

## Invited review

## Store-operated calcium channels and pro-inflammatory signals

Wei-chiao CHANG<sup>1</sup>*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK*

### Key words

calcium channels; store-operated Ca<sup>2+</sup> entry; capacitative Ca<sup>2+</sup> entry; calcium signaling; mast cells

<sup>1</sup> Correspondence to Dr Wei-chiao CHANG.  
Phn 44-18-6528-2177.  
Fax 44-18-6527-2488.  
E-mail wei-chiao.chang@physiol.ox.ac.uk

Received 2006-05-21

Accepted 2006-05-30

doi: 10.1111/j.1745-7254.2006.00395.x

### Abstract

In non-excitabile cells such as T lymphocytes, hepatocytes, mast cells, endothelia and epithelia, the major pathway for calcium (Ca<sup>2+</sup>) entry is through store-operated Ca<sup>2+</sup> channels in the plasma membrane. These channels are activated by the emptying of intracellular Ca<sup>2+</sup> stores, however, neither the gating mechanism nor the downstream targets of these channels has been clear established. Here, I review some of the proposed gating mechanisms of store-operated Ca<sup>2+</sup> channels and the functional implications in regulating pro-inflammatory signals.

### Introduction

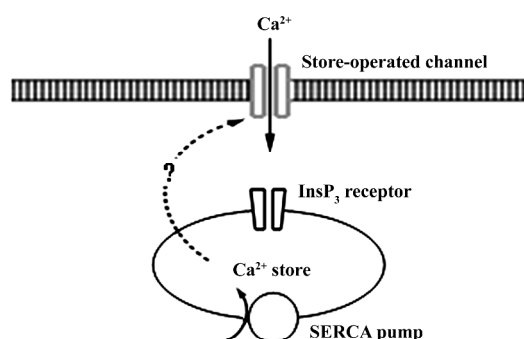
Cells use Ca<sup>2+</sup> as a key messenger to regulate a broad spectrum of vital processes. Changes in cytoplasmic Ca<sup>2+</sup> can trigger responses as diverse as exocytosis, muscle contraction, enzyme metabolism, gene transcription and cell proliferation<sup>[1]</sup>. To increase cytoplasmic Ca<sup>2+</sup> concentration, Ca<sup>2+</sup> is either released from intracellular stores or enters the cell by crossing the plasma membrane. In excitable cells, like nerve and muscle, calcium (Ca<sup>2+</sup>) entry is achieved largely through opening the voltage-gated and ligand-gated Ca<sup>2+</sup> channels<sup>[2,3]</sup>. The role of these ion channels is well established and the identity of amino acids in channel properties and gating is well known<sup>[2,3]</sup>. In non-excitabile cells, the main mechanism of Ca<sup>2+</sup> entry is a process known as “capacitative Ca<sup>2+</sup> entry” or “store-operated Ca<sup>2+</sup> entry”<sup>[4]</sup>. Non-excitabile cells do not fire action potentials and voltaged Ca<sup>2+</sup> channels are absent<sup>[4]</sup>. Instead, store-operated Ca<sup>2+</sup> entry is vital for driving most Ca<sup>2+</sup>-driven messengers. However, despite considerable research, neither the gating mechanism nor the molecular identity of these channels has been resolved. Here, I review some of the recent findings and theories of the store-operated Ca<sup>2+</sup> entry pathway.

### Store-operated Ca<sup>2+</sup> entry/capacitative Ca<sup>2+</sup> entry

The model of store-operated Ca<sup>2+</sup> entry in non-excitabile

cells was first proposed in 1986<sup>[5]</sup>. The fundamental idea of this model was that the Ca<sup>2+</sup> influx pathway could be activated by the amount of calcium in the internal store. As the Ca<sup>2+</sup> concentration falls, a signal is sent from the stores to open the Ca<sup>2+</sup> channels in the plasma membrane (Figure 1). The first direct evidence in support of the basic tenet of this idea was identified by discovery of a Ca<sup>2+</sup> current that was activated by store depletion. The underlying Ca<sup>2+</sup> channels were called calcium release-activated calcium channels (CRAC) and this calcium selective current was called  $I_{CRAC}$ <sup>[6]</sup>.

Store-operated Ca<sup>2+</sup> channels are the major route of Ca<sup>2+</sup> influx in non-excitabile cells and the best characterized store-operated current is  $I_{CRAC}$ . CRAC channels are non-voltage-



**Figure 1.** Scheme of capacitative calcium entry/store-operated calcium entry.

gated channels that are very selective to  $\text{Ca}^{2+}$  ( $P_{\text{Ca}^{2+}}/P_{\text{Na}^{+}} > 1000$ ) and have an extremely small conductance for  $\text{Ca}^{2+}$ [7]. The current is large at negative potentials and approaches the zero current level at positive potentials ( $>60 \text{ mV}$ )<sup>[7]</sup>. CRAC channels require extracellular  $\text{Ca}^{2+}$  to maintain their activity. Removal of extracellular  $\text{Ca}^{2+}$  results in a slow decline of channel activity<sup>[8]</sup>.

**Pharmacology of store-operated  $\text{Ca}^{2+}$  channels** Store-operated  $\text{Ca}^{2+}$  entry can be evoked by any strategy that lowers the  $\text{Ca}^{2+}$  content of the stores<sup>[6,7]</sup>. Physiologically, intracellular stores lose  $\text{Ca}^{2+}$  following a rise in the levels of inositol 1, 4, 5-trisphosphate [ $\text{Ins}(1,4,5)\text{P}_3$ ], which opens ligand-gated  $\text{Ca}^{2+}$  channels in the endoplasmic reticulum (ER) membrane. Experimentally, stores can be easily emptied following application of endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase (SERCA) inhibitors like thapsigargin or exposure of the cytoplasm to high concentrations of  $\text{Ca}^{2+}$  chelators such as EGTA or BAPTA that interfere with  $\text{Ca}^{2+}$  store refilling and cause passive stores to empty. Other methods of emptying intracellular stores include dialyzing the cytosol with  $\text{Ins}(1,4,5)\text{P}_3$  or permeabilizing the ER membrane to  $\text{Ca}^{2+}$  by applying  $\text{Ca}^{2+}$  ionophores like ionomycin.

Establishing a link between ion channel activity and activation of physiological functions relies on the use of pharmacological inhibitors. Thus, the issue of how best to block store-operated  $\text{Ca}^{2+}$  channels is an important theme in this field. Several pharmacological agents, such as  $\text{La}^{3+}$ , econazole and SK&F 96365 are known to inhibit store-operated  $\text{Ca}^{2+}$  channels. However, they can also block several other channels over a similar concentration range<sup>[9]</sup>. Thus, they should not be considered specific inhibitors for store-operated  $\text{Ca}^{2+}$  channels. 2-Aminoethoxydiphenyl borate (2-APB), an  $\text{InsP}_3$  receptor inhibitor, has become a popular tool to investigate store-operated  $\text{Ca}^{2+}$  channels<sup>[10–12]</sup>. 2-APB is used as an  $\text{InsP}_3$  receptor antagonist, but growing evidence suggests that it is not simply an  $\text{InsP}_3$  receptor blocker. For example,  $I_{\text{CRAC}}$  in mutant DT40 cells not expressing  $\text{InsP}_3$  receptors can be blocked by 2-APB, indicating that targets other than  $\text{InsP}_3$  receptor are affected<sup>[13,14]</sup>. Therefore, 2-APB seems to directly block the store-operated  $\text{Ca}^{2+}$  channels themselves. Another store-operated  $\text{Ca}^{2+}$  channel inhibitor is diethylstilbestrol. A synthetic estrogen agonist, diethylstilbestrol has been found to inhibit store-operated  $\text{Ca}^{2+}$  entry in human platelets, rat basophilic leukemia cells, and vascular smooth muscle cells but did not affect a whole-cell monovalent cation current mediated by TRPM7 channels<sup>[15]</sup>. *Trans*-stilbene, a close structural analog that lacks hydroxyl and ethyl groups, had no effect on store-operated  $\text{Ca}^{2+}$  influx<sup>[15]</sup>.

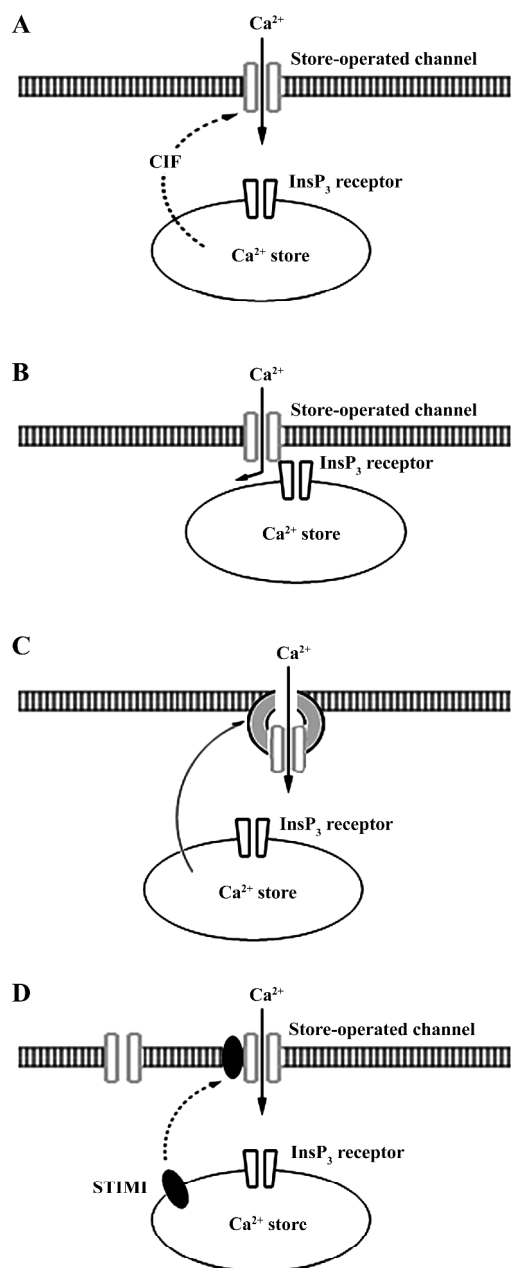
### Mitochondrial regulation of store-operated $\text{Ca}^{2+}$ channels

Mitochondria play an important role in production of ATP in eukaryotic cells. However, evidence has revealed that mitochondria can rapidly take up a great amount of calcium that has entered through different  $\text{Ca}^{2+}$  channels<sup>[16–19]</sup>. Can store-operated  $\text{Ca}^{2+}$  channels be regulated by the mitochondria? An elegant series of experiments establishing how mitochondrial  $\text{Ca}^{2+}$  uptake regulates store-operated  $\text{Ca}^{2+}$  channels has come from work on rat basophilic leukaemia (RBL) cells and T lymphocytes<sup>[20,21]</sup>. In RBL cells, mitochondria are also necessary for the activation of  $I_{\text{CRAC}}$  under physiological conditions of weak intracellular  $\text{Ca}^{2+}$  buffering. Whole cell dialysis with  $\text{Ins}(1,4,5)\text{P}_3$  fails to activate any detectable  $I_{\text{CRAC}}$  unless mitochondria are in an energized state<sup>[20,22]</sup>. Mitochondrial depolarization using electron transport chain blockers, such as antimycin A or rotenone, prevented  $I_{\text{CRAC}}$  from developing, and the size of  $I_{\text{CRAC}}$  to thapsigargin could be increased by energized mitochondria<sup>[20,22]</sup>. In T lymphocytes, moreover, energized mitochondria reduced  $\text{Ca}^{2+}$ -dependent slow inactivation, thereby prolonging the time-course of  $\text{Ca}^{2+}$  influx. Inhibition of mitochondrial  $\text{Ca}^{2+}$  uptake by CCCP or antimycin A enhance  $\text{Ca}^{2+}$ -dependent inactivation of  $I_{\text{CRAC}}$ <sup>[21]</sup>. Moreover, functional mitochondria are required to sustain CRAC channel activity, and downstream transcription factor NFAT translocation<sup>[21]</sup>.

### Mechanism of store-operated $\text{Ca}^{2+}$ channel activation

The activation mechanism of store-operated  $\text{Ca}^{2+}$  channels is one of the most intriguing mysteries. In the past few years, several models have been proposed to explain the link between store emptying and  $\text{Ca}^{2+}$  influx. Although there is evidence to support each model, there is also evidence that can not be easily fitted into any. In this review, I focus on different activation models (Figure 2) of store-operated  $\text{Ca}^{2+}$  channels.

**Diffusible messenger model** Randriamampita and Tsien were the first to propose the existence of a small diffusible molecule that activated store-operated  $\text{Ca}^{2+}$  channels<sup>[23]</sup>. This molecule was named calcium influx factor (CIF), and is released following  $\text{Ca}^{2+}$  store depletion and translocates to the plasma membrane to activate store-operated  $\text{Ca}^{2+}$  channels. CIF is a phosphorylated molecule that could be degraded by okadaic acid-sensitive protein phosphatases<sup>[24]</sup>. CIF produced by either mammalian cells or yeast with depleted  $\text{Ca}^{2+}$  stores directly activates store-operated  $\text{Ca}^{2+}$  channels in vascular smooth muscle cells<sup>[25]</sup>, and is able to accelerate the development of  $I_{\text{CRAC}}$  in Jurkat T lymphocytes



**Figure 2.** Four models proposed to explain the activation mechanism of store-operated calcium channels. (A) diffusible messenger model; (B) conformational coupling model; (C) vesicular fusion model; (D) calcium sensor model.

and RBL-2H3 cells<sup>[26,27]</sup>. Although recent work on CIF is encouraging, it is still not known how CIF activates store-operated Ca<sup>2+</sup> channels. Further evidence in support of the CIF model has come from work in smooth muscle cells<sup>[28]</sup>. Smani *et al*<sup>[28]</sup> reported that calcium-independent phospholipase A<sub>2</sub> was essential for CIF-mediated Ca<sup>2+</sup> influx. In this model, CIF disassociates inhibitory calmodulin from calcium-

independent phospholipase A<sub>2</sub> (iPLA<sub>2</sub>), leading to activation of iPLA<sub>2</sub> then LysoPLs, which in turn open store-operated Ca<sup>2+</sup> channels in the plasma membrane<sup>[28]</sup>. Importantly, both the pharmacological iPLA<sub>2</sub> inhibitor bromoenol lactone and antisense oligonucleotides directed against iPLA<sub>2</sub> suppress the activation of store-operated Ca<sup>2+</sup> channels following either with thapsigargin or CIF<sup>[28]</sup>. These recent studies support a role of iPLA<sub>2</sub> and LysoPLs in the CIF model.

**Conformational coupling model** Berridge and Irvine<sup>[29,30]</sup> first proposed a mechanism of connection between ER and store-operated Ca<sup>2+</sup> channels that involves a direct protein-protein interaction. According to this model, Ins(1,4,5)P<sub>3</sub> receptors in the ER could be physically associated to store-operated Ca<sup>2+</sup> channels in the plasma membrane. Store emptying could change the conformation of Ins(1,4,5)P<sub>3</sub> receptors, which then regulates the opening of store-operated channels through protein-protein interaction. This idea of conformational coupling was indirectly supported by works on Ins(1,4,5)P<sub>3</sub> receptors and the canonical TRP family, which are candidates for store-operated channels<sup>[31,32]</sup>. In HEK-293 cells, recombinant TRPC3 channels can be co-immunoprecipitated with Ins(1,4,5)P<sub>3</sub> receptors<sup>[33]</sup>. Kiselyov *et al*<sup>[31,34]</sup> also showed that the N-terminal domain of type 1 Ins(1,4,5)P<sub>3</sub> receptors is involved in the activation of TRPC3. Similarly, studies done in human platelets have revealed the interaction between TRPC1 and type II Ins(1,4,5)P<sub>3</sub> receptors<sup>[32]</sup>. However, there are some studies documenting that Ins(1,4,5)P<sub>3</sub> receptors are not essential for the activation of store-operated channels. For example, *I*<sub>CRAC</sub> in mutant DT40 cells not expressing all three types of InsP<sub>3</sub> receptors can still be activated by thapsigargin, indicating physical coupling might not be essential in the activation of CRAC channels<sup>[35]</sup>. These findings fit with observations by other groups, all of which reported that heparin, an IP<sub>3</sub> receptor antagonist, did not interfere with the activation of *I*<sub>CRAC</sub> in RBL cells or DT40 chicken B cells<sup>[36-38]</sup>. Thus, in some cell types, conformational coupling between Ins(1,4,5)P<sub>3</sub> receptor and store-operated channels might not be required for the activation of *I*<sub>CRAC</sub>.

**Vesicular fusion model** The third model to explain the activation mechanism of store-operated Ca<sup>2+</sup> channels is vesicular fusion. This hypothesis suggests that store emptying causes store-operated Ca<sup>2+</sup> channels to be inserted into the plasma membrane using an exocytotic mechanism. Yao *et al*<sup>[39]</sup> first reported the vesicular fusion model in *Xenopus* oocytes. They found *I*<sub>CRAC</sub> was disrupted by overexpression of a mutant of SNAP-25, indicating functional SNAP-25 is necessary to activate store-operated Ca<sup>2+</sup> current<sup>[39]</sup>. In rat megakaryocytes, the vesicular transport in-

hibitor primaquine was found to block the development of  $I_{\text{CRAC}}$ , suggesting the involvement of exocytotic mechanisms<sup>[40]</sup>. In HEK-293 cells, Alderton *et al*<sup>[41]</sup> reported that direct microinjection of botulinum neurotoxin A and tetanus neurotoxin, which cleaves SNAP-25 and specifically hydrolyzes vesicle-associated membrane protein 2, respectively, impaired store-operated  $\text{Ca}^{2+}$  entry. However, there is evidence indicating that SNAP-25 might not be involved in the activation of store-operated  $\text{Ca}^{2+}$  entry. The major challenge to the vesicular fusion model came from studies on the expression of SNAP-25 in nonexcitable cells. Scott *et al*<sup>[42]</sup> found that neither HEK-293 nor COS-1 cell lysates had detectable levels of SNAP-25. In contrast, both HEK-293 and COS-1 cells express high levels of botulinum neurotoxin A-insensitive SNAP-23 protein. In spite of the overexpression of mutant SNAP-23, store-operated  $\text{Ca}^{2+}$  entry was unaffected. In RBL cells, Bakowski *et al*<sup>[43]</sup> reported that recombinant protein alpha-SNAP1-285, an inhibitor of exocytosis, inhibited vesicular fusion but had no effect on the activation of  $I_{\text{CRAC}}$ . Hence, vesicular fusion does not seem to be involved in the activation of  $I_{\text{CRAC}}$ .

**Calcium sensor model** In 2005, some exciting results came from Roos *et al*<sup>[44]</sup> in the elusive mechanism of store-operated  $\text{Ca}^{2+}$  channels. They applied an RNAi-mediated silencing screen to 170 genes in *Drosophila* S2 cells, including a number of transient receptor potential genes, and used thapsigargin-evoked  $\text{Ca}^{2+}$  entry as a marker for store-operated channels. One gene coding for a protein called stromal interaction molecule (STIM) significantly reduced thapsigargin-induced store-operated  $\text{Ca}^{2+}$  influx. They also confirmed that STIM1 knockout in Jurkat T cells was abolished in  $I_{\text{CRAC}}$ <sup>[44]</sup>. Similarly, after screening 2,304 proteins, Liou *et al*<sup>[45]</sup> identified two proteins, STIM1 and STIM2, that are essential for maintaining the store-operated  $\text{Ca}^{2+}$  entry in HeLa cells.

The  $\text{NH}_2$ -terminal domain of STIM1 contains an  $\alpha$  helices structure called EF hand motif, and a protein-protein interaction domain called the sterile  $\alpha$  motif (SAM)<sup>[45]</sup>. Because of the EF hand motif, STIM1 is likely to be within the lumen of ER. STIM1 might function as the calcium sensor within stores<sup>[45]</sup>. By transfecting fluorescent fusion protein to detect the localization of STIM1, Liou *et al*<sup>[45]</sup> reported the redistribution of YFP-STIM1 into puncta near plasma membrane was triggered by thapsigargin-induced store depletion. Interestingly, when the calcium-binding aspartic acid residue in the EF hand motif was mutated to alanine, store emptying failed to trigger any store-operated  $\text{Ca}^{2+}$  influx<sup>[45]</sup>.

Zhang *et al*<sup>[46]</sup> were the first to report that stores depletion results in the translocation of endogenous STIM1 from

ER to plasma membrane. They showed that mutation of the EF hand motif that mimics store emptying triggered the activation and translocation of store-operated  $\text{Ca}^{2+}$  channels<sup>[46]</sup>. Further evidence supporting the STIM1 model showed that overexpression of STIM1 resulted in a substantial increase in  $I_{\text{CRAC}}$  and mutants in the EF hand motif and C terminal of STIM1 altered the basic features of CRAC channels<sup>[47]</sup>. The study indicates that, in addition to being a calcium sensor within ER, STIM1 within the plasma membrane might function as a regulatory component of store-operated  $\text{Ca}^{2+}$  channels<sup>[47]</sup>. Thus, a new model proposes that STIM1 is located in the membrane of ER when stores are full. Once stores are empty,  $\text{Ca}^{2+}$  dissociates from the EF hand motif of STIM1, and STIM1 translocates to plasma membrane to activate CRAC channels. More data supports the involvement of STIM1 in the regulation of store-operated  $\text{Ca}^{2+}$  channels. Thus, the goal for understanding the mechanism of store-operated  $\text{Ca}^{2+}$  channels activation seems to be a real possibility.

### Physiological function of store-operated $\text{Ca}^{2+}$ channels

There is growing evidence for a role of store-operated  $\text{Ca}^{2+}$  influx in human disease. Severe combined immunodeficiency<sup>[48,49]</sup> and acute pancreatitis<sup>[50,51]</sup> have been linked to a failure of store-operated  $\text{Ca}^{2+}$  entry. Actually, the increase of intracellular  $\text{Ca}^{2+}$  through CRAC channels can regulate several inflammatory processes. This review will focus on studies over the past several years that have been aimed at understanding the activation of pro-inflammatory signals such as transcription factor nuclear factor  $\kappa\text{B}$  (NF- $\kappa\text{B}$ )<sup>[52-54]</sup>, nuclear factor of activated T cells (NF-AT)<sup>[52-54]</sup>, endothelial nitric oxide synthase (eNOS)<sup>[55]</sup>, and leukotrienes<sup>[56]</sup> by store-operated  $\text{Ca}^{2+}$  channels.

**NF- $\kappa\text{B}$  and NF-AT** These two transcription factors play important roles in immunity, cell proliferation and proinflammatory cytokine gene activation<sup>[52-54,57-59]</sup>. NF- $\kappa\text{B}$  is considered to be a pro-inflammatory initiator that can be activated by different stimuli such as lipopolysaccharide<sup>[60,61]</sup>, ER stress<sup>[62]</sup>, Decoy receptor 3 signalling<sup>[63]</sup>, tumor necrosis factor<sup>[58,59]</sup> and variations of the  $\text{Ca}^{2+}$  oscillations amplitude<sup>[52-54]</sup>. Similarly, NF-AT is a family of transcription factors that regulate gene expression during the immune response. The activation of NF-AT is tightly regulated by the  $\text{Ca}^{2+}$ /calmodulin-dependent serine phosphatase calcineurin. In resting cells, phosphorylated NF-AT localizes in the cytoplasm but, after stimulation by calcium rise, calcineurin is activated, resulting in dephosphorylation of NF-AT. NF-AT translocates to the nucleus and stimulates gene transcription<sup>[64-66]</sup>. Delmetsch *et al*<sup>[52]</sup> first reported the

involvement of  $\text{Ca}^{2+}$  oscillations through CRAC channels in the regulation of transcription factors NF-AT, Oct/Oap and NF- $\kappa$ B in T cells. Different frequencies of  $\text{Ca}^{2+}$  oscillations resulted in different molecular mechanisms for transcription factor activation<sup>[52-54]</sup>. For example, high frequency of  $\text{Ca}^{2+}$  oscillations activated all three transcription factors, but low frequency  $\text{Ca}^{2+}$  oscillations only induced NF- $\kappa$ B activation. The changes in  $\text{Ca}^{2+}$  oscillations have been extended to control target gene activation, as well as to induce downstream immune response and inflammation. In Jurkat T cells, a sustained  $\text{Ca}^{2+}$  influx through CRAC channels is critical for transcription of NF-AT and the expression of the interleukin (IL)-2 gene<sup>[52-54]</sup>. YM-58483, a pyrazole derivative, potently inhibited thapsigargin-induced  $\text{Ca}^{2+}$  entry, NF-AT transcriptional activity and IL-2 production, but not AP-1-driven promoter activity, indicating that  $\text{Ca}^{2+}$  entry is able to drive different types of transcription factors<sup>[67]</sup>.

**eNOS** An increase in vascular permeability is an important sign of acute inflammatory process. Several mediators, such as histamine, bradykinin, prostaglandins and nitric oxide are known to be involved in vascular permeability changes<sup>[68]</sup>. eNOS is a calcium/calmodulin-dependent enzyme constitutively expressed in endothelial cells. eNOS-derived nitric oxide has an important role in some of the features of inflammation, such as cell rolling, vascular permeability and angiogenesis<sup>[68]</sup>. In human endothelial cells, eNOS can be regulated by lysophosphatidylcholine<sup>[69-71]</sup> through a dynamic interaction between casein kinase 2 and serine/threonine phosphatase 2A in Sp1 binding activity. In pulmonary artery endothelial cells, the SERCA inhibitor thapsigargin can activate nitric oxide production<sup>[72]</sup>. However, it is not known whether this activation is caused by  $\text{Ca}^{2+}$  release from intracellular stores or  $\text{Ca}^{2+}$  influx through store-operated  $\text{Ca}^{2+}$  channels. The first example of how store-operated  $\text{Ca}^{2+}$  influx can activate eNOS came from Lin *et al.*<sup>[73]</sup>. Their results indicated that membrane-associated wild-type eNOS enzymatic activity is more sensitive to  $\text{Ca}^{2+}$  entry through store-operated  $\text{Ca}^{2+}$  channels than the release from intracellular stores. Because the localization of wild-type nitric oxide synthase is very close to store-operated  $\text{Ca}^{2+}$  channels, this colocalization could contribute to the rapid activation of nitric oxide synthase by  $\text{Ca}^{2+}$  entry<sup>[73]</sup>.

**Arachidonic acid and leukotrienes** In rat basophilic leukemia cells,  $\text{Ca}^{2+}$  entry through CRAC channels driven exocytosis<sup>[74]</sup>. Among the influential molecules released from mast cells are the leukotrienes that regulate a variety of inflammatory reactions<sup>[75]</sup>. Chang and Parekh<sup>[56]</sup> first described the involvement of CRAC channels in the regulation of pro-

inflammatory signals by arachidonic acid and leukotriene  $\text{C}_4$  in RBL cells. Transient activation of CRAC channels following a 4 min stimulation by thapsigargin was sufficient to generate a significant increase in pro-inflammatory signals. Calcium influx through CRAC channels, but not calcium release from intracellular stores, stimulated arachidonic acid production<sup>[56]</sup>. Arachidonic acid can be metabolized by 5-lipoxygenase enzyme, leading to the formation of leukotrienes. In the regulation mechanism of  $\text{LTC}_4$  secretion, calcium entry through CRAC channels activates extracellular signal regulated kinase (ERKs) within minutes and this is necessary for stimulation of cPLA<sub>2</sub><sup>[76]</sup>.  $\text{Ca}^{2+}$  entry activates ERK indirectly, via stimulation of calcium-dependent protein kinase C isozymes  $\alpha$  and  $\beta$ <sup>[76]</sup>. Following opening of CRAC channels, protein kinase C isozymes  $\alpha$  and  $\beta$  translocate from the cytosol to plasma membrane. Acute inhibition of these isozymes or down regulation following chronic exposure to phorbol ester prevents  $\text{Ca}^{2+}$  entry from activating ERK, cPLA<sub>2</sub> and  $\text{LTC}_4$ <sup>[76]</sup>. Although phorbol ester (PKC activator) stimulation resulted in strong ERK phosphorylation, this was not associated with any arachidonic acid release nor  $\text{LTC}_4$  secretion. Therefore, activation of PKC and subsequent phosphorylation of ERK1/2 in the absence of a  $\text{Ca}^{2+}$  signal is not sufficient to activate cPLA<sub>2</sub>. Instead, both  $\text{Ca}^{2+}$  and PKC/ERK are needed. Neither stimulates alone, in the absence of the other, can trigger arachidonic acid and  $\text{LTC}_4$  secretion<sup>[76]</sup>. Importantly, mitochondrial depolarization, by impairing  $\text{Ca}^{2+}$  entry through CRAC channels, suppressed the phosphorylation of ERK, release of arachidonic acid and downstream  $\text{LTC}_4$  secretion. The results establish the importance of mitochondrial regulation of CRAC channel activity in determining subsequent downstream pro-inflammatory molecules<sup>[56]</sup>.

## Conclusion

Store-operated  $\text{Ca}^{2+}$  channels have an essential role in the short-term and long-term regulation of the inflammatory process. In light of the fact that NF- $\kappa$ B, NF-AT, eNOS activation and  $\text{LTC}_4$  secretion are very potent pro-inflammatory mediators and have been linked to chronic inflammatory disease such as arthritis, atherosclerosis and asthma, CRAC channels could be a rational therapeutic target for treating such disorders.

## Acknowledgements

I am grateful to Prof Anant PAREKH for reading this paper, and Wan-chen HUANG for help with the figures. Dr Wei-chiao CHANG is in receipt of an ORS studentship.

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