

## MINI-REVIEW

# Regulation of calcium entry in exocrine gland cells and other epithelial cells

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### STORE-OPERATED CALCIUM ENTRY : THE BEGINNINGS IN EXOCRINE GLANDS

Store-operated or capacitative calcium entry is a phenomenon whereby the depletion of intracellular  $\text{Ca}^{2+}$  stores, generally from the endoplasmic reticulum, leads to the activation of plasma membrane  $\text{Ca}^{2+}$  channels (1-3). The idea developed over a decade or so from studies of the relative roles and interactions of  $\text{Ca}^{2+}$  release and  $\text{Ca}^{2+}$  entry mechanisms in salivary and lacrimal gland cells. That many cell types utilize both intracellular release of  $\text{Ca}^{2+}$  together with influx of  $\text{Ca}^{2+}$  across the plasma membrane for the generation of cytoplasmic  $\text{Ca}^{2+}$  signals have been appreciated for some time (4, 5). In salivary gland cells, it was shown that the  $\text{Ca}^{2+}$  stores released by activation of autonomic receptors (muscarinic,  $\alpha$ -adrenergic, substance P) required  $\text{Ca}^{2+}$  influx through activated  $\text{Ca}^{2+}$  channels for their replenishment (6). In the late 1970's and early 1980's, two key findings influenced thinking on how these two modes of signaling might interact. First, it became clear that the source of intracellular  $\text{Ca}^{2+}$  for the release phase of responses was the endoplasmic reticulum (7, 8). Second, the signal for the release of  $\text{Ca}^{2+}$  from the endoplasmic reticulum was shown to be the soluble product of phospholipase C activation, inositol 1,4,5-trisphosphate ( $\text{IP}_3$ ) (9, 10).

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The first hint that intracellular stores might direct the activity of plasma membrane  $\text{Ca}^{2+}$  channels came from the observation in lacrimal gland cells that stores refilled rapidly following their emptying, and this rapid refilling did not require receptor activation (11). Casteels and Droogmans (12) speculated that in smooth muscle, this rapid refilling might occur through a direct route, not traversing the cytoplasm. Subsequent studies, however, showed that this could not be the case (13, 14). The general concept of store-operated entry was articulated in an hypothesis paper in *Cell Calcium* in 1986 (1). Subsequently, two key observations, both made using salivary gland cells, provided strong evidence for the concept.

The first was a publication essentially confirming the 1978 report showing by use of  $\text{Ca}^{2+}$  indicators that  $\text{Ca}^{2+}$  influx occurred in the absence of receptor activation, when  $\text{Ca}^{2+}$  stores were depleted (15). The second was the demonstration that depletion of  $\text{Ca}^{2+}$  stores by a mechanism independent of phospholipase C signaling quantitatively and qualitatively recapitulated the  $\text{Ca}^{2+}$  entry activated through phospholipase-linked receptors. This latter publication demonstrated for the first time the activation of  $\text{Ca}^{2+}$  entry by the SERCA pump inhibitor, thapsigargin (16). Since then, thapsigargin has come to represent the clearest pharmacological indicator for store-operated  $\text{Ca}^{2+}$  entry.

In 1992, the first demonstration of a store-operated  $\text{Ca}^{2+}$  current was published by Hoth and Penner (17). This current was measured by use of the whole-cell patch clamp mode in mast cells, and was subsequently shown to be similar in T-cells (18).

Hoth and Penner called the current  $I_{crac}$ , for calcium release-activated calcium current.  $I_{crac}$  was shown to develop rather slowly (10s of seconds) following  $Ca^{2+}$  store depletion, and to be highly  $Ca^{2+}$  selective and strongly inwardly rectifying. The single channel conductance is thought to be extremely small, estimated by noise analysis to be in the fS range (18, 19). As is the case for other  $Ca^{2+}$ -selective channels, the selectivity for  $Ca^{2+}$  is lost in low divalent cation solutions, permitting measurements of larger whole-cell  $Na^+$  currents (20).

## THE MOLECULAR COMPONENTS OF SOCE

For a full twenty years following the first formulation of the concept of store-operated  $Ca^{2+}$  entry, investigations moved in fits and starts attempting to resolve two fundamental questions: what is the nature of the signal from the endoplasmic reticulum, and what is the identity of the  $Ca^{2+}$  channel? Numerous candidates for the signaling mechanism came and went, including cyclic GMP, arachidonic acid metabolites, inositol 1,3,4,5-tetrakisphosphate, and the  $IP_3$  receptor to name a few (3). One idea, that a diffusible substance termed “calcium influx factor” or CIF has received continuing support from a limited number of laboratories (21-24). While the role of such a factor is possible within the context of the  $Ca^{2+}$  sensor STIM1 (discussed below), the major impediment to understanding the function of CIF is the lack of knowledge of its structure. This prevents the majority of laboratories from following up on the published findings of a few, since its formation and action can only be investigated through use of tedious methods of partial purification and reconstitution.

Remarkably, in 2005 the powerful use of RNAi-based genetic screens revealed the endoplasmic reticulum  $Ca^{2+}$  sensor, and one year later, the SOC channel. The  $Ca^{2+}$  sensor, STIM1, was reported by two laboratories within a few weeks of one another (25, 26). STIM1, and in vertebrates its close relative STIM2, are single pass membrane proteins. STIM1 is found in the endoplasmic reticulum and plasma membrane, while STIM2 appears to be exclusively in the endoplasmic reticulum (27). The function of STIM1 in the plasma membrane, at least in the context of SOCE, is unknown since constructs incapable of reaching the plasma membrane are fully capable of supporting SOCE (28). There is evidence

that STIM1 in the plasma membrane plays a role in the function of non-store-operated arachidonic acid gated channels (29). Much of the key domain structure of STIM1 is known (Fig. 1). The N-terminus is

## STIM1

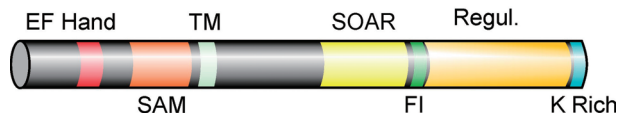


Figure 1. The domain structure of STIM1 includes a calcium sensing EF Hand, a sterile alpha motif (SAM), a single transmembrane domain (TM) and Orai-interacting SOAR domain, a regulatory (Regul.) domain in which numerous phosphorylation sites reside, and a C-terminal basic region that may be involved in interactions with plasma membrane acidic phospholipids.

directed towards the lumen of the endoplasmic reticulum. Therein lies the  $Ca^{2+}$  binding domain, an unpaired EF-hand. Immediately downstream is a sterile alpha motif (SAM) domain which is known to mediate protein-protein interactions, and interestingly also protein RNA interactions. When  $Ca^{2+}$  dissociates from the EF hand, this causes a conformational change in the EF-hand and SAM domains causing them to interact, initially dimerize and then to oligomerize (26, 30-32). STIM1 then aggregates in discrete subplasmalemmal sites where it apparently can interact directly with Orai channel molecules and activate them (33, 34). This is accomplished through a coiled-coiled domain first described by Yuan, *et al.* as a SOAR (STIM-Orai activating region) domain (35), and rapidly confirmed by three additional laboratories (34, 36, 37).

The evidence is very strong that Orai proteins constitute the pore forming subunits of the store-operated or CRAC channel. Overexpression of STIM1 and Orai1 produces huge  $I_{crac}$  (28, 38-40). Mutation of a glutamate at position 106 in human Orai1 to alanine or glutamine results in an inactive channel, while the more conservative mutation to aspartate results in a channel with altered selectivity (41-43).

## ROLE OF SOCE IN CALCIUM OSCILLATIONS

In most non-excitabile cells, including exocrine gland cells, activation of  $Ca^{2+}$ -mobilizing receptors

does not produce a sustained elevation in  $[Ca^{2+}]_i$ , but rather a series of  $Ca^{2+}$  spikes superimposed on a steady baseline. This phenomenon is generally referred to as  $Ca^{2+}$  oscillations (44, 45). The process of repetitive  $Ca^{2+}$  oscillations in epithelial cells was first inferred from fluctuations in chloride current by Berridge (46), and first directly demonstrated in hepatocytes by Cobbold (47). The major hallmark of these regenerative cytoplasmic  $Ca^{2+}$  spikes is their constant amplitude but variable frequency as a function of stimulus strength (45, 47). Such behavior is typical of an excitable process and requires some kind of positive feedback process to produce all-or-none rises in  $[Ca^{2+}]_i$  together with a shut-off or depletion mechanism to limit the size of the spikes. Despite decades of research, there is as yet no general consensus as to the nature of the key elements underlying cytoplasmic  $Ca^{2+}$  oscillations. This may be because multiple mechanisms exist that play different roles depending on the cell type and nature of the activating signal. There are two general models for cytoplasmic  $Ca^{2+}$  oscillations. One involves a positive feedback by  $Ca^{2+}$  on phospholipase C, causing fluctuations in  $IP_3$  levels (47-49). With this model,  $IP_3$  levels oscillate and  $Ca^{2+}$  signals reflect these changes in  $IP_3$ . In the alternative view,  $IP_3$  would remain constant, and the positive feedback would arise from  $Ca^{2+}$ -induced activation of the  $IP_3$  receptor.

The maintenance of  $Ca^{2+}$  oscillations requires influx of extracellular  $Ca^{2+}$  (45). In addition, it has been demonstrated that influx of  $Ca^{2+}$  during oscillations is primarily responsible for the activation of downstream responses, such as gene expression (50). Thus, it is important to understand the nature of this  $Ca^{2+}$  influx mechanism. There has been some controversy regarding this issue; although it is well accepted that maximal concentrations of agonists activate  $Ca^{2+}$  entry through the store-operated mechanism, it has been suggested that with low, more physiological concentrations of agonists, other non-store-operated entry pathways may be more significant (51).

We examined the  $Ca^{2+}$  entry supporting  $Ca^{2+}$  oscillations in a kidney cell line by using a combination of pharmacological and molecular criteria (52, 53). The data strongly indicate that it is the classical store-operated mechanism that supports these oscillations. Specifically: the oscillations were blocked by agents known to block store-operated channels, and in the same and unique concentration ranges wherein store-operated channels are affected (52);

and oscillations were blocked by RNAi knockdown of either the  $Ca^{2+}$  sensor, STIM1, or the SOC channel subunit, Orai1 (53). Interestingly, the oscillations were blocked by knockdown of STIM1, but were unaffected by knocking down STIM2, despite the fact that STIM2 is expected to be more active with small reductions in  $Ca^{2+}$  store content (54). This suggests that  $Ca^{2+}$  oscillations are capable of transiently lowering store content in critical sites into the range sensed by STIM1, and that STIM1 may thus be specially adapted to interacting with Orai channels to produce effective activation of downstream signals (54).

## CONCLUSION

In exocrine gland cells,  $Ca^{2+}$  signalling underlies the activation and control of secretory processes. A major component of these  $Ca^{2+}$  signals is the entry of  $Ca^{2+}$  across the plasma membrane through store-operated channels. In recent years, much has been learned of the molecular nature of store-operated channels, composed of Orai subunits as well as the  $Ca^{2+}$  sensors, STIM1 and 2, that initiate store-operated signaling. We look forward to continuing studies of the functions and regulation of these key  $Ca^{2+}$  signaling proteins and to a better understanding of their roles in exocrine physiology.

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