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Store-operated Ca^{2+} channels and microdomains of Ca^{2+} in liver cells

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Summary

1. Oscillatory increases in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) play essential roles in the hormonal regulation of liver cells. Increases in $[Ca^{2+}]_{cyt}$ require Ca^{2+} release from the endoplasmic reticulum (ER) and Ca^{2+} entry across the plasma membrane.

2. Store-operated Ca^{2+} channels (SOCs), activated by a decrease in Ca^{2+} in the ER lumen, are responsible for maintaining adequate ER Ca^{2+}. Experiments employing patch clamp recording and the fluorescent Ca^{2+} reporter fura-2 indicate there is only one type of SOC in rat liver cells. These SOCs have a high selectivity for Ca^{2+} and properties essentially indistinguishable from those of Ca^{2+} release-activated Ca^{2+} (CRAC) channels.

3. While Orai1, a CRAC channel pore protein, and Stim1, a CRAC channel Ca^{2+} sensor, are components of the liver cell SOCs, the mechanism of activation of SOCs, and in particular the role of subregions of the ER, are not well understood.

4. Recent experiments have employed the transient receptor potential V1 (TRPV1) non-selective cation channel, ectopically expressed in liver cells, and a choleretic bile acid to deplete Ca^{2+} from different ER subregions. The results have provided evidence that only a small component of the ER is required for STIM1 re-distribution and the activation of SOCs.

5. It is concluded that different Ca^{2+} microdomains in the ER and cytoplasmic space are important in both the activation of SOCs and in the signalling actions of Ca^{2+} in liver cells. Future experiments will further investigate the nature of these microdomains.

Introduction

Hormone-induced increases in the concentration of Ca^{2+} in the cytoplasmic space ($[Ca^{2+}]_{cyt}$) play a central role in intracellular signalling in animal cells.[1] In liver cells, one of the first types of animal cell in which oscillations in $[Ca^{2+}]_{cyt}$ were observed,[2] hormone-induced oscillations in $[Ca^{2+}]_{cyt}$ regulate pathways of intermediary and xenobiotic metabolism, bile acid secretion, cell proliferation and apoptosis and necrosis.[3] The maintenance of hormone-induced Ca^{2+} oscillations requires the constant replenishment of the endoplasmic reticulum (ER) Ca^{2+} stores by Ca^{2+} entering the cell through Ca^{2+} entry channels in the plasma membrane. While several types of Ca^{2+} entry channel might be involved in maintaining adequate Ca^{2+} in the ER, store-operated Ca^{2+} channels (SOCs) play a major role. The activation of SOCs is initiated by a decrease in Ca^{2+} in the ER. In hormonally-stimulated liver cells and in other animal cells, this ER Ca^{2+} decrease is mediated by inositol 1,4,5-trisphosphate receptors (IP_3Rs) (reviewed by Parekh & Putney[4]). Experimentally ER Ca^{2+} release can be induced by inhibition of the (Ca^{2+} + Mg^{2+})ATPase in the ER (SERCA) with thapsigargin, 2,5-di-(*tert*)-butyl)-1,4-benzohydro-quinone (DBHQ) and other SERCA inhibitors. The most extensively characterised SOCs are the Ca^{2+} release-activated Ca^{2+} channels (CRAC) in lymphocytes and mast cells (reviewed by Parekh & Putney[4]). The aim of this short review is to summarize the properties of SOCs in liver cells, current knowledge of their molecular components, likely mechanisms of activation, and the roles of Ca^{2+} microdomains in the activation mechanism and in the regulation of Ca^{2+} entry.

Characteristics of store-operated Ca^{2+} channels in liver cells

Many studies have shown that a SERCA inhibitor or IP_3 (introduced by micro injection or generated by addition of a hormone) will initiate the activation of Ca^{2+} entry to liver cells.[5-16] Since SOCs have often been functionally defined as channels which are activated by treatment of cells with SERCA inhibitor or IP_3[4] Ca^{2+} entry in response to these agents has often been attributed to SOCs. Moreover, it was suggested that more than one type of SOC may be expressed in hepatocytes and liver cell lines.[8,10,13,17] However, in the majority of these studies the nature of the Ca^{2+} entry pathway involved was not clearly defined. In recent patch clamp experiments with rat liver cells only one type of SOC, a highly Ca^{2+}-selective channel similar to CRAC channels, could be detected.[18-20] It is possible that in some earlier studies IP_3 and SERCA inhibitors may have initiated the activation of non-SOCs (reviewed by Barritt *et al.*[3]).

Ca^{2+}-permeable channels activated by thapsigargin and IP_3 in H4-IIE rat liver cells and rat hepatocytes have been characterised using patch clamp recording.[18-20] These SOCs exhibit a high selectivity for Ca^{2+} compared with monovalent cations and exhibit properties similar, or identical, to those of the CRAC channels found in

lymphocytes and mast cells.[18-20] Time courses of activation, current amplitudes, dependence on the extracellular Ca^{2+} concentration ($[Ca^{2+}]_{ext}$), conductance for Ba^{2+} compared with Ca^{2+}, and inhibition by La^{3+}, Gd^{3+} and 2-aminoethyl diphenylborate (2-APB) are similar for liver cell SOCs and CRAC channels. Ca^{2+} entry, measured by whole cell patch clamp recording, through SOCs in rat hepatocytes can be activated by physiological concentrations of vasopressin and ATP.[20] The permeability sequence for the movement of cations through liver cell SOCs is $Ca^{2+} > Ba^{2+} > Sr^{2+} > Na^{+} > Cs^{+}$.[18]

Liver cell SOCs are partially blocked by Co^{2+} and Cd^{2+} and completely blocked by Zn^{2+}, Gd^{3+} and La^{3+}. The most potent blocking agents are Gd^{3+} and La^{3+} which give complete block at about 2 μM in the presence of 10 mM Ca^{2+}_{ext}.[18,20] The concentration of 2-APB which gives half-maximal inhibition of Ca^{2+} entry is approximately 10 μM.[21] Ca^{2+} entry through liver cell SOCs is also inhibited by SK&F 96365,[15,22] arachidonic acid,[23] the phospholipase C (PLC) inhibitor U73122,[24,25] and by isotetrandrine and tetrandrine.[23]

There is some evidence to indicate that calmodulin is involved in the regulation of liver cell SOCs.[5,22] The results of patch clamp studies with H4-IIE rat liver cells have provided evidence that the fast inactivation of the SOC Ca^{2+} current, I_{SOC}, is a calmodulin- and Ca^{2+}-dependent process, similar to the Ca^{2+}-dependent fast inactivation of CRAC channels.[19] Thus over-expression of either a calmodulin inhibitor peptide or a mutant form of calmodulin lacking functional EF hand domains reduced the fast component of liver cell I_{SOC} inactivation. However, no effect of the calmodulin antagonists Mas-7 and calmidazolium was detected. It is possible that calmodulin is tethered to the rat liver SOC protein which shields it from the actions of calmodulin inhibitors.[26]

Molecular components of store-operated Ca^{2+} channels in liver cells

Experiments conducted during the past 3 years have shown that a member of the Orai (CRACM) family of proteins constitutes the pore of SOCs in mast cells, lymphocytes and in many other types of animal cells while STIM1 (stromal interaction factor 1) located in the ER constitutes the Ca^{2+} sensor. STIM1 is thought to detect the decrease in $[Ca^{2+}]_{er}$ and convey this information to Orai at the plasma membrane leading to activation of the channel and Ca^{2+} entry. This involves the movement of some STIM to ER-plasma membrane junctions leading to an interaction between STIM and Orai.[27-31] Further experiments are required to determine whether there is a direct interaction between STIM and Orai, or whether additional proteins are involved. The localisation of Orai and STIM and the Ca^{2+} entry channel may create domains of increased $[Ca^{2+}]_{cyt}$ at specific locations under the plasma membrane.[28]

It is likely that STIM is required in the mechanism of activation of liver cell SOCs. Knockdown of STIM1 in H4-IIE liver cells using siRNA caused a substantial reduction in the amplitude of I_{SOC} initiated by IP_3 or thapsigargin.[25] Treatment of H4-IIE cells with thapsigargin led to a redistribution of STIM1 to puncta, as assessed using cells transfected with GFP-STIM1 and by imaging endogenous STIM1 by immunofluorescence.[32]

The proposed mechanism of activation of liver cell SOCs involving the interaction of STIM1 with Orai1 at ER-plasma membrane junctions requires that such junctions are normally present in hepatocytes or are formed upon depletion of Ca^{2+} in the ER. Evidence for a close association of some ER with the plasma membrane in hepatocytes comes from previous subcellular fractionation experiments which generated highly purified plasma membrane fractions and provided evidence that specialised subregions of the ER are located close to the plasma membrane.[33,34]

In some other types of animal cells, TRP (transient receptor potential) proteins, including TRPC1, TRPC3, TRPC4, TRPV5 and/or TRPV6, are thought to constitute the pores of SOCs.[4,35,36] Some of these TRP proteins are expressed in liver cells (reviewed by Barritt *et al.*[3]). In H4-IIE rat liver cells, ectopic expression of hTRPC1 or knockdown of endogenous TRPC1 proteins using siRNA did not substantially change thapsigargin-stimulated Ca^{2+} entry (assessed using a fluorescent Ca^{2+} sensor and patch clamp recording), indicating that it is unlikely that the TRPC1 peptide is a component of SOCs in rat liver cells.[37,38] As described above, in patch clamp recording experiments only one type of SOC can be detected in rat liver cells and this has a high selectivity for Ca^{2+}, comparable to that of CRAC channels in lymphocytes and mast cells. The Ca^{2+}-permeable channels formed by TRPC1 polypeptides and by most other TRP polypeptides have a relatively low selectivity for Ca^{2+} compared with Na^{+}.[39,40] This suggests that it is unlikely that any of the known TRP polypeptides constitutes the Ca^{2+}-selective SOCs found in rat hepatocytes and liver cells.

Trimeric GTP-binding protein G_{i2}, F-actin and phospholipase Cγ1 may play permissive roles in the activation of liver cell SOCs

While STIM1 and Orai1 proteins are likely to be the major proteins which constitute liver cell SOCs, several other proteins appear to be required. Knockdown of PLCγ1 in H4-IIE rat liver cells using siRNA was found to be associated with a substantial decrease in the amplitude of I_{SOC} initiated by either IP_3 or thapsigargin. No interaction between PLCγ1 and STIM1 was detected in immunoprecipitation experiments.[25] It was concluded that PLC-γ1 is required to couple ER Ca^{2+} release to the activation of SOCs independently of any PLCγ1-mediated generation of IP_3 and independently of a direct interaction between PLCγ1 and STIM1.

ADP-ribosylation of the trimeric GTP-binding protein, $G_{i2\alpha}$, by treatment of livers with pertussis toxin, or the inhibition of $G_{i2\alpha}$ function using an inhibitory antibody or an inhibitory peptide, were each found to inhibit thapsigargin- and IP_3-induced Ca^{2+} entry (measured using fura-2) to freshly-isolated rat hepatocytes.[41-45] ADP-

ribosylation of $G_{i2\alpha}$ was associated with inhibition of the formation of the band of cortical F-actin around the canaliculus in isolated hepatocyte doublets when spatial polarity was regained, and with some disruption of the ER.[46] Moreover, studies with hepatocytes have shown that $G_{i2\alpha}$ interacts with F-actin.[46] Disruption of F-actin with cytochalasin D, within a narrow concentration range, inhibited thapsigargin- and IP_3-induced Ca^{2+} entry.[47] Taken together, these results indicate that the normal functions of $G_{i2\alpha}$ and F-actin are required for the activation of hepatocyte SOCs. The results of other studies suggest that, in addition to G_{i2}, a monomeric G-protein, possible ARF-1, is also required for the activation of SOCs in hepatocytes.[48]

Since the interventions described above inhibited the activation of SOCs when this was initiated by thapsigargin as well as by IP_3, it was concluded that the requirements for $G_{i2\alpha}$ and F-actin are downstream of the step in which Ca^{2+} is released from the ER. Thus, it was proposed that $G_{i2\alpha}$ is not involved in the formation of IP_3, catalysed by PLC_β linked to a G protein-coupled receptor, but rather that the role of $G_{i2\alpha}$ in the activation of SOCs represents a "receptor independent" function of $G_{i2\alpha}$ (*cf.* the role of the G_{i3} in vesicle trafficking and in other receptor-independent functions of G-proteins.[49] G_{i2} may function to maintain hepatocyte spatial polarity since it has been shown that trimeric G-proteins are involved in determining cell polarity in other cell types.[50] PLCγ1, $G_{i2\alpha}$, the monomeric G protein and F-actin may play "permissive" roles, such as maintenance of the integrity of the ER and the putative ER-plasma membrane junctions, in SOC activation in spatially polarised hepatocytes.

Roles of IP_3 receptor subtypes and putative subregions of the endoplasmic reticulum in the activation of liver cell SOCs

Two questions concerning the roles of IP_3R and the ER in the activation of SOCs in liver cells have been addressed. The first is whether a specific subtype of IP_3R is required for SOC activation, and the second is whether all of the ER or only a sub-component of the ER is required for the activation of SOCs. Rat hepatocytes express type 1 (20%), type 2 (80%) and a small proportion (<1%) of type 3 inositol 1,4,5-trisphosphate receptors (IP_3Rs).[51-53] In hepatocytes, type 2 IP_3Rs are expressed chiefly in the pericanalicular region and are responsible for the initiation of waves of increased $[Ca^{2+}]_{cyt}$ originating from this region.[51,53,54] When microinjected into freshly-isolated hepatocytes, a monoclonal anti-type 1 IP_3R antibody, which in other studies was shown to inhibit Ca^{2+} release mediated by type 1 IP_3R, was found to inhibit hormone- and thapsigargin-induced Ca^{2+} entry with little effect on the release of Ca^{2+} from intracellular stores.[55] The microinjection of a relatively low concentration of adenophostin A, which has a high affinity for IP_3Rs relative to that of IP_3, induced near-maximal activation of Ca^{2+} entry with little detectable release of Ca^{2+} from intracellular stores.[55] The results of experiments in which IP_3 analogues selective for either type 1 or type 2 IP_3R were microinjected to rat hepatocytes suggest that type 1 IP_3R are preferentially involved in SOC activation.[56]

As mentioned above, type 2 IP_3R are predominantly located in the ER near the bile canaliculus while type 1 IP_3R are distributed throughout most regions of the ER with some type 1 IP_3R concentrated in ER close to the plasma membrane in the sinusoidal and canalicular domains.[51,54,56] The results of subcellular fractionation studies indicate that type 1 IP_3R are found in regions of the ER very close to the plasma membrane, and are held in this location by F-actin.[33,34,57] Taken together, the results obtained using these different experimental approaches suggest that a small subregion of the ER enriched in type 1 IP_3Rs is required for SOC activation.

The question of whether the activation of liver cell SOCs requires the whole or only a small component of the ER has been further investigated using the non-selective cation channel TRPV1 and the choleretic bile acid taurodeoxycholic acid (TDCA) to release Ca^{2+} from different regions of the ER. It has previously been shown that TDCA activates SOCs in liver cells by releasing Ca^{2+} from the ER and causing a redistribution of STIM1.[32] When ectopically expressed in H4-IIE rat liver cells, TRPV1 was found to be localised in the ER as well as in the plasma membrane (Castro J, Aromataris EC, Rychkov, G & Barritt GJ, unpublished results). In liver cells expressing TRPV1, the amount of Ca^{2+} released from the ER by a TRPV1 agonist (RTX), measured using the cytoplasmic fluorescent Ca^{2+} reporter fura-2, was found to be the same as that released by a SERCA inhibitor (DBHQ), indicating that TRPV1 agonist-sensitive stores substantially overlap with SERCA inhibitor-sensitive stores (results not shown). However, in contrast to SERCA inhibitors, TRPV1 agonists did not activate Ca^{2+} entry measured using fura-2 or patch clamp recording (Castro J, Aromataris EC, Rychkov, G & Barritt GJ, unpublished results). In cells expressing TRPV1, the release of Ca^{2+} from the ER could readily be detected using fura-2, but could not be detected using the fluorescent Ca^{2+} reporter FFP-18, which detects increases in intracellular Ca^{2+} concentration beneath the plasma membrane[58] (Figure 1A,B). By contrast, Ca^{2+} release caused by SERCA inhibitors could be detected by both fura-2 and FFP-18 (results not shown). Taken together these results indicate that in cells expressing TRPV1, the region of the ER from which TRPV1 agonists release Ca^{2+} is some distance from the plasma membrane.

In liver cells incubated in the absence of agonist, STIM1 is distributed in the ER, as shown by the fluorescence images of STIM1-Cherry and YFP-tagged ER tracker (ER-YFP) in Figure 2A. In contrast to the effect of the SERCA inhibitor thapsigargin (Figure 2B middle panel (Tg) *cf.* Figure 2B left panel (control)) the TRPV1 agonist RTX did not cause a redistribution of STIM1 (Figure 2B right-hand panel (RTX) *cf.* Figure 2B left-hand panel (control)). In cells expressing TRPV1, incubated at zero Ca^{2+}_{ext}, the release of Ca^{2+} from the ER induced by TDCA could be detected by FFP-18 but not by fura-2 (Figure 1D *cf.* Figure 1C). Moreover, in TRPV1-expressing cells

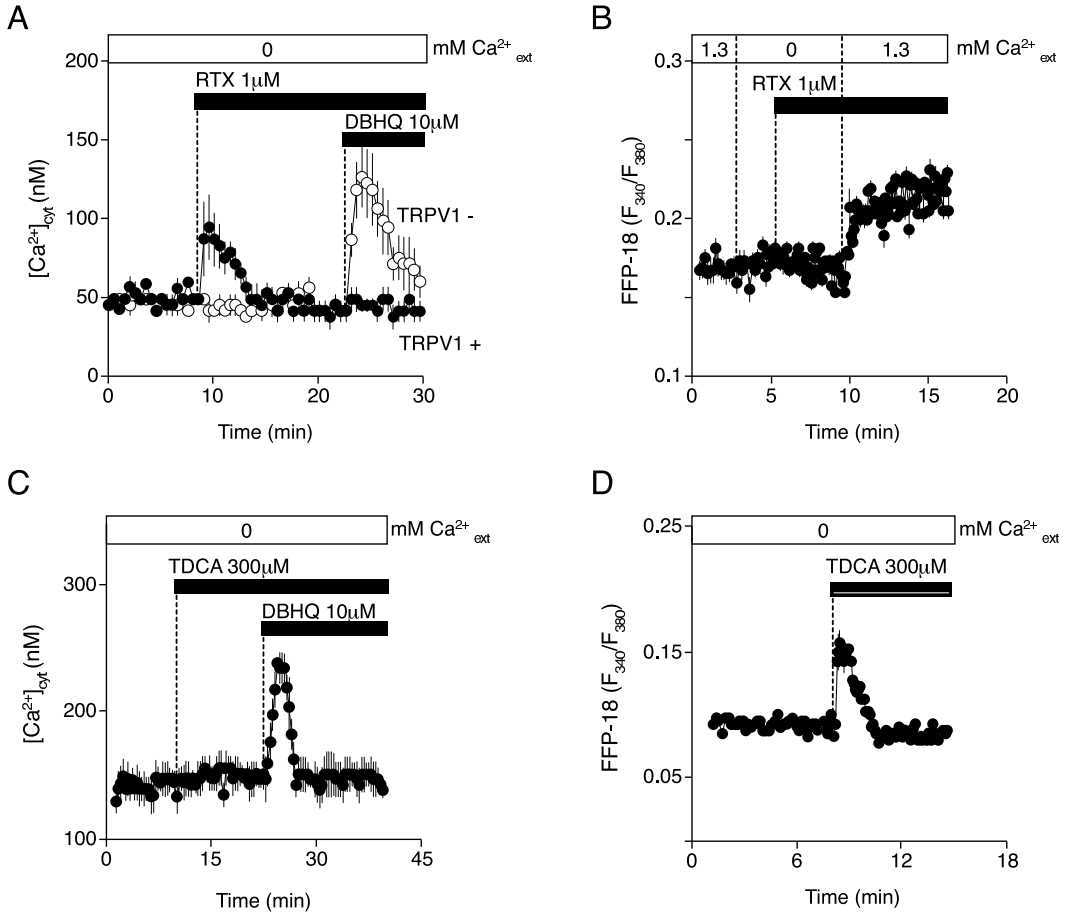

Figure 1. *Ca^{2+} release from the ER of H4-IIE liver cells induced by the TRPV1 agonist resiniferatoxin (RTX) is detected by the cytoplasmic Ca^{2+} reporter fura-2 (**A**) but not by the near plasma membrane intracellular Ca^{2+} reporter FFP-18 (B), whereas Ca^{2+} release induced by the choleretic bile acid TDCA is not detected by fura-2 (C) but is detected by FFP-18 (D). **A**: In the absence of Ca^{2+}_{ext}, RTX induces the release of Ca^{2+} from the ER in H4-IIE cells ectopically expressing TRPV1 (TRPV1(+) cells) but not in cells which do not express TRPV1 (TRPV1(-)) cells. Subsequent addition of DBHQ releases no further Ca^{2+} in TRPV1(+) cells but does release Ca^{2+} in the TRPV1(-) cells. **B:** In cells loaded with FFP-18 and incubated in the absence of Ca^{2+}_{ext} (after prior incubation at 1.3 mM Ca^{2+}_{ext}), RTX does not cause a detectable increase in $[Ca^{2+}]_{cyt}$. Subsequent addition of Ca^{2+}_{ext} does lead to an increase in $[Ca^{2+}]_{cyt}$. **C:** In cells loaded with fura-2 incubated in the absence of Ca^{2+}_{ext}, taurocholic acid (TDCA) (300 μM) does not induce any detectable increase in $[Ca^{2+}]_{cyt}$, while the subsequent addition of 10 μM DBHQ causes a substantial transient increase in $[Ca^{2+}]_{cyt}$. **D:** In cells loaded with FFP-18 incubated in the absence of Ca^{2+}_{ext}, TDCA induces a detectable increase in $[Ca^{2+}]_{cyt}$. H4-IIE cells were loaded with fura-2 or FFP-18 and changes in $[Ca^{2+}]_{cyt}$ were measured by confocal fluorescence microscopy. The times of addition of reagents are indicated by the horizontal bars (from Castro J, Aromataris EC, Rychkov, G & Barritt GJ, unpublished results).*

incubated at zero Ca^{2+}_{ext}, TDCA caused a redistribution of STIM1 to puncta similar to that caused by the SERCA inhibitor thapsigargin (Figure 2D (TDCA) *cf.* Figure 2C (Tg)). These results have provided further evidence that in liver cells Ca^{2+} release from a small component of the ER, which is presumably located near the plasma membrane, is required to induce STIM1 redistribution and SOC activation (Castro J, Aromataris EC, Rychkov, G & Barritt GJ, unpublished results).

Ca^{2+} microdomains and the regulation of Ca^{2+} entry through liver cell SOCs

Current ideas for the mechanism of activation of SOCs in liver cells are summarized in Figure 3. This shows in schematic form the proposed subregion of the ER which is enriched in IP_3R and located in ER-plasma membrane junctions, the roles of the Orai1 and STIM1 proteins as plasma membrane channel pore and ER Ca^{2+} sensor, respectively, and the proposed permissive roles of PLCγ1, G_{i2} and F-actin. The activation mechanism involves several microdomains of intracellular Ca^{2+} in the cytoplasmic space and in the ER. The results obtained using ectopically-expressed TRPV1 and TDCA, described above, suggest that Ca^{2+} release from the bulk of the ER is not required, or at least is not critical, for SOC activation. It is proposed that the essential component of the ER, as far as SOC activation

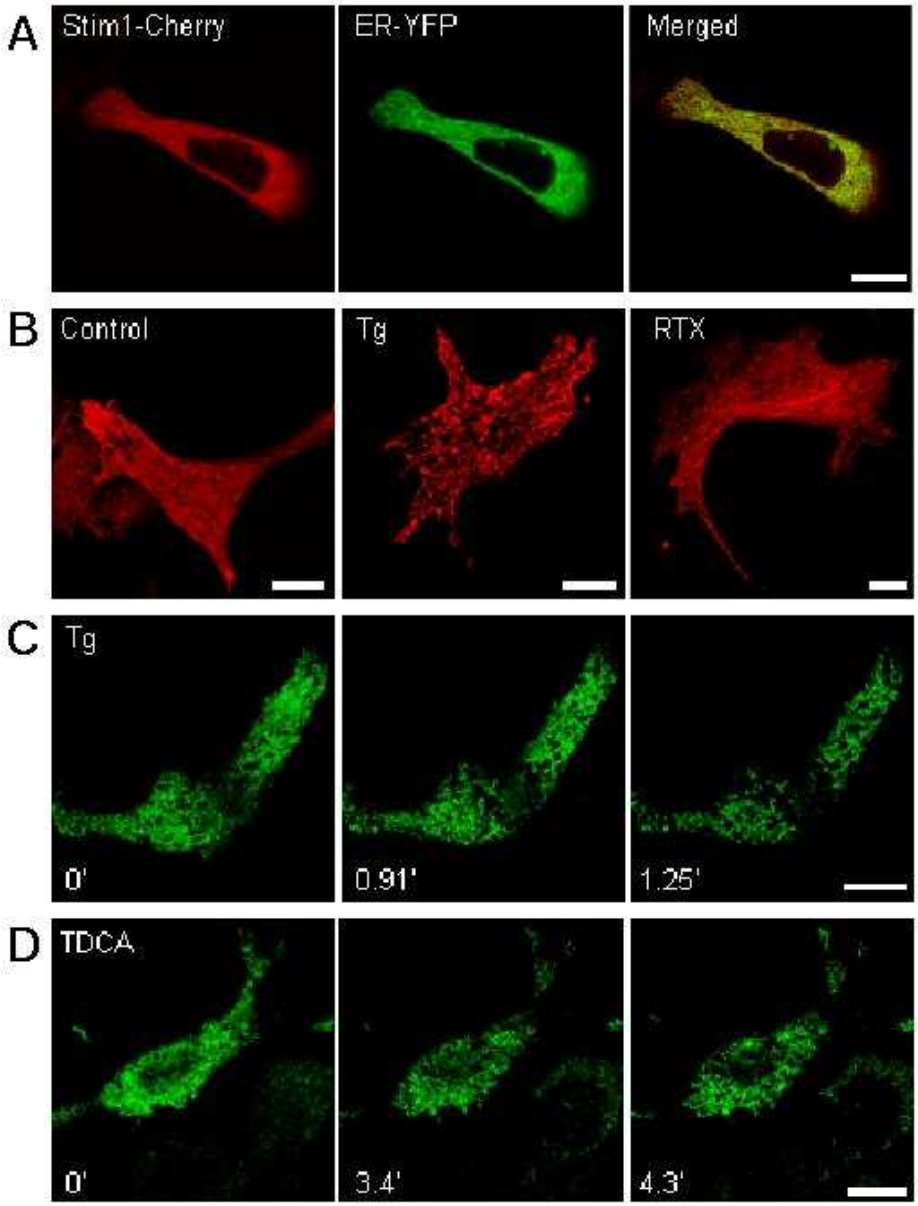

Figure 2. *In contrast to SERCA inhibitor thapsigargin (Tg), the TRPV1 agonist RTX does not induce a redistribution of STIM1 in H4-IIE liver cells ectopically expressing TRPV1, whereas taurodeoxycholic acid (TDCA) does induce a redistribution of STIM1 under experimental conditions similar to those where no TDCA-induced increase in* $[Ca^{2+}]_{cyt}$ *is detected with fura-2 (*cf. *Figure 1C).* ***A:*** *Images obtained by confocal fluorescence microscopy of the distribution of STIM1, observed using STIM1-Cherry (STIM1), and the ER, observed using a YFP-tagged ER marker (ER-YFP) in the same cell, and the merged image (Merged). The STIM1-Cherry construct is a fluorescent reporter constructed by inserting the fluorescent mCherry protein after the signal sequence of hSTIM1.*[63] ***B:*** *Cells ectopically expressing TRPV1 and STIM1-Cherry treated with vehicle (Control), 1 μM thapsigargin for 10 min (Tg), or 1 μM RTX for 10 min (RTX).* ***C,D:*** *TRPV1(+) cells expressing STIM1-Cherry were treated with 1 μM thapsigargin (Tg) (**C**) or 300 μM TDCA (TDCA) (**D**). The time elapsed (seconds) after addition of agonist is shown at the bottom of each frame. The scale bars represent 10 μm (from Castro J, Rychkov, G & Barritt GJ, unpublished results).*

is concerned, is a putative ER subregion which is enriched in IP_3R1 and is presumably located at ER-plasma membrane junctions. It is implied, but yet to be tested directly, that luminal movement of Ca^{2+} between the bulk of the ER and this ER subregion close to the plasma membrane is slow relative to the timescale of SOC activation. Further, that another property of the ER subregion which differentiates it from the bulk of the ER is that ectopically-expressed TRPV1 is not localised in this subregion.

Results obtained from studies with other cell types indicate that the activation of SOCs and regulation of the

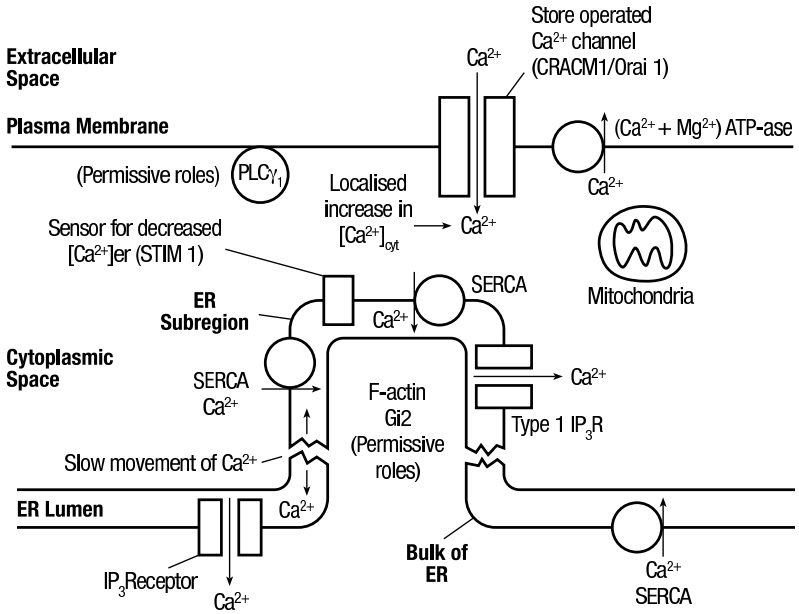

Figure 3. *A schematic representation of the proteins and organelles thought to be involved in the activation of store-operated Ca^{2+} channels in liver cells. The proposed mechanism of activation of SOCs can be summarized as follows. SOC activation requires a decrease in Ca^{2+} in the lumen of the ER in a subregion of the ER which is in close proximity to the plasma membrane and which forms ER-plasma membrane junctions. The ER subregion is enriched in type 1 IP_3Rs. While the ER subregion communicates with the bulk of the ER, the movement of Ca^{2+} between the subregion and the bulk of the ER is slow. The steps in the activation of SOCs are: the initiating decrease in $[Ca^{2+}]$ in the lumen of the ER induced by IP_3 (physiological) or a SERCA inhibitor (experimental), dissociation of Ca^{2+} from the luminal domain of the Ca^{2+} sensor STIM1, a conformational change in STIM1, oligomerisation of STIM1, relocalisation of STIM1 in the ER, interaction of STIM1 in close proximity to ER-plasma membrane junctions with CRACM1/Orai1, leading to a conformational change and increase the probability of opening of the Orai1 channel. Other proteins (as yet unidentified) are likely to be involved. The structure of the F-actin cytoskeleton, regulated in part by $G_{i2\alpha}$ and PLCγ1, is thought to play permissive roles in the activation pathway. Ca^{2+} which moves through SOCs into the ER-plasma membrane junction may cause a local increase in $[Ca^{2+}]_{cyt}$ at the mouth of the channel, before being transported directly to the lumen of the ER* via *SERCA pumps, and to mitochondria (adopted from Barritt* et al.*, 2008[3]).*

flow of Ca^{2+} through SOCs involves at least one microdomain of Ca^{2+} in the cytoplasmic space. This is a local increase in Ca^{2+} in the ER-plasma membrane junctional space at the mouth of the SOC (Orai) channel which occurs after channel activation. This, in part, is responsible for feedback inhibition of the channel itself, and may be responsible for the regulation of some enzymes such as adenylate cyclase.[4,59-62] The transport of Ca^{2+} from this putative microdomain to the ER and mitochondria plays an important role, not only in refilling the ER Ca^{2+} stores, but also in regulating the feedback inhibition by Ca^{2+} of the SOC channel (reviewed by Parekh & Putney[4]). Interpretation of some results obtained in studies investigating the role of Ca^{2+} in this microdomain in regulating the activities of enzymes such as adenylate cyclase is complex as often the experiments were conducted in the presence of a SERCA inhibitor (*e.g.* thapsigargin) which would cause a much larger increase in Ca^{2+} in this microdomain than would occur under physiological conditions.[59]

Conclusions

It can be concluded that liver cells express SOCs with a high selectivity for Ca^{2+} and with properties essentially similar to those of CRAC channels in lymphocytes and mast cells. Orai polypeptides and STIM1 polypeptides constitute the pore and Ca^{2+} sensor of the liver cell SOC, respectively. The activation mechanism involves Ca^{2+} release from a putative small subregion of the ER which is enriched in IP_3R1 and likely close to the plasma membrane. Further experiments might be directed to investigating the nature of the ER subregion and its relationship with the bulk of the ER, especially in connection with the steps involved in STIM1 movement, and oligomerisation, and the interaction of STIM1 with Orai1 and other proteins.

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