STIM proteins: integrators of signalling pathways in development, differentiation and disease

Lorna S. Johnstone a, *, Sarah J. L. Graham a, Marie A. Dziadek b, c

a School of Biological Sciences, University of Auckland, Auckland, New Zealand
b Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
c Faculty of Medicine, The University of New South Wales, Sydney, NSW, Australia

Received: February 24, 2010; Accepted: March 29, 2010

Abstract

The stromal interaction molecules STIM1 and STIM2 are endoplasmic reticulum \( \text{Ca}^{2+} \) sensors, serving to detect changes in receptor-mediated ER \( \text{Ca}^{2+} \) store depletion and to relay this information to plasma membrane localized proteins, including the store-operated \( \text{Ca}^{2+} \) channels of the ORAI family. The resulting \( \text{Ca}^{2+} \) influx sustains the high cytosolic \( \text{Ca}^{2+} \) levels required for activation of many intracellular signal transducers such as the NFAT family of transcription factors. Models of STIM protein deficiency in mice, Drosophila melanogaster and Caenorhabditis elegans, in addition to the phenotype of patients bearing mutations in STIM1 have provided great insight into the role of these proteins in cell physiology and pathology. It is now becoming clear that STIM1 and STIM2 are critical for the development and functioning of many cell types, including lymphocytes, skeletal and smooth muscle myoblasts, adipocytes and neurons, and can interact with a variety of signalling proteins and pathways in a cell- and tissue-type specific manner. This review focuses on the role of STIM proteins in development, differentiation and disease, in particular highlighting the functional differences between STIM1 and STIM2.

Keywords: STIM1 • STIM2 • adipocyte • myoblast • lymphocyte • differentiation • cancer • Drosophila • C. elegans • store-operated \( \text{Ca}^{2+} \) entry

Introduction

Calcium is a ubiquitous intracellular signalling molecule implicated in the control of an impressive variety of cell behaviours, from immediate responses such as contraction and secretion, to longer-term responses including proliferation, differentiation and apoptosis. Consequently, it is not surprising that deregulation of \( \text{Ca}^{2+} \) signalling is a feature of multiple diseases [1–3] and several aspects of \( \text{Ca}^{2+} \) signalling regulation have been successfully targeted therapeutically [4, 5]. The maintenance of intracellular \( \text{Ca}^{2+} \) concentration ([\( \text{Ca}^{2+} \)]) at around 100 nM, at least 10,000-fold lower than the \( \text{Ca}^{2+} \) concentration in the extracellular milieu [6], is key to these ions’ ability to act as a second messenger. A rise in [\( \text{Ca}^{2+} \)] mediated by cell surface signalling molecules can be rapidly detected, decoded by the cell and translated into an appropriate cellular response. In many cells, receptor stimulation...
results in rapid release of Ca\(^{2+}\) from the endoplasmic reticulum (ER), the main intracellular Ca\(^{2+}\) storage organelle [7]. The resulting increase in [Ca\(^{2+}\)]\(_i\) is short lived, but sufficient to activate many downstream Ca\(^{2+}\)-sensitive enzymes and transcription factors. For some responses, however, a sustained increase in [Ca\(^{2+}\)]\(_i\) is required, which is achieved by the activation of a second Ca\(^{2+}\) influx pathway through plasma membrane Ca\(^{2+}\) channels [8]. First conceptualized over 20 years ago as a method to maintain ER Ca\(^{2+}\) store content at optimum levels, and named capacitative calcium entry [9], later store-operated Ca\(^{2+}\) entry (SOCE), this pathway is activated exclusively following emptying of ER Ca\(^{2+}\) stores [10]. The molecular identity of the channel proteins themselves and the mechanism by which ER Ca\(^{2+}\) store content was relayed to SOCs remained unclear until seminal studies in 2005 and 2006 converged upon members of the stromal interaction molecule (STIM) family and ORAI proteins as comprising the critical ER Ca\(^{2+}\) sensor and channel component, respectively [11–14].

The STIM family is an evolutionarily conserved gene family which in mammals comprises two closely related family members, STIM1 and STIM2 [15, 16]. Mouse models of STIM deficiency and the phenotypes of patients bearing mutations in STIM1 that serve to abrogate SOCE [17], together with analyses of the effects of modulating STIM proteins on cellular physiology in vitro, have given great insight into the role of these proteins in the development and functioning of complex organisms. Several recent excellent reviews have described in detail the mechanisms of STIM–ORAI coupling [18] and experimental studies of STIM protein function in a number of organ systems, in particular the immune system, and in skeletal and smooth muscle [17, 19, 20]. Therefore, in this review, we focus on the cell and molecular mechanisms by which STIM proteins control developmental processes and cell function, with a particular focus on studies that shed light on the functional differences between STIM1 and STIM2 in cell physiology.

Part I: STIM proteins

STIM1 and SOCE

The function of STIM1 in SOCE is 2-fold — (i) to sense and respond to [Ca\(^{2+}\)]\(_{i}\) and (ii) to activate the plasma membrane localized ORAI proteins. The protein architecture of the STIM1 polypeptide reflects these functional attributes, with the N-terminal domain specialized for Ca\(^{2+}\) sensing, and the C-terminus for directing the interactions that activate ORAI proteins (Fig. 1). The ability of STIM1 to control the level of Ca\(^{2+}\) entry through SOCs has been described in many cell types (for review see [21]). In store replete cells, STIM1 likely exists as a dimer, stabilized via homodimeric C-terminal coiled-coil interactions [22–24] and appears diffusely arranged within the ER tubule system [11]. Strikingly, once [Ca\(^{2+}\)]\(_{i}\) drops below about 300 μM, STIM1 dimers aggregate laterally in the peripheral ER membrane in multimeric foci, or puncta [25, 26], in areas of the ER that are underneath, yet within 10–25 nM of, the plasma membrane [11, 23, 27, 28]. The STIM1 EF hand domain possesses weak Ca\(^{2+}\) binding affinity in vitro, such that the drop in [Ca\(^{2+}\)]\(_{i}\) promotes Ca\(^{2+}\) dissociation and results in oligomerization and the formation of puncta [29, 30]. Indeed mutations in the EF hand domain that abrogate Ca\(^{2+}\) binding ability render STIM1 constitutively active, behaving as if ER stores were constantly depleted [11, 31]. An inherent ORAI1-interaction domain within the distal coiled-coil of STIM1 is exposed within the oligomers, which is both necessary and sufficient to interact with ORAI1 proteins in the plasma membrane and activate SOCE [32–35]. These interactions induce clustering of ORAI1 tetramers into large aggregates [32, 36], which are thought to concentrate local Ca\(^{2+}\) influx at the particular regions of the cell where STIM1 and ORAI1 cluster [21].
Setting the level of SOCE: expression levels of STIM1 and ORAI1

The expression levels of both STIM1 and ORAI1 appear to be critical regulators of the amplitude of SOCE in cells. STIM1 is widely expressed in embryonic and adult cells and tissues [37] and in virtually all cells tested thus far, abrogation of STIM1 expression abolishes SOCE in a dose-dependent manner (e.g. [11, 12, 38–41]), suggesting that a minimum number of STIM1 molecules must be contained within puncta to activate ORAI1 tetramers. Conversely, co-overexpression of STIM1 and ORAI1 induces ‘monster’ Ca\(^{2+}\) entry, yet is still store operated, [42], indicating that these two components together comprise the functional SOCE unit. Although the exact stoichiometry required for optimal activation of ORAI1 by STIM1 is not yet known, it is clear that a tetrameric arrangement of ORAI1 polypeptides comprises a functional channel unit [24, 36, 43]. Whether this tetrameric arrangement is present in resting cells, [36, 43], or requires STIM1-induced assembly from ORAI dimers [24] is still unresolved, and may well be tissue and species specific. When overexpressed alone, STIM1 increases the magnitude of SOCE markedly in some cell types [41, 44], but has little or no effect in others [12, 31]. In contrast, overexpression of ORAI1 alone strongly reduces SOCE, which can be rescued by increasing STIM1 protein levels [42, 45]. This suggests that, at least in some cells, the amount of STIM1 available to couple to ORAI1 channels sets the magnitude of SOCE, whereas in others, it is the number of ORAI1 channels that sets the maximum level. However, there is clearly an ‘optimal’ ORAI : STIM ratio which, when perturbed, has dramatic consequences for the magnitude of SOCE.

Because the expression level of STIM1 directly corresponds to the level of SOCE (up to a maximum set by the expression level of ORAI1 proteins), this potentially provides a cell with an autonomous mechanism for controlling Ca\(^{2+}\) entry through SOCs that is independent of extracellular signals. We and others have shown that the expression levels of STIM1 and ORAI1 are up-regulated during differentiation. STIM1 protein levels increase early in the differentiation of the mouse 3T3-L1 adipocyte cell line [44], and likely result in increased levels of SOCE in differentiated adipocytes, similar to that seen in differentiated brown adipocytes from mouse and squirrel [46]. SOCE also increases during myoblast differentiation concomitant with an increase in the expression of STIM1 and ORAI1 [47, 48]. However, the transcriptional and translational regulation of STIM1 has received little attention thus far.

STIM1 and the regulation of other proteins at the plasma membrane

A proportion (possibly 15–30%, [49]) of endogenous STIM1 is able to bypass the ER retention machinery and traffic to the plasma membrane, where its role is less well characterized. This cell surface expression is not due to insertion of STIM1 into the plasma membrane following store depletion [28, 41]. In cells expressing mutated STIM1 constructs bearing bulky N-terminal insertions such as EGFP, STIM1 is unable to reach the plasma membrane, yet is still able to promote Ca\(^{2+}\) influx via ORAI1 [23, 28, 50, 51]. Thus plasma membrane STIM1 is thought to have a role distinct from mediating store-operated Ca\(^{2+}\) influx via ORAI1. Shuttleworth and colleagues demonstrated that plasma membrane STIM1 was essential for Ca\(^{2+}\) entry through arachidonic acid regulated (ARC) channels that are distinct from SOCs and are not affected by ER store depletion [49]. Antibodies directed to the N-terminus of STIM1 abolish the current through ARC channels and overexpression of STIM1 increases the current, in a manner that is independent of Ca\(^{2+}\) binding to the STIM1 EF hand domain [49]. Inhibiting STIM1 glycosylation renders STIM1 incapable of both reaching the plasma membrane and of increasing current through ARC channels [22, 49].

STIM1 has also been implicated in activating Ca\(^{2+}\)-permeable TRPC1–6 and of regulating their heteromultimerization directly via its C-terminus (for review see [21]). Moreover, a recent report describes the ability of STIM1 to positively regulate adenylate cyclase isoforms independently of the well-described route of Ca\(^{2+}\)-mediated activation [52]. ER store-depletion mediated interactions between STIM1 and adenylyl cyclase isoforms in several cell types were shown to increase CAMP generation, leading to protein kinase A activation and an increase in CAMP-response-element binding protein (CREB)-mediated transcription activity.

The orientation of STIM1 at the plasma membrane with the N-terminal domain projecting outside of the cell [53] presents the potential for mediating cell–cell interactions. Indeed, early studies demonstrated that a recombinant protein comprising the entire STIM1 N-terminal domain could bind to the surface of murine pre B-cells and stimulate their proliferation [54]. We have shown that the human STIM1 N-terminal domain additionally binds to a number of different cell types (L. Johnstone, M. Dziadek, unpublished results). These findings suggest that STIM1 situated on the plasma membrane may have the potential to mediate cell–cell communication or cell adhesion, and this possibility warrants further exploration.

STIM2 and SOCE

The second STIM family member, STIM2, is very similar to STIM1 in domain architecture but the mature protein is longer by 69 amino acids than STIM1, 12aa of which are located N-terminal to the EF hand domain, and the remainder C-terminal to the distal coiled coil (Fig. 1). The proline-rich region of STIM2 contains multiple histidine residues, in addition to the serine residues mainly seen in STIM1 [37]. Unlike STIM1, biotinylation and FACs analysis suggest that STIM2 does not traffic to the plasma membrane, even when overexpressed at a similar level as STIM1 (M. Dziadek, unpublished results and [41]). Although STIM2 is widely expressed in cell lines and tissues it is usually present at lower
steady state levels than STIM1 [15]. STIM2 transcription is also dynamically regulated according to differentiation status, being up-regulated upon differentiation of naïve T cells into Th1 or Th2 T lymphocytes [50] and down-regulated upon reaching confluence in adipocytes and C2C12 myoblasts (L. Johnstone, unpublished results). The expression pattern of STIM2 thus often opposes that of STIM1, whose expression levels do not change upon T-lymphocyte differentiation [50] or increase during the differentiation of adipocytes and myoblasts [44, 47, 48]. This suggests that cells may utilize either STIM2 or STIM1 Ca^{2+} sensing abilities depending on the cellular context.

The role of STIM2 in SOCE is somewhat complex, and still controversial. Although STIM2 was identified together with STIM1 in the initial dsRNAi studies as a positive regulator of SOCE in HeLa cells [11], in other cell types a reduction in STIM2 expression has no effect on SOCE [12, 55]. Interestingly, in situations where a suboptimal ratio of STIM1:ORAI1 is present, such as when ORAI1 is overexpressed [56, 57] or in STIM1-deficient embryonic fibroblasts and T cells [50], overexpression of STIM2 can reconstitute SOCE. Nevertheless, both the amplitude of Ca^{2+} entry and the kinetics of ORAI1 activation are greatly reduced compared to reintroduction of STIM1. In fact a number of studies have shown that STIM2 inhibits endogenous SOCE and a calcium release-activated calcium current (ICRAC) when overexpressed alone in cells, suggesting that it can negatively regulate SOCE, unlike the positive regulation by STIM1 [25, 41, 56, 57]. Curiously, Brandman and colleagues found that STIM2 overexpression decreased endogenous SOCE when assayed 24 hrs after transfection, but if analysed at 8 hrs after transfection, SOCE was in fact increased [25]. Biophysical studies have shown that STIM2 has a slightly lower affinity for Ca^{2+} than does STIM1, and STIM2 EF-SAM domain monomers are more stable than the equivalent STIM1 monomers in the absence of Ca^{2+}, with a lower tendency for aggregation, particularly at low protein concentrations [29, 58]. These differences in Ca^{2+} sensing and oligomerization properties would account for the kinetic and functional differences between STIM1 and STIM2. Cells overexpressing STIM2 have increased resting intracellular Ca^{2+} levels, which can be blocked by SOC channel inhibitors [56], consistent with STIM2 mediating the persistent signalling of SOC channels at a low level. Both constitutive Ca^{2+} entry and SOCE mediated by STIM2 appear to occur through ORAI channels because STIM2 interacts with ORAI1, 2 and 3 [56].

Together, these studies suggest a model in which STIM2 has a role in basal Ca^{2+} regulation, allowing Ca^{2+} to enter cells in an apparently constitutive fashion. However, when the amount of STIM2 is increased in a background of ORAI1 excess or STIM1 depletion, STIM2-mediated SOCE becomes apparent, but with different kinetics and amplitude of activation to STIM1. A recent study suggests that amino acids residing between the signal peptide and EF hand domain of STIM2 are important for increased stability of the N-terminal domain and acting as a brake to limit its activation [57]. Overall, it appears that STIM2, like ORAI1, is exquisitely sensitive to the ratio of STIM1 : STIM2 : ORAI1 and the overall effects of increased STIM2 expression on SOCE will depend on the endogenous levels of the other proteins.

Part II: STIM proteins and the regulation of cell physiology and pathology

The importance of SOCE in cell physiology, and its likely contribution to the pathology of several diseases were recognized two decades ago (for review see [59]). However, not until the identification of the molecular components of this pathway has a precise and in depth study of the actual role of SOCE been possible. In human beings, a nonsense mutation that results in STIM1 deletion has been linked to immunodeficiency, autoimmune haemolytic anaemia, thrombocytopenia, muscular hypotonia and abnormal enamel dentition [60], a clinical phenotype that is similar to that caused by mutations in ORAI1 [14]. In mice, STIM1 deficiency is perinatally lethal, with the majority of STIM1-deficient mice created by homologous recombination displaying severe growth retardation and dying shortly before, or just after birth [50, 61] and those created by gene-trap insertion dying around 4–6 weeks following birth [62]. STIM2-deficient mice display a less severe growth retardation phenotype, and begin to die spontaneously from around 4–8 weeks after birth depending on the genetic background, with few survivors beyond 30 weeks [50, 63]. Human and mouse cells isolated from STIM-deficient individuals as well as genetically manipulated cell lines have provided important information regarding the mechanisms by which STIM proteins might regulate cell physiology and result in pathologies exhibited by STIM deficiency.

Immune system

The importance of SOCE in immune function is underpinned by the identification of at least four families of patients with severe combined immunodeficiency (SCID) associated with a severe reduction in SOCE in T lymphocytes, B lymphocytes and fibroblasts [60, 64–66]. In the two families that have been analysed, immunodeficiency is caused by either homozygous mutations in the pore forming region of ORAI1 or a homozygous nonsense mutation in STIM1, both of which result in abrogation of SOCE and CRAC currents [14, 60]. The STIM1 nonsense mutation results in a frameshift and premature termination, which would result in a truncated STIM1 protein containing only the N-terminal EF-hand domain and a small segment of the adjacent SAM domain [60]. However, the predicted truncated protein was not detected in patient fibroblast cells, and strongly reduced transcript levels of STIM1 suggested that the mutation likely results in nonsense-mediated mRNA decay and a STIM1 null phenotype.

The SCID phenotype in STIM1-deficient patients is characterized by life-threatening viral, bacterial and fungal infections from an early age. Total lymphocyte counts were within the normal range in these patients, and the percentages of CD4^{+} and CD8^{+} T cells and of CD19^{+} B cell subsets were also normal in all three patients [60]. In contrast, a lack of antigen-specific antibody
responses and impairment in T-cell activation is evident by a marked reduction in proliferation of CD4+ T cells in vitro. Together these data indicate that the immunodeficiency in STIM1-deficient patients is likely not due to aberrant lymphocyte development, but rather is caused mainly by impaired lymphocyte function.

Analysis of immune cells in STIM1-deficient mice bearing a T-cell specific deletion in CD4+ T cells demonstrated normal lymphocyte development despite undetectable SOCE and CRAC channel currents in naive and stimulated T cells [50]. In common with human STIM1-deficient patients, T-cell activation was severely compromised in STIM1-deficient CD4+ T cells, as assessed by a failure to produce interleukin (IL)-2, interferon (IFN)-γ and IL-4 in response to T-cell receptor stimulation. The expression of these critical immunomodulatory cytokines are under complex regulation, and their promoters are rich in binding sites for Ca2+-sensitive transcription factors such as NF-κB, NFAT and CREB which act in a combinatorial fashion to ensure optimal transcriptional output [67]. In SCID patients bearing mutations in ORAI1, nuclear extracts from T cells contain virtually no NFAT binding activity, implicating this transcription factor as a key mediator of the disease [68]. In resting T cells, NFAT normally resides in the cytoplasm in an inactive phosphorylated form, and T-cell receptor activation induces SOCE and activation of the serine/threonine phosphatase calcineurin [69]. Calcineurin dephosphorylates NFAT and mediates its translocation into the nucleus where it exerts transcriptional regulation on the IL-2 promoter [68]. Calcineurin-mediated nuclear import is balanced by nuclear export, involving nuclear kinases such as GSK3β, casein kinase I and DYRK1A that rephosphorylate NFAT [70]. In stimulated STIM1-deficient CD4+ T cells, nuclear translocation of NFAT is severely reduced when compared to control T cells, resulting in diminished cytokine production [50].

In contrast to STIM1-deficient CD4+ T cells, SOCE and lCRAC in resting STIM2-deficient CD4+ T cells is virtually normal, and only slightly affected in activated CD4+ T cells, where STIM2 expression is normally up-regulated [50]. Despite this, T-cell activation-induced production of IL-2 and IFN-γ is reduced, correlating with reduced NFAT nuclear residency. However, in stark contrast to STIM1-deficient CD4+ T cells, the ability to drive NFAT to the nucleus is hardly affected in STIM2-deficient CD4+ T cells, but these cells are completely unable to retain NFAT nuclear localization. Together, these results clearly suggest different, but synergistic, roles for STIM1 and STIM2 in the regulation of NFAT transcriptional activity: STIM1 as a driver of NFAT nuclear localization, and STIM2 as an inhibitor of NFAT nuclear export. Both activities appear necessary for optimal NFAT transcriptional activity in CD4+ T cells.

In support of the proposition that STIM1 and STIM2 synergeize in regulating lymphocyte function, mice double deficient for STIM1 and STIM2 in CD4+ T cells develop a lymphoproliferative disorder characterized by splenomegaly, lymphadenopathy, dermatitis and blepharitis, or inflammation of the eyelid margins, and infiltration of leucocytes into many organs [50]. Whereas all T- and B-lymphocyte subsets are normal in single STIM-deficient mice, double STIM1/STIM2-deficiency results in a 90% reduction in CD4+ CD25+ FoxP3 regulatory T cells (Tregs). The resulting lymphoproliferative disorder is ameliorated by supplementation of STIM1-/STIM2-deficient mice with wild-type Tregs [50].

Tregs function to dampen immune responses to self-antigens. Naturally occurring Tregs are thought to develop from CD4+ CD8+ T cells, mediated by FoxP3, a member of the forkhead family of transcription factors [71]. Notably, binding sites for NFAT2 reside in the FOXP3 promoter and can positively regulate FOXP3 expression in vitro [72]. Furthermore, a complex consisting of NFAT and FoxP3 proteins is recognized to bind to the IL-2 promoter [73]. It appears to be the combined STIM1-/STIM2-mediated reduction in activity of NFAT in developing double-deficient Treg cells that result in the vastly reduced number of Treg cells in double-deficient mice [17, 50, 74]. However, this is unlikely to be the only mechanism involved, because complete abrogation of NFAT activity in T cells of STIM1-deficient mice does not affect Treg numbers [50] and mice that are double deficient in NFAT1 and NFAT4, or NFAT1 and NFAT2 also have normal Treg numbers [75]. Moreover, several studies indicate that it is in fact a low level of NFAT activity that is required for Treg function because CsA treatment in patients can increase Treg-like function in some instances, and FoxP3, whilst interacting with NFAT at the IL-2 promoter, acts as a repressor of NFAT-mediated IL-2 transcription [76]. Thus it seems plausible that combined STIM1/STIM2 deficiency impinges not only on the NFAT signalling pathway, but also on additional signalling pathways critical for mediating the development or homeostasis of Treg cells.

The function, but not the development, of other immune cell subsets is also impaired in STIM1-deficient mice, in addition to T lymphocytes, including mast cell degranulation, macrophage-mediated phagocytosis and platelet activation (for review see [77]). The other clinical symptoms of patients bearing mutations in STIM1 and ORAI1, however, suggest that these proteins are important for the development of other tissues, most notably skeletal and smooth muscle, and neural tissue [17].

**Skeletal muscle**

Several studies now point to a critical role for STIM1 in controlling the Ca2+-mediated events leading to correct muscle development and function. Patients with STIM1 deficiency suffer from congenital non-progressive muscle weakness [60] and STIM1-deficient mice bearing a gene trap in the C-terminus of STIM1 have reduced skeletal muscle cross sectional area concomitant with a marked increase in inter-fibre connective tissue [47]. A number of the fibres have centrally, rather than peripherally, placed nuclei, and a great increase in large swollen mitochondria between myofibres and in the subsarcolemma. These features are considered hallmarks of centronuclear myopathy, a subtype of congenital myopathies characterized by weak muscles, abundant mitochondria and an increase in fat and connective tissue [78]. Mutations in several genes encoding proteins involved in cytoskeletal remodelling and endocytosis, including myotubulin, dynamin and amphiphysin, have been identified as the cause of these centronuclear
myopathies [78]. Individual myotubes prepared from STIM1-deficient mice display essentially no SOC entry, and the lack of myosin heavy chain expression indicates defective muscle development [47]. Indeed, siRNA-mediated knockdown of STIM1 prior to the onset of differentiation in human myoblasts significantly reduces differentiation capacity in a dose-dependent manner [48]. Effects on muscle differentiation require an early reduction of SOCE during development, because inhibition of SOCE at time-points after the onset of differentiation fails to influence myotube formation. Thus the amplitude of SOCE critically determines a differentiation signal that drives the early differentiation of myoblasts, potentially at a stage prior to hyperpolarization of the plasma membrane to –70 mV [48]. Hyperpolarization occurs by dephosphorylation-mediated up-regulation of Kir2.1 K+ channel activity, which is maximal 6 hrs after induction of differentiation [79]. Of note, inhibition of myoblast fusion decreases Kir2.1 K+ channel-mediated hyperpolarization, and STIM1-deficient myoblast cells fail to cluster their nuclei, a hallmark of human myoblast fusion. Moreover, STIM1 overexpression accelerates differentiation in human myoblasts [48] and in C2C12 mouse myoblasts (Fig. 2; S. Graham, L. Johnstone, unpublished results), likely by increasing fusion events. A role for the Ca2+ activated calcineurin/NFAT pathway in the regulation of myoblast fusion has been demonstrated by the diminished muscle mass in NFAT2-deficient mice [80] and the abrogation of myoblast fusion in vitro by inhibitors of calmodulin activity [81]. Moreover, increasing intracellular Ca2+ induces myoblast fusion and buffering intracellular Ca2+ levels inhibits myoblast fusion [82]. Together these observations suggest that STIM1-mediated Ca2+ dependent signalling processes are likely to regulate processes necessary for myoblast fusion.

Although STIM1 expression is necessary at the early stages of muscle development, up-regulation of STIM1 expression at subsequent stages of myoblast differentiation in vitro and in vivo indicate that STIM1 has additional roles in mature muscle function [47, 48, 83]. It has long been appreciated that skeletal muscle contraction and relaxation are controlled by the precise release and re-uptake of Ca2+ from the specialized sarcoplasmic reticulum (SR) Ca2+ store within skeletal muscle fibres. These highly specialized cells are designed with specific tubular invaginations of the plasma membrane, T tubules, which penetrate deep into the cytoplasm. Terminal portions of the SR flank each T tubule, and are physically coupled to it by interactions between L-type calcium channels located on the T tubule membrane and Ryanodine receptors (Ryrs) on the opposing SR membrane.

Muscle contraction is initiated by action potentials that depolarize the T tubules causing a conformational change in L-type calcium channels and a subsequent activation of RyRs, which release Ca2+ from the terminal SR. The Ca2+ signal is rapidly propagated via RyRs through the adjacent longitudinal SR to activate the contractile machinery. Recently, elegant experiments using mechanically skinned muscle fibres, where Ca2+ indicator dyes are ‘trapped’ in the T tubule system, have indicated that a fraction of Ca2+ released via RyRs is extruded into the T tubule, and is quickly followed by Ca2+ influx via SOCs back into the muscle fibre [84]. These data raised the possibility that reuptake of Ca2+ from the T tubule back into muscle cells by SOCs could comprise an integral part of skeletal muscle contraction–relaxation, by providing the means to sustain the SR load of Ca2+ required for persistent contractions.

In normal muscle fibres, STIM1 is localized to regions of the terminal SR that significantly overlap with RyR expression [47], and is either pre-localized to areas adjacent to ORAI1 or perhaps pre-coupled to the channel, and thus able to rapidly respond to the Ca2+-depletion induced conformational change that activates the channel. A recent study suggests that the C-terminus of STIM1 and STIM2 have particular abilities to bind to plasma membrane phosphoinositides [85]. The synthesis of PtdInsP2 in vertebrate muscle is limited mainly to the T-tubule membrane [86, 87], and PtdInsP2 synthesis increases during rat myoblast differentiation [88]. This localized synthesis of PtdInsP may serve to target the STIM1 C-terminus to the terminal SR membrane where it would be in position to rapidly activate ORAI1.
Myofibrils isolated from homozygous STIM1-deficient mice display essentially no SOCE and, when subjected to repeated depolarizations, show a rapid decrease in Ca\(^{2+}\) transient amplitude which is a hallmark of muscle fatigue [47]. Thus, STIM1 appears to be required in mature muscle for sustained KCl-induced Ca\(^{2+}\) transients. This is supported by other studies which, in disrupting the triad structure, have at the same time disrupted SOCE, and have shown an increased propensity to muscle fatigue [89]. In addition, increased muscle fatigue in ageing skeletal muscle is associated with decreased SOCE [83]. Muscle fibres from heterozygous STIM1-deficient mice also showed an increased propensity to fatigue earlier than wild-type mice, suggesting that reduced STIM1 expression can disrupt SOCE in mature muscle fibres. The role of STIM2 in muscle development and function has not been explored.

**Smooth muscle**

Store-operated channels with similar characteristics to that of kICRAC have been described in various smooth muscle preparations, including vascular smooth muscle cells (VSMC) isolated from aorta [90, 91], portal vein [92] and pulmonary arteries [93], as well as smooth muscle cell (SMC) lines [94]. The detailed biophysical characteristics of SOCs in SMCs have recently been reviewed [95]. Much less is known about the regulation of these channels during smooth muscle differentiation. Unlike skeletal or cardiac muscle, whose cells are terminally differentiated, SMCs retain remarkable plasticity and can revert from a differentiated, contractile phenotype back to a de-differentiated, proliferative phenotype in response to local environmental cues.

STIM1 and ORAI1 expression has been demonstrated in VSMC from several species and reduction of STIM1 or ORAI1 expression shown to impair SOCE and whole-cell SOC currents [91, 96–99]. However, despite robust expression of STIM2, ORAI2 and 3 in VSMC cultures, knockdown of these genes appears to have little effect on VSMC SOCE [55, 91]. In proliferating rat VSMCs, SOCE mediated by STIM1 and ORAI1 has an unusual phenotype, being inhibited by low concentrations of 2-ABP, whereas in all other cells tested, low concentrations of 2-ABP potentiate SOCE. These observations suggest that the molecular composition of SOCs in VSMCs may differ to that of other cells, or, that unique, VSMC-specific post-translational modifications of ORAI1 or STIM1 contribute to the characteristics of SOCE in this cell type [91].

Although the role of STIM1 in VSMC differentiation has not been examined directly, several studies have noted that SOCE is increased in proliferating SMCs when compared with quiescent, more differentiated SMCs [91, 93, 96, 100], suggesting that down-regulation of SOCE is associated with the acquisition of a differentiated phenotype. Moreover, in a model of VSMC de-differentiation, STIM1 expression levels are rapidly up-regulated, concomitant with an increase in SOCE, consistent with a role for STIM1 in either the acquisition or maintenance of a de-differentiated, and therefore proliferative and migratory, VSMC phenotype. Indeed, depletion of STIM1 reduces the proliferative and migratory capabilities of de-differentiated VSMCs [91, 101–104]. A failure of these cells to progress to S phase of the cell cycle is associated with up-regulation of p21 and reduction in Rb phosphorylation [101] and a reduction in CREB phosphorylation [102] and NFAT transcriptional activity [103], suggesting that multiple signalling pathways may be regulated by STIM1 in VSMCs.

De-differentiation of VSMCs plays a major pathophysiological role in the development of atherosclerosis, restenosis following stenting or bypass surgery, and hypertension. In a model of neointima formation following balloon angioplasty, characterized by excessive proliferation of SMCs in the intima layer of the blood vessel, increased SMC proliferation is associated with increased STIM1 expression. Adenovirus-mediated delivery of shRNA directed to STIM1 in vivo following balloon injury significantly reduced neointima formation, suggesting that STIM1 could represent a viable therapeutic target to reduce restenosis following injury [101, 103], possibly utilizing surgical implantation of stents impregnated with drugs [105]. Exercise can prevent VSMC proliferation and the associated up-regulation of STIM1 and TRPC1 in SMCs following stenting in swine [106]. SOCE is elevated in freshly isolated preglomerular VSMC from hypertensive rats compared to normal rats [107], and STIM1 and ORAI1 expression is increased in vessels from spontaneously hypertensive rats [108], which have augmented vessel tone and are more sensitive to constrictor stimuli, key markers of arterial hypertension. This augmented phenotype could be abolished by intracellular delivery of antibodies targeting either STIM1 or ORAI1 [108]. These studies demonstrate the important role of STIM1 in mediating patho-physiological events associated with atherosclerosis and hypertension, and the potential for therapeutic intervention through modulation of STIM1 function or expression.

**Nervous system**

Early immunolocalization studies demonstrated STIM1 expression in the foetal and adult central and peripheral nervous system, particularly in Purkinje cells of the cerebellum [37]. Reporter gene activity is also preferentially high in this region in STIM1 gene-trap mice [47], and high levels of STIM1 are detected in protein extracts from the cerebellum of mouse brains [37, 109]. However, a more recent study has demonstrated that mouse brain is the only tissue analysed where STIM2 is expressed much more highly than STIM1 [63]. Comparison of SOCE in cortical neurons isolated from STIM1- and STIM2-deficient mice showed no defects in STIM1-deficient neurons, whereas STIM2-deficient neurons had lower basal Ca\(^{2+}\) levels and abrogated SOCE [63]. These observations suggest a crucial role for STIM2 rather than STIM1 in regulation of SOCE in the nervous system. Ischemia-induced increases in intracellular Ca\(^{2+}\) play a critical role in triggering neuronal cell death and in STIM2-deficient neurons the increase in Ca\(^{2+}\) after *in vitro* ischemia is slower and neuronal recovery faster than in wild-type or STIM1-deficient neurons [63]. This neuroprotective effect of STIM2 deficiency was also observed *in vivo*, with lower infarct volumes found in STIM2-deficient mice after an ischemic
episode. Inhibitors of STIM2 function may thus have therapeutic value as neuroprotective agents to treat ischemic injury and potentially other neurodegenerative disorders involving Ca²⁺ homeostasis [63].

No gross neurological deficits or structural abnormalities were visible in STIM2-deficient mice, suggesting that reduced SOCE does not influence brain development in a major way [63]. However, a pronounced cognitive impairment became apparent when mice were tested for hippocampus-dependent spatial memory using the Morris water maze test [63]. Several Ca²⁺-sensitive signalling pathways operate to modulate hippocampus-dependent regulation of spatial learning and memory. CAMKII, activated at high levels of Ca²⁺/calmodulin activity, is required for optimal performance in spatial memory tests [110] and sustained CREB activation is additionally required for hippocampus-dependent spatial memory formation [111–113]. The effects of STIM2 deficiency on synaptic transmission and plasticity have not yet been explored.

Adipose tissue

The role of Ca²⁺ signalling in regulation of adipogenesis was indicated from observations of the obesogenic effects of the calcineurin inhibitor CsA in patients requiring immune suppression following transplant surgery, suggesting that the Cn/NFAT pathway negatively regulates adipocyte differentiation [114]. The inhibitory effects of PGE₂, on adipogenesis were later found to be almost exclusively calcineurin-dependent [115] and adipocyte differentiation was enhanced by inhibiting calcineurin activity in mouse 3T3-L1 pre-adipocytes [114]. We demonstrated that STIM1 expression increases progressively during differentiation of 3T3-L1 cells into mature, triglyceride-laden adipocytes, suggesting that either STIM1 contributes to the development of mature adipocytes from pre-adipocytes, or that the functionality of mature adipocytes requires STIM1 [44]. Depletion of STIM1 in 3T3-L1 pre-adipocytes markedly enhances differentiation, associated with increased levels of CCAAT/enhancer-binding protein alpha (C/EBPα), one of the ‘master transcriptional regulators’ of adipocyte differentiation. Conversely, overexpression of STIM1, associated with an increased magnitude of SOCE, decreases triglyceride accumulation in a dose-dependent manner. C/EBPα expression is also abolished by STIM1 overexpression, suggesting that STIM1 negatively regulates adipocyte differentiation via a mechanism that is critical for the maintenance of C/EBPα expression.

The role of NFATs in regulation of adipogenesis in vivo was revealed by the phenotype of compound NFAT2-/NFAT4-deficient mice, which exhibit defects in fat accumulation, remain lean and are resistant to diet-induced obesity [116]. An increased resting [Ca²⁺]i is characteristic of adipocytes from overweight human beings and rodents, which develops in as little as 3 weeks following initiation of a high fat diet in rats [117–120]. Maneuvres which reduce these high [Ca²⁺]i can reverse weight gain, and improve insulin responses [121, 122], suggesting therapeutic manipulation of SOCE as a potential strategy in treatment of obesity.

Cancer

The demonstrated role of STIM proteins in the physiology of the variety of cell types summarized above indicates that quantitative changes in STIM protein expression can influence proliferation, differentiation, and cell death in a cell specific manner, processes that are intimately involved in tumorigenesis. STIM1 maps to a region of 11p15.5 implicated in several childhood and adult tumours, including Wilms’ tumour, rhabdomyosarcoma, adrenal carcinoma, hepatoblastoma, bladder, breast, lung, ovarian and testicular cancers [123, 124], and was originally proposed as a candidate tumour suppressor in this region [125, 126]. Expression of STIM1 is barely detectable in rhabdomyosarcoma tumours [126]. Tumour suppressor function is supported in studies showing that depletion of STIM1 expression transforms the weakly metastatic B16F0 mouse melanoma cell line into a more aggressive form, accelerating cell mobility in vitro and increasing metastasis in a mouse model of tumorigenesis [127]. STIM2 maps to chromosome 4p15.1, a region implicated in invasive carcinomas of the head and neck, breast and lung [128–130].

An oncogenic rather than a tumour suppressive function has been demonstrated for STIM1 and STIM2 in glioblastoma multiforme, where both proteins have increased expression and/or increased copy number [131, 132]. The increased expression of both STIM1 and STIM2 in this tumour may correlate with the increased basal [Ca²⁺]i and SOCE measured in primary cultures from glioblastoma tumours, and in glioblastoma cell lines compared with normal human astrocytes [133], and potentially contribute to the highly malignant behaviour of this tumour type [131].

An oncogenic role for STIM proteins is also implicated in other cancers. Overexpression of STIM1 increases the migration of MCF-10A cells, a non-tumorigenic mammary gland cell line which expresses moderate endogenous levels of STIM1 [134]. Conversely, siRNA-mediated depletion of STIM1 in the tumorigenic breast cancer cell line MDA-MB-231, which expresses higher levels of STIM1, reduces migratory capacity in vitro and dramatically decreases their ability to metastasize in vivo [134]. Our own studies have demonstrated that overexpression of either STIM1 or STIM2 in rat PC12 pheochromocytoma cells does not affect the size of solid tumours formed after subcutaneous injection into nude mice, but causes significant invasion of cells into the body wall skeletal musculature, which was not evident after injection of parental PC12 cells (Fig. 3; M. Dziadek, unpublished results).

MDA-MB-231 cells in which STIM1 levels were depleted had decreased focal adhesion turnover, resulting in larger focal adhesions, and consequently stronger adhesion, implicating STIM1-mediated SOCE in regulation of components of the focal adhesion machinery. Overexpression of focal adhesion kinase, the critical regulator of focal adhesion turnover, is associated with cancer grade in some, but not all, cancers, suggesting that focal adhesion kinase may play alternative roles in different tumours and/or in different stages of tumour progression [135]. Because STIM1 and STIM2 likely regulate a diverse number of downstream signalling
cascades, the pathways and partners involved in STIM1- and STIM2-mediated cancer progression are likely to be tumour cell type dependent. Further studies on the role of STIM proteins in different cancers are now required to determine in which cases therapeutic agents designed to either increase or decrease the activity of STIM1 and STIM2, and thus SOCE, would be useful for cancer treatment.

Part III: invertebrate animal models of STIM deficiency

Unlike vertebrate species which have two STIM genes, STIM1 and STIM2, invertebrate organisms such as Caenorhabditis elegans and Drosophila melanogaster have a single STIM gene [15, 16], and also have a single ORAI gene [136]. The feasibility of forward and reverse genetics in these invertebrate organisms provides a powerful means to unravel the signalling pathways that may intersect both upstream and downstream of STIM-mediated SOCE and aid in the analysis of pathologies associated with aberrant SOCE.

**D. melanogaster**

D-Stim codes for a protein that is significantly shorter than mammalian STIM1 and STIM2 but retains the important functional domains in the extracellular region (Fig. 1). The cytoplasmic region of D-Stim shares the two conserved coiled-coil domains situated within the larger ERM domain, but is then truncated such that the proline- and lysine-rich regions and the polybasic tail present in mammalian STIM proteins are absent [37]. The mechanisms by which D-Stim activates D-Orai appear highly conserved with vertebrates [24]. A recent study has shown that D-Stim, like STIM2 in embryonic neurons [63], appears to regulate both [Ca$^{2+}$]$_{ER}$ and [Ca$^{2+}$]$_{i}$ [137].

D-Stim is widely expressed in embryonic and larval tissues, including imaginal discs [138], dsRNA-mediated knockdown of D-Stim in early embryogenesis results in late larval lethality, despite apparently normal early patterning of the embryo, similar to the effects of STIM1- and STIM2- deficiencies in mice [50]. Tissue-specific knockdown in imaginal discs, however, revealed that D-Stim has a specific role in cell fate decisions and tissue patterning. D-Stim deficiency in wing discs resulted in significant vein thickening, and in mesothoracic discs resulted in loss of mechanosensory bristles on the notum. The thick vein phenotype was enhanced when D-Stim deficiency was combined with Notch deficiency, and rescued by co-expression of an activated Notch allele. Notch signalling normally acts to prevent wing cells adopting a vein cell fate [139] and also plays a role in the specification of mechanosensory bristles on the notum, which arise from the asymmetric division of sensory organ precursor cells. In the notum, Notch-deficiency results in aberrant division and specification of both daughter cells towards a neural fate and absence of mechanosensory bristles. In the Drosophila eye,

![Fig. 3 Haematoxylin and eosin stained sections of subcutaneous tumours derived from STIM1 or STIM2 overexpressing PC12 cells injected into nude mice. Control (vector) PC12 tumour cells (t) are aligned against connective tissue and skeletal muscle fibres (m) of the body wall, with only very limited infiltration of cells between muscle fibres seen in two of nine tumours. In STIM1 (n = 9) and STIM2 (n = 9) tumours, invasive tumour-derived cells have infiltrated between skeletal muscle fibres (m), with extensive infiltration evident in >50% of tumours of both types.](image-url)
D-Stim overexpression interferes with the development of the repeating units of the compound eye, and inhibits the formation of interommatidial bristles, processes also regulated by Notch signalling. These phenotypes suggest that D-Stim function intersects with regulators of Notch signalling during cell fate specification in the Drosophila embryo. In addition, overexpression of D-Stim in the wing disc causes wing margin defects associated with decreased expression of arm (β-catenin) and other wingless (Wg) signalling outputs, demonstrating inhibition of canonical Wg signalling. The effects of D-Stim overexpression in other cell types are consistent with activation of the non-canonical Wg pathway in which Ca^{2+} is utilized as a second messenger [140].

Evidence of interactions between D-Stim and Notch and Wg (Wnt) signalling pathways in Drosophila provide clues to potential molecular interactions involving mammalian STIM proteins. Ca^{2+}-dependent proteins interact with, and exert regulation over, the wider Notch signalling pathway, and vice versa, in mammals [141, 142]. In immune cells, Notch signalling can impose a regulatory phenotype on T cells [143]. Activating mutations in NOTCH1 are implicated in T-ALL/lymphoma, and are associated with increased activity of calcineurin/NFAT signalling [144]. It is interesting to note that glioblastoma multiforme is frequently associated with increased expression and activation of NOTCH1 and other members of this pathway [145, 146], and is also associated with increased expression of STIM1 and STIM2 [131, 132]. Further exploration of potential interactions between STIM-mediated Ca^{2+} signalling and Notch and Wnt signalling should be undertaken, given the known deregulation of these developmental pathways in many cancer types [147].

**C. elegans**

The single C. elegans STIM orthologue, C-Stim, encodes a protein of similar size and structure to D-Stim [148] (Fig. 1) that interacts with the single C. elegans Orai orthologue, C-Orai, to induce Ca^{2+} entry, and ORAC-like currents upon store depletion, recapitulating the effects of the mammalian homologues [149].

The effects of C-Stim depletion on the late larval stages were studied by delivering C-Stim specific dsRNAi via the gut, where it is absorbed and distributed to somatic tissues and the germline. C-Stim-deficient worms appear normal and have no defects in external morphology, movement or feeding behaviour, but are completely sterile due to defective sheath cells that surround the oocytes. These myoepithelial cells normally contract weakly to expel oocytes from the gonad into the spermatheca. C-Stim-depletion results in a reduced rate and force of sheath contractions causing failure of the gonad-spermatheca valve to open and trapping oocytes within the gonad. Because mutations in C elegans calcineurinB display similar defects [150], it is likely that C-Stim activates calcineurin, but this and other downstream effects of C-Stim function need further analysis.

Somewhat surprisingly, posterior body wall muscle contractions, known to be controlled by IP3-dependent Ca^{2+} oscillations in intestinal epithelial cells, are not affected by either C-Orai or C-Stim depletion [148]. This has been interpreted to indicate that IP3-mediated ER Ca^{2+} release does not necessary result in store-depletion mediated Ca^{2+} entry through SOCs under physiological conditions, and that Orai1-STIM1-mediated SOCE may be a ‘last resort’ failsafe mechanism to ensure Ca^{2+} stores are not totally depleted by pathophysiological insults [149]. Because STIM1 in particular appears to play an important role in SMCs in vertebrates, further analysis of contractile function of sheath cells in C. elegans may better define the function of STIM and ORAI proteins in these cells.

**Part IV: conclusions and future perspectives**

Identification of STIM and ORAI proteins as the key molecular regulators of SOCE has led to intensive research over the last 4 years that has elucidated the mechanisms that regulate Ca^{2+} influx into cells after ER Ca^{2+} store depletion and revealed important roles of SOCE in the development and function of a variety of cell types. An important consequence of this research has been the demonstration that mutations in STIM1 and ORAI1 are responsible for human immunodeficiency syndromes. Additional analyses of cell function in mouse and cell models of STIM deficiency and STIM overexpression have also implicated STIM proteins in myopathy, atherosclerosis, hypertension, ischemic stroke, cognitive impairment, obesity and cancer. These studies, together with analysis of developmental models such as D. melanogaster, indicate that STIM proteins are capable of interacting with a variety of signalling proteins and pathways. Understanding the mechanisms by which STIM proteins exert their physiological roles in the array of seemingly unrelated pathologies is the major challenge for future research. STIM proteins have already been proposed as potential targets for development of therapeutics against some of the disorders associated with deregulation of SOCE, and exciting developments are expected in this area over the coming years.

**Acknowledgements**

The research work in the authors’ laboratory was supported by the National Health and Medical Research Council, Australia, Health Research Council and Auckland Medical Research Foundation, New Zealand, and the University of Auckland Faculty Research and Development Fund. The unpublished data presented here was generated by Richard Williams and Sarah Graham.

**Conflict of interest**

The authors confirm that there are no conflicts of interest.
References

1. Marambaud P, Dresses-Werrinroer U, Vingdeux V. Calcium signaling in neurodegeneration. Mol Neurodegener. 2009; 4: 20–30.

2. Petersen OH, Tepikin AV, Gerasimenko JV, et al. Fatty acids, alcohol and fatty acid ethyl esters: toxic Ca²⁺ signal generation and pancreatitis. Cell Calcium. 2009; 45: 634–42.

3. Lebecque D, Davidoff AJ, Hajjar RJ. Interplay between impaired calcium regulation and insulin signaling abnormalities in diabetic cardiomyopathy. Nat Clin Pract Cardiovasc Med. 2008; 5: 715–24.

4. Mohan GC, Gandhi T. Therapeutic potential of voltage gated calcium channels. Mini Rev Med Chem. 2008; 8: 1285–90.

5. Ferraccioli GF, Tomietto P, De Santis M. Rationale for T cell inhibition by cyclosporin A in major autoimmune diseases. Ann N Y Acad Sci. 2005; 1051: 658–65.

6. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. J Biol Chem. 1985; 260: 3440–50.

7. Streb H, Irvine RF, Berridge MJ, et al. Release of Ca²⁺ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature. 1983; 306: 67–9.

8. Feske S, Gilmann J, Dolschew R, et al. Gene regulation mediated by calcium signals in T lymphocytes. Nat Immunol. 2001; 2: 316–24.

9. Putney JW Jr. A model for receptor-regulated calcium entry. Cell Calcium. 1986; 7: 1–12.

10. Takemura H, Hughes AR, Thasstrup O, et al. Activation of calcium entry by the tumor promoter thapsigargin in parotid acinar cells. Evidence that an intracellular calcium pool and not an inositol phosphate regulates calcium fluxes at the plasma membrane. J Biol Chem. 1989; 264: 12266–71.

11. Liu J, Kim ML, Heo WD, et al. STIM is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. Curr Biol. 2005; 15: 1235–41.

12. Roos J, DiGregorio PJ, Yeromin AV, et al. STIM1, an essential and conserved component of store-operated Ca²⁺ channel function. J Cell Biol. 2005; 169: 435–45.

13. Vig M, Peinelt C, Beck A, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca²⁺ entry. Science. 2006; 312: 1220–3.

14. Feske S, Gwack Y, Prakriya M, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature. 2006; 441: 179–85.

15. Williams RT, Manji SS, Parker NJ, et al. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. Biochem J. 2001; 357: 673–86.

16. Cai X. Molecular evolution and functional divergence of the Ca²⁺ sensor protein in store-operated Ca²⁺ entry: stromal interaction molecule. PLoS One. 2007; 2: e609–15.

17. Feske S. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca²⁺ entry in the immune system and beyond. Immunol Rev. 2009; 231: 19–290.

18. Fahrner M, Muik M, Derler I, et al. Mechanistic view on domains mediating STIM1-Orai coupling. Immunol Rev. 2009; 231: 99–112.

19. Dirkens RT. Checking your SOCCs and feet: the molecular mechanisms of Ca²⁺ entry in skeletal muscle. J Physiol. 2009; 587: 3139–47.

20. Wang Y, Deng X, Hewavitharana T, et al. Stim, ORAI and TRPC channels in the control of calcium entry signals in smooth muscle. Clin Exp Pharmacol Physiol. 2008; 35: 1127–33.

21. Cahalan MD. STIMulating store-operated Ca(2+) entry. Nat Cell Biol. 2009; 11: 669–77.

22. Williams RT, Senior PV, Van Stekelenburg L, et al. Stromal interaction molecule 1 (STIM1), a transmembrane protein with growth suppressor activity, contains an extracellular SAM domain modified by N-linked glycosylation. Biochim Biophys Acta. 2002; 1596: 131–7.

23. Baba Y, Hayashi K, Fujii Y, et al. Coupling of STIM1 to store-operated Ca²⁺ entry through its constitutive and inducible movement in the endoplasmic reticulum. Proc Natl Acad Sci USA. 2006; 103: 16704–9.

24. Penna A, Demuro A, Yeromin AV, et al. The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. Nature. 2008; 456: 116–20.

25. Brandman O, Liu J, Park WS, et al. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca²⁺ levels. Cell. 2007; 131: 1327–39.

26. Luik RM, Wang B, Prakriya M, et al. Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation. Nature. 2008; 454: 538–42.

27. Liou J, Fivaz M, Inoue T, et al. Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca²⁺ store depletion. Proc Natl Acad Sci USA. 2007; 104: 9301–6.

28. Wu MM, Buchanan J, Luik RM, et al. Ca²⁺ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. J Cell Biol. 2006; 174: 803–13.

29. Stathopulos PB, Zheng L, Ikura M. Stromal interaction molecule (STIM) 1 and STIM2 calcium sensing regions exhibit distinct unfolding and oligomerization kinetics. J Biol Chem. 2009; 284: 728–32.

30. Stathopulos PB, Zheng L, Li GY, et al. Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. Cell. 2008; 135: 110–22.

31. Zhang SL, Yu Y, Roos J, et al. STIM1 is a Ca²⁺ sensor that activates CRAC channels and migrates from the Ca²⁺ store to the plasma membrane. Nature. 2005; 437: 902–5.

32. Park CY, Hoover PJ, Mullins FM, et al. STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. Cell. 2009; 136: 876–90.

33. Muik M, Fahrner M, Derler I, et al. A cytosolic homomerization and a modulatory domain within STIM1 C terminus determine coupling to Orai1 channels. J Biol Chem. 2009; 284: 8421–6.

34. Muik M, Frischau F, Derler I, et al. Dynamic coupling of the putative coiled-coil domain of Orai1 with STIM1 mediates Orai1 channel activation. J Biol Chem. 2008; 283: 8014–22.

35. Yuan JP, Zeng W, Dorwart MR, et al. SOAR and the polybasic STIM1 domains gate and regulate Orai channels. Nat Cell Biol. 2009; 11: 337–43.

36. Ji W, Xu P, LiZ, et al. Functional stoichiometry of the unitary calcium-release-activated calcium channel. Proc Natl Acad Sci USA. 2008; 105: 13668–73.

37. Dziadek MA, Johnstone LS. Biochemical properties and cellular localization of STIM proteins. Cell Calcium. 2007; 42: 123–32.
38. Wedel B, Boyles RR, Putney JW Jr, et al. Role of the store-operated calcium entry proteins Stim1 and Orai1 in muscarinic cholinergic receptor-stimulated calcium oscillations in human embryonic kidney cells. J Physiol. 2007; 579: 679–89.

39. Jousset H, Frieden M, Demaurex N. Stim1 knockdown reveals that store-operated Ca^{2+} channels located close to sarco/endoplasmic Ca^{2+} ATPases (SERCA) pumps silently refill the endoplasmic reticulum. J Biol Chem. 2007; 282: 11456–64.

40. Thompson MA, Pabelick CM, Prakash YS. Role of Stim1 in regulation of store-operated Ca^{2+} influx in pheochromocytoma cells. Cell Mol Neurobiol. 2009; 29: 193–202.

41. Soboloff J, Spassova MA, Hewavitharana T, et al. Stim2 is an inhibitor of Stim1-mediated store-operated Ca^{2+} Entry. Curr Biol. 2006; 16: 1465–70.

42. Soboloff J, Spassova MA, Tang XD, et al. Orai1 and Stim1 reconstitute store-operated calcium channel function. J Biol Chem. 2006; 281: 20661–5.

43. Mignen O, Thompson JL, Shuttleworth TJ. Orai1 subunit stoichiometry of the mammalian CRAC channel pore. J Physiol. 2008; 586: 419–25.

44. Graham SJ, Black MJ, Soboloff J, et al. Stim1, an endoplasmic reticulum Ca^{2+} sensor, negatively regulates STI-L1 pre-adipocyte differentiation. Differentiation. 2009; 77: 239–47.

45. Peinelt C, Wig M, Koomoa DL, et al. Amplification of CRAC current by Stim1, an endoplasmic reticulum Ca^{2+} ATPases selectively (ARC) pumps silently refill the endoplasmic reticulum. J Biol Chem. 2007; 282: 11456–64.

46. Dolgacheva LP, Konakov MV, Agafonova Peinelt C, Vig M, Koomoa DL, et al. Orai1 and Stim1 reconstitute store-operated calcium channel function. J Biol Chem. 2008; 283: 20661–5.

47. Mignen O, Thompson JL, Shuttleworth TJ. Orai1 subunit stoichiometry of the mammalian CRAC channel pore. J Physiol. 2008; 586: 419–25.

48. Darbellay B, Arnaudeau S, Konig S, et al. Stim1 and Orai1-dependent store-operated calcium entry regulates human myoblast differentiation. J Biol Chem. 2009; 284: 5370–80.

49. Mignen O, Thompson JL, Shuttleworth TJ. Stim1 regulates Ca^{2+} entry via arachidonate-regulated Ca^{2+}-selective (ARC) channels without store depletion or translocation to the plasma membrane. J Physiol. 2007; 579: 703–15.
88. Keresztes M, Haggblad J, Heilbronn E, et al. Initiation of human myoblast differentiation via dephosphorylation of Kir2.1 K⁺ channels at tyrosine 242. Development. 2008; 135: 859–67.

89. Horsley V, Friday BB, Matteson S, et al. Regulation of the growth of multinucleated muscle cells by an NFATC2-dependent pathway. J Cell Biol. 2001; 153: 329–38.

90. Ercan E, Momburg F, Engel U, et al. Compromised store-operated Ca²⁺ entry in aged skeletal muscle. Aging Cell. 2008; 135: 859–67.

91. Potier M, Gonzalez JC, Motiani RK, et al. Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: role in proliferation and migration. FASEB J. 2009; 23: 2425–37.

92. Albert AP, Large WA. A Ca²⁺-permeable non-selective cation channel activated by depletion of internal Ca²⁺ stores in single rabbit portal vein myocytes. J Physiol. 2002; 558: 117–28.

93. Golovina VA, Platsoshyn O, Bailey CL, et al. Upregulated TRP and enhanced capacitative Ca(2+) entry in human pulmonary artery myocytes during proliferation. Am J Physiol. 2001; 280: H746–55.

94. Brueggenmann LI, Markun DR, Henderson KK, et al. Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca(2+) entry in vascular smooth muscle cells. J Pharmacol Exp Ther. 2006; 317: 488–99.

95. Leung FP, Yung LM, Yao X, et al. Store-operated calcium entry in vascular smooth muscle cells. Br J Pharmacol. 2008; 153: 846–57.

96. Berra-Romani R, Mazzocco-Spezzia A, et al. Fiber FA deficiency alters plasma membrane STIM1 and TRPC1 in vascular smooth muscle cells. FASEB J. 2009; 23: 3547–54.

97. Csutora P, Peter K, Kilic H, et al. Novel role for STIM1 as a trigger for calcium influx factor production. J Biol Chem. 2001; 276: 7782–90.

98. Potier M, Gonzalez JC, Motiani RK, et al. Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: role in proliferation and migration. FASEB J. 2009; 23: 2425–37.

99. Albert AP, Large WA. A Ca²⁺-permeable non-selective cation channel activated by depletion of internal Ca²⁺ stores in single rabbit portal vein myocytes. J Physiol. 2002; 558: 117–28.

100. Golovina VA, Platsoshyn O, Bailey CL, et al. Upregulated TRP and enhanced capacitative Ca(2+) entry in human pulmonary artery myocytes during proliferation. Am J Physiol. 2001; 280: H746–55.

101. Brueggenmann LI, Markun DR, Henderson KK, et al. Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca(2+) entry in vascular smooth muscle cells. J Pharmacol Exp Ther. 2006; 317: 488–99.

102. Leung FP, Yung LM, Yao X, et al. Store-operated calcium entry in vascular smooth muscle cells. Br J Pharmacol. 2008; 153: 846–57.

103. Berra-Romani R, Mazzocco-Spezzia A, et al. Fiber FA deficiency alters plasma membrane STIM1 and TRPC1 in vascular smooth muscle cells. FASEB J. 2009; 23: 3547–54.

104. Csutora P, Peter K, Kilic H, et al. Novel role for STIM1 as a trigger for calcium influx factor production. J Biol Chem. 2001; 276: 7782–90.

105. Potier M, Gonzalez JC, Motiani RK, et al. Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: role in proliferation and migration. FASEB J. 2009; 23: 2425–37.

106. Albert AP, Large WA. A Ca²⁺-permeable non-selective cation channel activated by depletion of internal Ca²⁺ stores in single rabbit portal vein myocytes. J Physiol. 2002; 558: 117–28.

107. Golovina VA, Platsoshyn O, Bailey CL, et al. Upregulated TRP and enhanced capacitative Ca(2+) entry in human pulmonary artery myocytes during proliferation. Am J Physiol. 2001; 280: H746–55.

108. Brueggenmann LI, Markun DR, Henderson KK, et al. Pharmacological and electrophysiological characterization of store-operated currents and capacitative Ca(2+) entry in vascular smooth muscle cells. J Pharmacol Exp Ther. 2006; 317: 488–99.

109. Leung FP, Yung LM, Yao X, et al. Store-operated calcium entry in vascular smooth muscle cells. Br J Pharmacol. 2008; 153: 846–57.

110. Berra-Romani R, Mazzocco-Spezzia A, et al. Fiber FA deficiency alters plasma membrane STIM1 and TRPC1 in vascular smooth muscle cells. FASEB J. 2009; 23: 3547–54.

111. Csutora P, Peter K, Kilic H, et al. Novel role for STIM1 as a trigger for calcium influx factor production. J Biol Chem. 2001; 276: 7782–90.
exhibit deficits in hippocampal synaptic plasticity and memory storage. Learn Mem. 2005; 12: 111–9.

114. Neal JW, Clipstone NA. Calcineurin mediates the calcium-dependent inhibition of adipocyte differentiation in 3T3-L1 cells. J Biol Chem. 2002; 277: 49776–81.

115. Liu L, Clipstone NA. Prostaglandin P2alpha inhibits adipocyte differentiation via a G alpha q-calcium-calcineurin-dependent signaling pathway. J Cell Biochem. 2007; 100: 161–73.

116. Yang TT, Suk HY, Yang X, et al. Role of transcription factor NFAT in glucose and insulin homeostasis. Mol Cell Bioi. 2006; 26: 7372–87.

117. Byyny RL, LoVerde M, Lloyd S, et al. Cytosolic calcium and insulin resistance in elderly patients with essential hypertension. Am J Hypertens. 1992; 5: 459–64.

118. Draznin B, Sussman K, Kao M, et al. The existence of an optimal range of cytosolic free calcium for insulin-stimulated glucose transport in rat adipocytes. J Biol Chem. 1987; 262: 14385–8.

119. Draznin B, Sussman KE, Ecker RH, et al. Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. J Clin Invest. 1988; 82: 1848–52.

120. Kim JH, Mynatt RL, Moore JW, et al. The effects of calcium channel blockade on agouti-induced obesity. FASEB J. 1996; 10: 1646–52.

121. Jang YJ, Ryu HJ, Choi YO, et al. Improvement of insulin sensitivity by chelation of intracellular Ca(2+) in high-fat-fed rats. Metabolism. 2002; 51: 912–8.

122. Jang YJ, Ryu HJ, Choi YO, et al. Effects of an intracellular Ca(2+) chelator on insulin resistance and hypertension in high-fat-fed rats and spontaneously hypertensive rats. Metabolism. 2004; 53: 269–72.

123. Sotelo-Avila C, Gooch WM, 3rd. Neoplasms associated with the Beckwith-Wiedemann syndrome. Persp Ped Path. 1976; 3: 255–72.

124. Ruano Y, Mollejo M, Ribalta T, et al. Identification of novel candidate target genes in amiglions of Glioblastoma multiforme tumors detected by expression and real-time quantitative PCR. J Neurooncol. 2008; 88: 281–91.

125. Kovacs GG, Zsembery A, Anderson SJ, et al. Changes in intracellular Ca2+ and pH in response to thapsigargin in human glioblastoma cells and normal astrocytes. Am J Physiol Cell Physiol. 2005; 289: C361–71.

126. Yang S, Zhang JH, Huang XY. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. Cancer Cell. 2009; 15: 124–34.

127. van Nimwegen MJ, van de Water B. Focal adhesion kinase: a potential target in cancer therapy. Biochem Pharmacol. 2007; 73: 597–609.

128. Cai X. Molecular evolution and structural analysis of the Ca(2+)/calmodulin-dependent protein kinase C. J Biol Chem. 2007; 282: 1284–91.

129. Venkiteswaran G, Hasan G. Intracellular Ca2+ signaling and store-operated Ca2+ entry are required in Drosophila neurons for flight. Proc Natl Acad Sci USA. 2009; 106: 10326–31.

130. Eid JP, Arias AM, Robertson H, et al. The Drosophila STIM1 orthologue, dSTIM, has roles in cell fate specification and tissue patterning. BMC Dev Biol. 2008; 8: 104–20.

131. Blair SS. Wing vein patterning in Drosophila and the analysis of intercellular signaling. Ann Rev Cell Dev Biol. 2007; 23: 293–319.

132. Freisinger CM, Schneider I, Westfall TA, et al. Calcium dynamics integrated into signalling pathways that influence vertebrate axial patterning. Phil Trans R Soc B. 2006; 363: 1377–85.

133. Tokumitsu H, Hatano N, Inuzuka H, et al. Phosphorylation of Numb family proteins. Possible involvement of Ca2+/calmodulin-dependent protein kinases. J Biol Chem. 2005; 280: 35108–18.

134. Sullivan KM, Robin GM. The Ca(2+)-calmodulin-activated protein phosphatase calcineurin negatively regulates EGF receptor signaling in Drosophila development. Genetics. 2002; 161: 183–93.

135. Campese AF, Grazioli P, Colantonio S, et al. Notch3 and pTalpha/pre-TCR sustain the in vivo function of naturally occurring regulatory T cells. Int Immunol. 2009; 21: 727–43.

136. Medyouf H, Ghysdael J. The calcineurin/NFAT signaling pathway: a novel therapeutic target in leukemia and solid tumors. Cell cycle. 2008; 7: 297–303.

137. Kanamori M, Kawaguchi T, Negro JM, et al. Contribution of Notch signaling activation to human glioblastoma multiforme. J Neurosurgery. 2007; 106: 417–27.

138. Margareto J, Leis O, Larrarte E, et al. Gene expression profiling of human gliomas reveals differences between GBM and LGA related to energy metabolism and notch signaling pathways. J Mol Neurosci. 2007; 32: 53–63.

139. Katoh M. Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. Stem Cell Rev. 2007; 3: 30–8.

140. Yan X, Xing J, Lorin-Nebel C, et al. Function of a STIM1 homologue in C. elegans: evidence that store-operated Ca2+ entry is not essential for oscillatory Ca2+ signaling and ER Ca2+ homeostasis. J Gen Physiol. 2006; 128: 443–59.

141. Strange K, Yan X, Lorin-Nebel C, et al. Physiological roles of STIM1 and Orai1 homologs and CRAC channels in the genetic model organism Caenorhabditis elegans. Cell Calcium. 2007; 42: 193–203.

142. Bandyopadhyay J, Lee J, Lee J, et al. Calcineurin, a calcium/calmodulin-dependent protein phosphatase, is involved in movement, fertility, egg laying, and growth in Caenorhabditis elegans. Mol Biol Cell. 2002; 13: 3281–93.