69, 70). This initially led to the belief in early research that STIM2 is unrelated to the Ca^{2+} influx mediated by the CRAC.
- Lipid binding affinity: The polybasic domain (PB) of STIM proteins interacts with lipids, which is the structural basis for their recruitment to the ER-PM region after activation. STIM1 and STIM2 have different affinities for different lipids. STIM2 can bind to two types of lipids, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3), while STIM1 primarily binds to PI(4,5)P2 (71, 72). In addition, a recent discovery has identified the STIM1 Orai Activating Region (SOAR) domain of STIM1, capable of binding to phosphoinositol-4-phosphate (PI(4)P) and facility targeting STIM1 to ER-PM junction (73).

Three characteristics regions of STIM proteins:

- ER Intraluminal Region: This section serves as the pivotal component for detecting changes in Ca^{2+} levels within the ER. It encompasses two helix-loop-helix structural (EF hand) domains and a sterile alpha motif (SAM) domain (28) (Fig. 2). The EF hand domain is frequently present in the structure of calcium-regulated proteins, featuring a helix-coil-helix structure where Ca^{2+} can bind to the coil structure (74). Two EF hand domains exist in this segment: the canonical EF hand (cEF) domain and the noncanonical EF hand (nEF) domain (75). Although these two domains have slightly distinct functions, collectively, they enable STIM to effectively perceive alterations in Ca^{2+} concentration within the ER (76). The cEF domain, with its cyclic structure, forms a stable octahedral coordination with Ca^{2+}, primarily responsible for binding Ca^{2+} when abundant, thereby maintaining STIM in an inhibited state. Conversely, the nEF, with its cyclic structure containing only three oxygen atoms, cannot form a stable octahedral coordination with Ca^{2+}, resulting in unstable binding (77–79). When the signal-inducing Ca^{2+} release from the ER is transmitted, Ca^{2+} bound to the nEF is the first to dissociate from STIM (80). Depletion of ER Ca^{2+} results in Ca^{2+} dissociation from the nEF. The resulting conformational changes induce STIM1 dimerization (*via* the SAM domain) and translocation to the ER-PM junction to activate Orai1 (3). The SAM domain which has a hydrophobic region, serves as the structural foundation for STIM to sustain dimerization after the Ca^{2+} depletion in the ER (68, 81).
- Single Transmembrane Domain (TM): STIM positions itself on the ER membrane through the α-helical TM domain. However, STIM is not statically anchored to the ER; instead, it exhibits dynamic movement (82). When STIM detects signals indicating a decline in Ca^{2+} levels within the ER, it can translocate from the ER to the ER-PM junction. The activation signal, initiated by the deformation of the STIM N-terminus situated in the ER lumen, is transmitted along the TM domain to the cytoplasmic segment. This transmission induces conformational changes in the STIM cytoplasmic segment, consequently influencing its interaction with Orai and the subsequent opening of the CRAC (83, 84). Therefore, the length and amino acid sequence of this domain significantly impact the functionality of STIM proteins (85).
- Cytoplasmic Region: The cytoplasmic segment of STIM extends from near the ER membrane to its final end, featuring three coiled-coil domains (CC1, CC2, CC3), an additional domain for inactivation (ID), and a PB domain located at the C-terminus (86) (Fig. 2). The trio of coiled-coil domains constitutes a fundamental structural foundation for the functional role of STIM. Among them, CC2 and CC3 together form the SOAR domain, a crucial functional domain of STIM and the smallest domain capable of activating Orai (87–89). Upon activation, the two coiled-coil domains within the SOAR domain adopt a U-shaped structure, binding to the C-terminus of Orai, thereby initiating the opening of the CRAC and inducing Ca^{2+} influx (90). However, CC1 functions somewhat differently from the CC2 and CC3 domains. It typically serves as a self-inhibition lock, binding to CC3 in the inhibited state of STIM to obstruct the SOAR domain (91). When STIM undergoes activation, CC1 undergoes conformational changes, releasing the SOAR domain to bind with Orai, consequently opening the CRAC channel (92). Following the SOAR domain is the ID domain, rich in acidic amino

acids. Its primary role is to bind to the C-terminus of Orai, facilitating the calcium-dependent inactivation (CDI) of the CRAC channel (93). The ID domain acts as a negative feedback regulatory element of the CRAC channel, preventing excessive activation and maintaining a balanced Ca^{2+} level within the cell (94). The PB at the C-terminus of STIM is rich in lysine, featuring a substantial positive charge (87). This domain interacts with the negatively charged PI(4,5)P2 on the PM (95). While it can also bind to other phosphatidylinositol analogs, its highest affinity is with PI(4,5)P2 (72). This electrostatic attraction is believed to play a role in transporting STIM to the ER-PM junction (73, 96). Consequently, the PB domain is crucial for the translocation and aggregation of STIM, although it alone is insufficient to induce activation (29).

The activation mechanism of STIM

Research indicates that oxidative stress, temperature fluctuations, hypoxic conditions, and pH variations all have the potential to activate STIM (25). However, the primary mechanism for STIM activation remains the depletion of Ca^{2+} stores. Extracellular signaling molecules, including growth factors, signal peptides, neurotransmitters, and hormones, can bind to receptors on the plasma membrane, leading to the phosphorylation and activation of phospholipase C (PLC) proteins, rendering them catalytically active (97). Activated PLC hydrolyzes PI(4,5)P2 on the PM, generating inositol trisphosphate (IP_3) and diacylglycerol (DAG). Notably, IP_3 binding to the IP_3R on the ER membrane triggers the rapid release of stored Ca^{2+} from the ER, depleting Ca^{2+} stores (98). As Ca^{2+} depletion occurs in the ER, the Ca^{2+} originally bound to the EF hand domain dissociates, activating the EF-SAM domain (99). This unstable state generates an activation signal transmitted to the cytoplasmic segment through the TM domain. The cytoplasmic portion of STIM undergoes conformational changes upon receiving the activation signal, leading to the release of the interaction between CC1 and CAD/SOAR. Consequently, the cytoplasmic region shifts from a tight to a loose configuration. This structural alteration causes the C-terminus of STIM to adopt an extended conformation, and the positively charged PB domain at the terminus protrudes outward. This outward extension induces STIM aggregation toward the ER-PM junction through electrostatic attraction. Concurrently, the SOAR domain, freed from structural inhibition, becomes exposed and binds to Orai, serving as the structural basis for the CRAC. This interaction assists in the influx of extracellular Ca^{2+} (100–102) (Fig. 3).

Calcium/STIM-Orai signaling plays a crucial role in the pathogenesis and treatment of various immune-related diseases, encompassing T cell activation (103), autoimmune diseases (40), infectious diseases (104), and tumor immunity (105). By controlling Ca^{2+} signaling, we can not only promote T cell activation but also potentially induce tumor cell death through Ca^{2+} overload. Consequently, the modulation of Ca^{2+} signaling holds promise for advancing clinical therapeutic approaches (106, 107). It is imperative to regulate the function of STIM proteins in a controlled manner within cells to maintain cellular Ca^{2+} homeostasis and prevent genetic diseases arising from Ca^{2+} imbalances. Founded on theoretical principles, optogenetics presents a fresh avenue for the controlled modulation of Ca^{2+} entry. Distinguished by its rapidity, sensitivity, reversibility, and exceptional spatiotemporal precision, this approach outperforms conventional

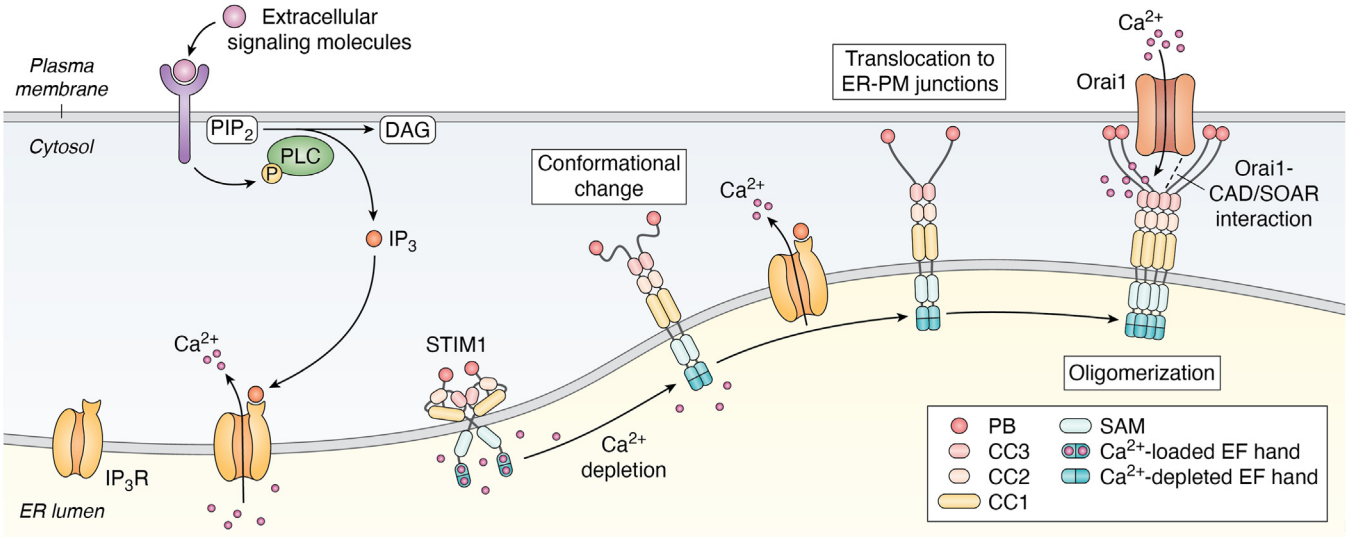

Figure 3. The process of the activation and coupling of STIM1 to activate the Orai1 channel. Upon extracellular signaling molecules binding to receptors on the PM, it leads to the phosphorylation and activation of PLC within the cell. The activated PLC then separates PI(4,5)P2 on the PM into IP_3 and DAG. IP_3, binding to the Ca^{2+} channel IP_3R located on the ER membrane, triggers the release of Ca^{2+} from the ER, depleting calcium stores within the ER. As the Ca^{2+} concentration inside the ER decreases, the STIM1 protein, functioning as a calcium sensor, detects this change and undergoes conformational changes to become activated. Activated STIM1 gradually forms oligomers and translocates to the ER-PM junctions, where its released SOAR/CAD domain bind to Orai1 located on the PM, facilitating Ca^{2+} influx. CC1, coiled-coil domain 1; CC2, coiled-coil domain 2; CC3, coiled-coil domain 3; DAG, diacylglycerol; ER, endoplasmic reticulum; IP_3, inositol triphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor; Orai1, calcium release-activated calcium channel protein 1; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; PM, plasma membrane; SAM, sterile alpha motif; STIM1, stromal interaction molecule 1.

methods of pharmacological, chemical, and genetic regulation (108–110). With continuous advancements in optogenetic technology, numerous studies have integrated it into the SOCE activation. These endeavors aim to achieve precise light control over SOCE or govern the formation of ER-PM membrane contact sites (111–113). These efforts hold considerable significance in advancing basic scientific research, clinical investigations, and the development of novel therapeutic approaches rooted in the imbalances of Ca^{2+} homeostasis.

Engineering Ca^{2+} homeostasis *via* optogenetic manipulation of STIM protein

In the early stages of optogenetic research, the widely recognized protein for such studies was channelrhodopsin-1 (ChR1), a light-gated ion channel activated by blue light, allowing non-specific cations to enter the cell (114, 115). ChR1 originates from unicellular green algae, and experiments have shown that it can be correctly expressed in various biological cells (116). ChR1 facilitated the initial understanding of using light to control electrical excitability, intracellular acidity, and Ca^{2+} influx, among other cellular processes (117). However, as science progressed, challenges arose that ChR1 and related photosensitive proteins couldn't address, particularly its non-selective nature for K^+, Na^+, and Ca^{2+} (118). This limitation hindered specific regulation of Ca^{2+} entry. Additionally, the reliance on blue light for regulation posed sensitivity issues due to its poor penetration (114, 119).

To overcome these challenges and achieve precise control over Ca^{2+} homeostasis using light, it became essential to develop and refine methods for regulating Ca^{2+} entry into cells. Building on ChR1, researchers harnessed natural, non-opsin-based photosensitive proteins like cryptochrome-2 (CRY2) from *Arabidopsis thaliana* and the light-oxygen-voltage sensing domain 2 (LOV2) from oat phototropin-1 (120, 121). CRY2 undergoes oligomerization when exposed to blue light, while LOV2 undergoes a structural change by unwinding its C-terminal Jα helix, separating it from the photosensitive period-ARNT-single-minded domain (PAS) in response to blue light exposure (122, 123). There are also some light-controlled dimerization systems such as improved light-induced dimer/SspB (iLID/SspB) and CRY2/CIBN. iLID integrates the *Escherichia coli* (*E. coli*) SsrA sequence into the Jα helix of optimized AsLOV2, featuring 10 mutations across the PAS domain, hinge loop, and Jα helix. Upon blue light irradiation, SsrA undergoes uncaging and binds to its adaptor protein SspB, also of *E. coli* origin, resulting in the formation of a light-inducible heterodimer pair (124). In CRY2/CIBN system, CIBN acts as the ligand of the photosensitive protein CRY2, which can also dimerize with CRY2 upon blue light irradiation. Additionally, certain photosensitive proteins can also undergo processes like oligomerization, dissociation (125), and conformational changes when exposed to specific excitation light wavelengths.

Relying on these non-opsin photosensors, researchers chose to employ optogenetics to manipulate the CRAC channel, the primary conduit for Ca^{2+} entry in non-excitable cells, allowing for light-regulated control over Ca^{2+} influx. Given that STIM1 is the earliest identified member of the STIM family proteins, and its associated mechanism has been extensively studied, scientists predominantly developed a range of optogenetic tools based on STIM1. Moreover, Ma *et al.* have also analyzed the kinetic characteristics of CRY2-STIM2$_{(324–833)}$ with Ca^{2+} influx, which showed a very mild increase in light-elicited Ca^{2+} response (126). Drawing from earlier investigations, the molecular mechanism through which STIM1 activates the CRAC channel can be primarily delineated into two stages (127): (1) Upon detecting a decrease in Ca^{2+} concentration in the ER, the luminal segment of STIM1 initiates activation by undergoing oligomerization through the EF-SAM domain. (2) Following the transmission of the activation signal to the cytoplasmic segment of STIM1, the structure of the cytoplasmic segment becomes more relaxed, migrates to the ER-PM junction, releases contact inhibition between the CC1 domain and the SOAR domain, and allows the SOAR domain to bind to Orai, thereby activating the CRAC.

Utilizing naturally occurring photosensitive proteins, CRY2 and LOV2, researchers have designed optogenetic systems for the optical control of Ca^{2+} entry (Fig. 4*A*). CRY2-based Ca^{2+} modulator is a fusion protein that combines the photolyase homology (PHR) domain of CRY2 with the cytoplasmic segment of STIM1 (128). Leveraging the oligomerization characteristic of the CRY2 PHR domain activated by blue light, it mimics the oligomerization of the STIM1 luminal segment in response to a decrease in Ca^{2+} concentration. After being activated by blue light, STIM1$_{ct}$ oligomerizes through CRY2, relieving the inhibition of the CC1 domain on the SOAR domain. Subsequently, the exposed SOAR interacts with Orai1 on the PM, opening the CRAC channel and allowing the influx of Ca^{2+}. This system exhibits rapid activation kinetics of less than 1 min and a deactivation half-life of 4 to 6 min after light irradiation cessation, enabling quick and sensitive small-scale light activation (129) (Fig. 4*B*, left panel). Substituting other optogenetic tools, such as iLID/SspB or CRY2/CIBN, which also oligomerize post-light irradiation, can further enhance the kinetics of Ca^{2+} influx with a shorter activation time. Studies have demonstrated that the sensitivity of the optogenetic tool CRY2 can be modified through genetic engineering, such as point mutations or adding amino acids at the C-terminus, thereby enhancing the sensitivity of the light control system (130). Building upon these optimizations, systems with improved kinetic effects, such as monSTIM1 and enhanced OptoSTIM1 (eOS1), have been developed (129, 131) (Table 1).

Leveraging the caging function of the C-terminus of LOV2, a range of light-controlled LOV2-based Ca^{2+} modulators, including Opto-CRAC1 (132), BACCS (133), and LOVS1K (134), were created to simulate the self-inhibition process of CC1 and SOAR domains in the cell. In the dark state, LOV2 replaces CC1 to inhibit SOAR (Table 1). Upon being activated by blue light, the photo-excitation leads to the formation of a covalent photo-adduct. This event induces the unwinding and separation of the Jα helix from the core body, facilitating the release of the locked SOAR domain (135). Subsequently, the free SOAR domain can bind to Orai on the PM, triggering the

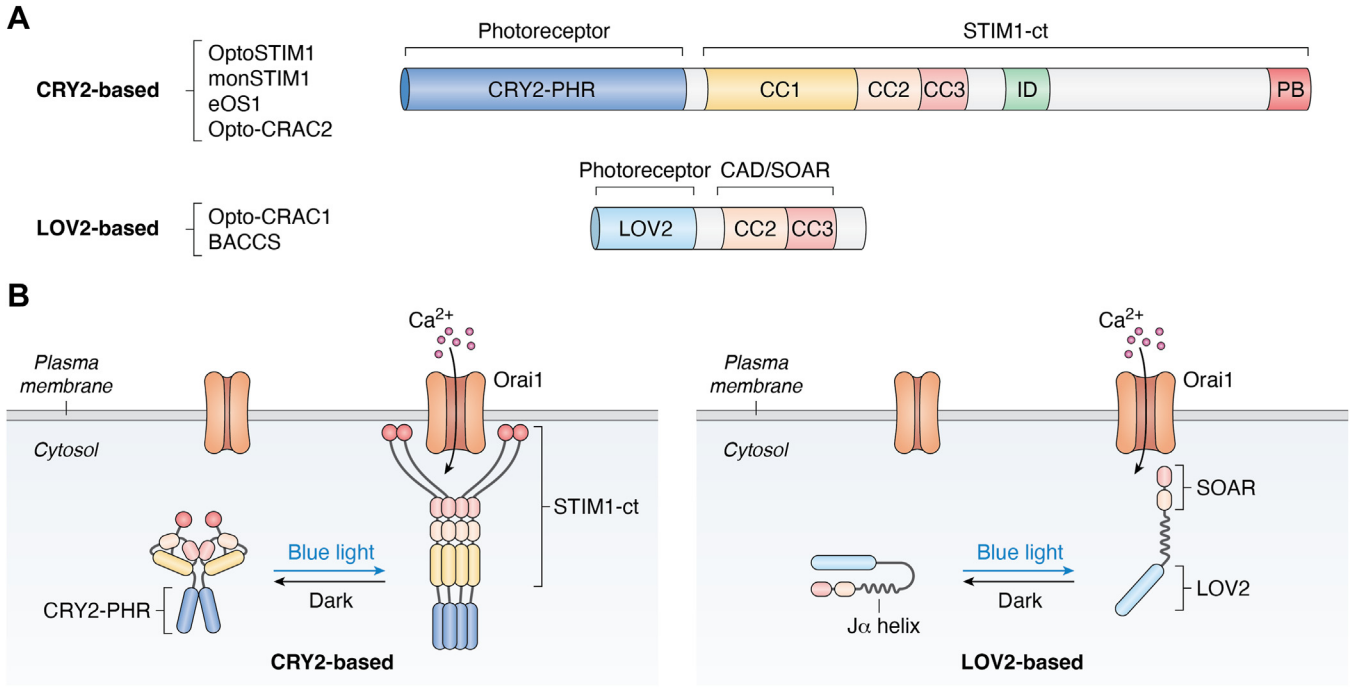

Figure 4. Illustration depicting two primary types of optogenetic Ca^{2+} modulator derived from STIM1 for photo-controlling Ca^{2+} influx. *A*, the structural domains of CRY2-based and LOV2-based Ca^{2+} modulators. CRY2-based Ca^{2+} modulators utilize the CRY2-PHR domain as a light sensor, with the cytoplasmic segment of STIM1 serving as the functional protein. LOV2-based Ca^{2+} modulators employ LOV2 as the light sensor and the SOAR region of STIM1 as the functional protein. *B*, the mechanism of CRY2-based Ca^{2+} modulators involves replacing the original ER luminal segment of STIM1 with CRY2-PHR (*left panel*). Under 470 nm *blue* light illumination, CRY2 undergoes oligomerization, mimicking the oligomerization effect of STIM1 in the ER lumen. This allows the remaining STIM1 structure to respond to the oligomerization signal, leading to the release of the SOAR region, which then binds to Orai1, facilitating Ca^{2+} influx; The mechanism of LOV2-based modulators involves tethering the SOAR region to the C-terminus of the optogenetic protein LOV2 (*right panel*). In the dark, LOV2 blocks the SOAR region. Upon illumination with 470 nm *blue* light, the Jα helix at the LOV2 C-terminus undergoes a conformational change, releasing the SOAR region. This enables it to bind to Orai1, promoting Ca^{2+} influx. CRY2, cryptochrome 2; ID, inactivation Domain; LOV2, light-oxygen-voltage-sensing domain 2; PB, polybasic domain; PHR, photolyase homology region; SOAR, STIM1 Orai1 activating region; STIM1, stromal interaction molecule 1; STIM1-ct, STIM1 cytoplasmic tail.

opening of the Ca^{2+} influx channel (Fig. 4*B*, right panel). These systems demonstrate activation kinetics of 10 to 30 s and a deactivation half-life of 30 to 50 s. Due to the shorter length of the SOAR domain compared to the cytoplasmic region or full-length STIM1, these light-controlled systems are more sensitive and faster (132).

Theoretically, optogenetic tools derived from either STIM1 or Orai1 can potentially regulate calcium influx, given their involvement in the process of SOCE. Hence, optogenetic tools centered on Orai1, termed light-operated Ca^{2+} (LOCa) channel, have been developed. LOCa involves fusing LOV2 to various transmembrane domains of Orai1, thereby enabling optical control over conformational changes in the Orai1 protein. This manipulation serves as a switch for the CRAC channel, thus regulating calcium influx (136). Currently, the optogenetic tools developed for regulating Ca^{2+} entry based on STIM1 are mainly based on the mechanism of the cytosolic region of STIM1. In the future, new mechanisms can be

Table 1
Summary of STIM1-based optogenetic tools

Based physiological process	Tools name	Functional domain	Photosensitive domain	Refs
Light-induced Oligomerization	OptoSTIM1	$STIM1^{238–685}$	$CRY2_{PHR}$	(128, 149)
		$STIM1^{238–463}$	$CRY2_{PHR}$	(128, 149)
		$STIM1^{342–685}$	$CRY2_{PHR}$	(128, 149)
	monSTIM1	$STIM1^{238–685}$	$CRY2_{PHR\text{-}E281A\text{-}9A}$	(129)
	eOS1	$STIM1^{238–685}$	$CRY2_{PHR\text{-}E490G}$	(131)
	Opto-CRAC2	$STIM1^{233–685}$	$CRY2_{PHR}$/CIBN	(126)
		$STIM1^{233–685}$	iLID/SspB	(126)
		ER-tethered-$STIM1^{233–685}$	$CRY2_{PHR}$	(126)
Light-induced unfolding	Opto-CRAC1	$STIM1^{336–685}$	LOV2	(132)
	BACCS	$STIM1^{347–448}$	LOV2	(133)
		$STIM1^{347–448}$	2*LOV2	(133)
	LOVS1K	$STIM1^{233–450}$	LOV2	(134)

BACCS, blue light-activated Ca^{2+} channel switch; CIBN, the N-terminal domain of CIB1; CRY2, cryptochrome 2; eOS1, enhanced OptoSTIM1; iLID, improved light-induced dimer(LOV2-SsrA); LOV2, light-oxygen-voltage sensing domain 2; LOVS1K, LOV2 fused STIM1 fragment (233–450); monSTIM1, monster-OptoSTIM1; Opto-CRAC1, optogenetic Ca^{2+} release-activated Ca^{2+} channel 1; Opto-CRAC2, optogenetic Ca^{2+} release-activated Ca^{2+} channel 2; OptoSTIM1, optogenetic stormal interaction molecule 1; PHR, photolyase homology region; SspB, stringent starvation protein B; STIM1, stromal interaction molecule 1.

adopted, such as controlling the binding of EF-hands and Ca^{2+} inside the ER lumen or controlling the conformational change of the TM domain through optogenetics, thereby regulating the activity of STIM1 to achieve the purpose of regulating the SOCE process.

In conclusion, a range of optogenetic tools has been developed. When compared to conventional pharmacological, chemical, and genetic regulatory approaches, this method of optically controlling Ca^{2+} entry exhibits swifter kinetics, heightened sensitivity, and reversibility.

Potential applications of STIM1-derived optogenetic tools

The preceding content has highlighted the crucial role of Ca^{2+} signaling in governing life processes, emphasizing the potential development of clinical applications due to the relationship between Ca^{2+} homeostasis imbalance and various diseases.The optogenetic system anchored in STIM1 presents promising clinical significance (137). Currently, met these CRY2-based and LOV2-based light-specific Ca^{2+} regulation optogenetic systems have undergone extensive validation (127). Researchers have successfully overexpressed this system in diverse cell types derived from various organisms, demonstrating its efficacy in regulating Ca^{2+} influx in cells such as neurons (128), embryonic stem cells (138), T cells (139), and macrophages (140) (Fig. 5).

Furthermore, investigations have explored the applicability of OptoSTIM1 and Opto-CRAC in fields such as immunotherapy and gene therapy (135). NFAT, a pivotal transcription factor in T cell activation, undergoes activation when intracellular Ca^{2+} levels increase. The activation of calmodulin phosphatase in the cytoplasm, triggered by elevated Ca^{2+}, leads to NFAT dephosphorylation. Once dephosphorylated, NFAT translocates to the nucleus, initiating a cascade of downstream cytokines like interleukin-2 (IL-2) and interferon gamma (IFN-γ), ultimately activating T cells (40, 141) (Fig. 5*A*). Consequently, the integration of Opto-CRAC1 with NFAT proteins in T cells offers a means for optical control of T cell activation, thereby enhancing the effectiveness of immunotherapy. In addition, studies have shown that Ca^{2+} levels within macrophages are critical for activating the inflammasome nucleotide-binding and oligomerization domain (NOD)-like receptor thermal protein domain associated protein 3 (NLRP3) (142).

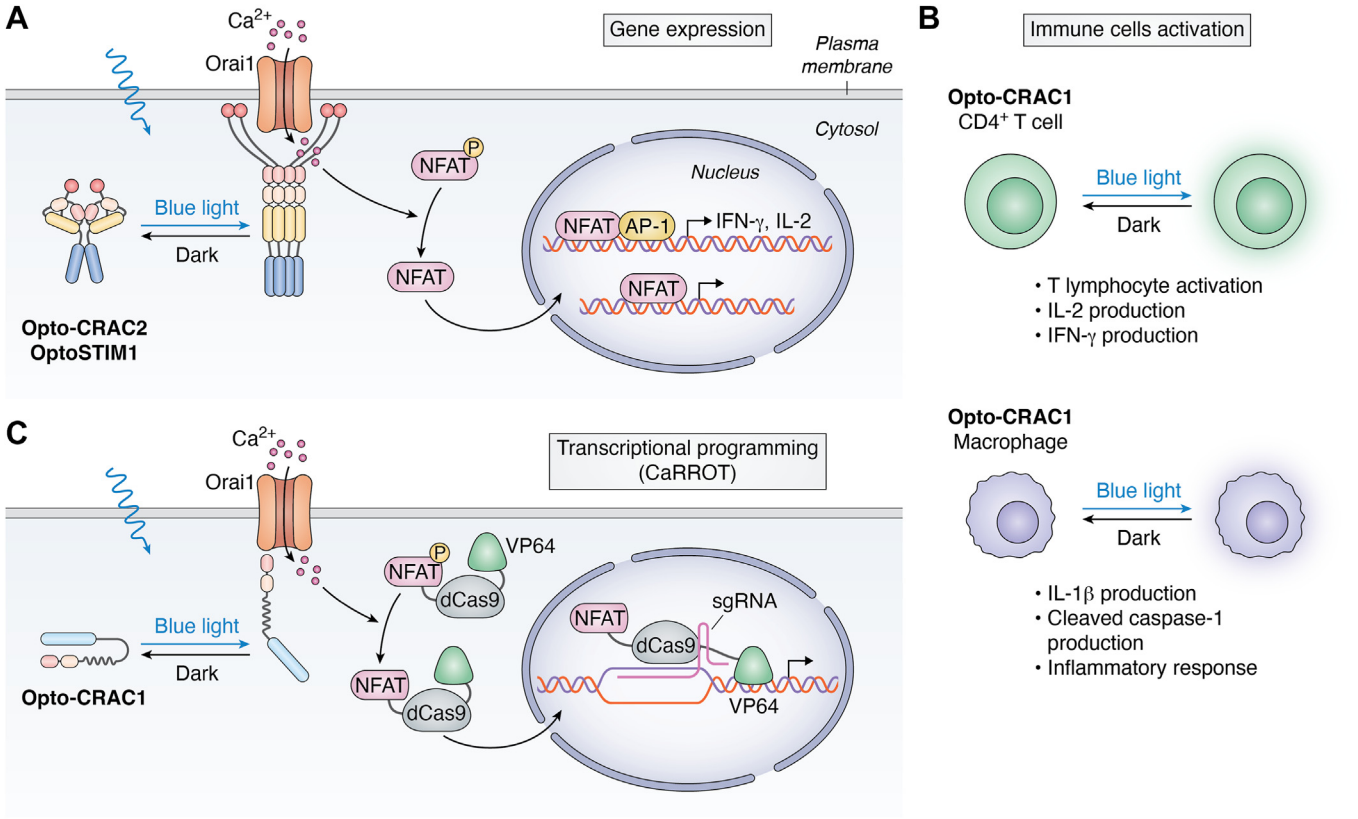

Figure 5. Applications of STIM1-based optogenetic Ca^{2+} modulators. *A*, exogenous expression of OptoSTIM1 or Opto-CRAC2 activates SOCE under blue light irradiation, leading to Ca^{2+} influx. As a result, NFAT is dephosphorylated, translocates into the nucleus, and binds to corresponding DNA regions, promoting downstream transcription. *B*, in T cells, Ca^{2+} influx mediated by Opto-CRAC1 induces the production of IL-2 and IFN-γ, thereby promoting T cell activation and exerting immunotherapeutic effects. In macrophages, Ca^{2+} influx mediated by the Opto-CRAC1 leads to the secretion of IL-1β and processed caspase-1 fragments, triggering an inflammatory response. *C*, the N-terminus (1∼460) of NFAT facilitates nuclear translocation after dephosphorylation. sgRNA, working in conjunction with the dCas9, enables specific binding to DNA sequences. VP64, serving as a transcriptional enhancement element, facilitates downstream transcription. The CaRROT system integrates these components. Co-expression of Opto-CRAC1 and CaRROT systems enables light-controlled expression or inhibition of specific genes, holding potential for gene therapy. AP-1, activator protein 1; CaRROT, calcium-responsive transcriptional reprogramming tool; dCas9, deactivated Cas9 protein; IFN-γ, interferon γ; IL-1β, interleukin 1β; IL-2, interleukin 2; NFAT, nuclear factor of activated T-cells; sgRNA, single guide RNA; SOCE, store-operated calcium entry; VP64, a tetramer of VP16 (a transcriptional activation domain).

NLRP3 activation often leads to the release of the immune factor Interleukin-1 beta (IL-1β) and the processed caspase-1 (p20 subunit) inside the cell, indicating that increased intracellular Ca^{2+} can significantly promote the inflammatory response mediated by macrophages (143, 144). Experiments using the Opto-CRAC1 system on macrophages demonstrated an increase in IL-1β and p20, confirming that light can control the extracellular immune processes of macrophages. This finding has significant potential for expanding the possibilities of immune therapy in clinical settings (Fig. 5*B*).

Simultaneously, recognizing the distinct functionality of the N-terminus (1∼460) of NFAT, which facilitates nuclear translocation post-dephosphorylation, led to the creation of the NFAT (1∼460)-dCas9-VP64 system, known as CaRROT for "calcium-responsive transcriptional reprogramming tool" (145) (Fig. 5*C*). In this system, single guide RNA, which works in conjunction with the deactivated Cas9 protein (dCas9), enables it to bind to specific DNA sequences (146). Concurrently, VP64, serving as a transcriptional enhancement element, facilitates the transcription of downstream sequences of single guide RNA (147). Previous studies have indicated that CaRROT has the capability to associate with certain transcriptional repressors, like Kruppel-associated Box (KRAB), leading to the suppression of oncogene expression in tumors. This mechanism enables gene therapy for cancer (148). Consequently, the integration of the CaRROT system with Opto-CRAC1 enables optical control for promoting or inhibiting gene expression without genomic alterations, thereby augmenting the effectiveness of gene therapy (Fig. 5*C*).

Broadly, this light-regulated system for Ca^{2+} entry not only holds promise for addressing diseases arising from disruptions in Ca^{2+} homeostasis but also exhibits potential for treating malignant tumors. Through synergistic combinations with other systems or proteins, it can bolster therapeutic approaches like immunotherapy and gene therapy, offering spatiotemporally precise and targeted treatments with significant clinical research implications.

Conclusion

While experimentally validated optogenetic systems capable of light-controlled regulation of Ca^{2+} entry into cells have been designed to enhance sensitivity and efficiency, these systems face challenges. Firstly, the overexpression of optogenetic tools in cells can impact function due to expression efficiency variations. And, the overexpression of exogenous genes may disrupt normal physiological functions, trigger immune responses, or induce cellular toxicity. Secondly, precise targeting of the light source to the desired cell or tissue is crucial for activating the optogenetic system, but issues like light scattering, absorption, and reflection can limit penetration. Careful control of light intensity and duration is essential to avoid over-activation or inhibition of Ca^{2+} channels, as well as to prevent light damage and photobleaching. Thirdly, the presence of proteins similar to ERp57 in the cell, capable of binding to STIM and exerting inhibitory effects, poses a challenge to the stability and efficiency of the optogenetic system. To enhance system efficiency, it is crucial to select photosensitive proteins that interact effectively with the STIM protein while minimizing interference with other cellular factors. Ongoing efforts are directed towards optimizing the optogenetic control of Ca^{2+} entry. Some research teams employ techniques like adenovirus delivery to ensure optimal expression efficiency and levels of the optogenetic system in the body. Additionally, laboratories explore the use of upconversion nanoparticles (UCNPs) *in vivo* to address the challenge of insufficient penetration of specific excitation light sources. UCNPs are nanomaterials with optical properties capable of converting near-infrared radiations with low-energy light into visible radiations with high-energy light. They serve to increase the depth of light penetration, particularly in deeper tissues, thus enhancing the effectiveness of *in vivo* optogenetic methods. In conclusion, the combination of optogenetic tools and STIM proteins offers an optically controllable approach to regulate Ca^{2+} entry into cells, presenting novel theories and methods for maintaining calcium homeostasis in the body and treating diseases arising from abnormal calcium concentrations.

Data availability

All data are in the article.

Acknowledgments—We express our appreciation to all researchers for their insightful explorations of STIM1 in the context of Ca^{2+} homeostasis. We are grateful to the National R&D Program of China (2023YFC3403400) and the National Natural Science Foundation of China (32271485) to J.J., Natural Science Foundation of Zhejiang Province (YXD23H0302) to J.J., and Zhejiang Key Laboratory of Prevention, Diagnosis and Therapy of Upper Gastrointestinal Cancer (2022E10021). We would like to extend our gratitude to the developers at BioRender for providing the platform used to create our figures at BioRender.com.

Author contributions—X. C., J. J. supervision; X. C., J. J. funding acquisition; J. J., Y. M., and Z. Z. conceptualization; Y. X., Y. W., Y. M., and Z. Z. writing–review & editing; Y. X., Y. M., and Z. Z. writing–original draft.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: Ca^{2+}, Calcium ions; STIM1, Stromal interaction molecule 1.

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