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Lipid rafts/caveolae as microdomains of calcium signaling

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Summary

Ca^{2+} is a major signaling molecule in both excitable and non-excitable cells, where it serves critical functions ranging from cell growth to differentiation to cell death. The physiological functions of these cells are tightly regulated in response to changes in cytosolic Ca^{2+} that is achieved by the activation of several plasma membrane (PM) Ca^{2+} channels as well as release of Ca^{2+} from the internal stores. One such channel is referred to as store-operated Ca^{2+} channel that is activated by the release of endoplasmic reticulum (ER) Ca^{2+} which initiates store operated Ca^{2+} entry (SOCE). Recent advances in the field suggest that some members of TRPCs and Orai channels function as SOCE channels. However, the molecular mechanisms that regulate channel activity and the exact nature of where these channels are assembled and regulated remain elusive. Research from several laboratories has demonstrated that key proteins involved in Ca^{2+} signaling are localized in discrete PM lipid rafts/caveolar microdomains. Lipid rafts are cholesterol and sphingolipid enriched microdomains that function as unique signal transduction platforms. In addition lipid rafts are dynamic in nature which tends to scaffold certain signaling molecules while excluding others. By such spatial segregation, lipid rafts not only provide a favorable environment for intra-molecular cross talk but also aid to expedite the signal relay. Importantly, Ca^{2+} signaling is shown to initiate from these lipid raft microdomains. Clustering of Ca^{2+} channels and their regulators in such microdomains can provide an exquisite spatiotemporal regulation of Ca^{2+} mediated cellular function. Thus in this review we discuss PM lipid rafts and caveolae as Ca^{2+} signaling microdomains and highlight their importance in organizing and regulating SOCE channels.

Keywords

Lipid rafts/caveolae; Membrane microdomains; Caveolin; Ca^{2+}signaling; Store operated Ca^{2+} entry (SOCE); TRPC channels; STIM; Signal transduction

1. Introduction

Ca^{2+} is one of the simplest, yet perhaps the most versatile cellular messenger. Cellular Ca^{2+} signals are initiated primarily as an increase in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) levels, but in different compartments, which are transduced to regulate a myriad of complex processes such as gene transcription, metabolism, muscle contraction, neural transmission, hormonal/fluid secretion, inflammation, cell proliferation and apoptosis [1,2]. It is now widely accepted that the

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specificity, reliability and accurate execution of many of these processes depend on tightly regulated spatiotemporal Ca^{2+} signals restricted to precise microdomains that contain Ca^{2+}-permeable channels and their modulators [3,4]. Restriction of Ca^{2+} influx pathways to specific microdomains favors efficient communication of the channel with its upstream regulators and downstream targets. In addition such microdomains facilitate dynamic cellular signaling by providing a perfect niche for clustering receptors, channels and bringing together signaling components that were previously isolated [5–7]. Elucidation of these microdomains is not only essential for understanding the biology of Ca^{2+} signaling, but will also provide insight into the regulation of diverse physiologically process.

Stimulation of cells by various agonists/pharmacological agents results in release of Ca^{2+} from intracellular stores as well as Ca^{2+} influx across the plasma membrane, both of which contribute to the control of physiological functions [2,8–10]. In most cells types, IP3 mediated release of Ca^{2+} via the IP3R is the major mechanism, which activates plasma membrane SOCE channels [4,9]. Although, most fundamental processes requires the release of Ca^{2+} from intracellular ER stores, it is the influx of external Ca^{2+} that is essential to sustain many physiological responses. Thus, SOCE is not only essential for the refilling of internal ER stores, but also critically contributes towards regulating/fine tuning of biological processes. Two proteins, TRPCs and Orai's have been suggested as putative SOCE channels [10–12]; however the mechanism by which SOCE channels are regulated in order to modulate Ca^{2+} signaling in various cell types is not yet established. Also, the microdomains where the channels are assembled as well as their interactions with key cellular regulators are still elusive. Lipid rafts have been shown to group signaling complexes [5,13–15] and many of the regulators and ion channels involved in Ca^{2+} signaling have been found to be present in lipid raft domains [16], suggesting that these domains have a significant role in modulating Ca^{2+} signaling. In addition, recent research has demonstrated the association of TRPCs with caveolar/lipid raft microdomains [17–20]. The critical role of lipid rafts/caveolae in Ca^{2+} signaling has been further established by many interesting studies where pharmacological and/or genetic disruption of these domains have been shown to significantly decrease SOCE [2,17,18,21],

2. Molecular components of Calcium Signaling

One of the most critical questions in the field is how a multiplicity of environmental cues are translated to generate Ca^{2+} signals that are choreographed to execute the appropriate physiological response. While recent research have provided many answers as to how Ca^{2+} signaling occurs, as noted above, the exact molecular components/composition of the channel (s) have not yet been identified. As depicted in figure 1, the concerted efforts of various channels pumps, transporters, and exchangers are required to maintain cellular Ca^{2+} homeostasis. Modalities of Ca^{2+} entry into cells can be broadly classified into three distinct categories – (i) Ca^{2+} entry activated by depolarization of the plasma membrane, this occurs via the voltage gated Ca^{2+} channels; (ii) Ca^{2+} entry activated by the binding ligand to the channel; (iii) Ca^{2+} entry activated by the depletion of internal Ca^{2+} stores, which is mediated via the SOCE channels and is the focus of this review. In SOCE channel activation, the first step is the binding of agonists (hormone or growth factors) to the cell surface receptors (ex-GPCR/RTKs). Activation of the cell surface receptor in turn engages a cascade of signaling events that culminates in the PLC mediated hydrolysis of PIP2 and the generation of the membrane bound diacylglycerol (DAG) and the diffusible messenger - inositol 1,4,5-trisphosphate (IP_3) (Figure 1). IP_3 binds to ER localized IP_3R and mediates Ca^{2+} release from the intracellular ER stores. Depletion of ER Ca^{2+} initiates a signal that ultimately results in the activation of SOCE channels, thus, SOCE activation is determined by the Ca^{2+} content of the ER Ca^{2+} stores.

The concept of SOCE was introduced almost three decades ago; however the ion channel(s) that regulate Ca^{2+} entry upon store depletion was not identified until recently [10,12,22]. Identification of TRPCs (especially TRPC1, and TRPC4 and in certain conditions TRPC3 and TRPC6) and Orai's (especially Orai1) as putative SOCE channels opened new and exciting directions towards elucidation of the mechanism of SOCE [23,24]. Although the precise composition and molecular identity of SOCE channels in different cell types is still an open question, evidence from several investigators indicate that some TRPCs and Orai proteins can together or independently function as SOCE channel/components [11–13,25–32]. TRP family of ion channels comprises of 27 human members which are divided into seven sub families [22]. All of these channels have six predicted transmembrane domains with both N and C terminus in the cytosol [10,11,22]. Whereas, only 3 mammalian isoforms of Orai (Orai1, 2, and 3) have been identified. Orai, are small proteins with four transmembrane domains, whose N and C-termini are also located in the cytosol [12].

RNA interference screens have recently identified STIM proteins (stromal interaction molecule 1 and 2) as the most important regulators of SOCE [33–35]. STIM1 and STIM2 are both single-pass transmembrane proteins with N-terminal Ca^{2+} binding EF hands and SAM (sterile alpha motif) domain located in the ER lumen and the cytosolic C-terminus containing the ERM (ezrin-radixin-moesin) and coiled-coiled protein interacting domains [28,30]. STIM1 has been shown to be associated with PM [33,36], however the precise role of PM STIM1 in SOCE remains debatable, although plasma membrane STIM1 has shown to be critical for the activation of arachidonic-acid-regulated Ca^{2+}-selective (ARC) channels channel [37,38]. Similarly STIM2, which is ~45% identical with STIM1, was also identified as second modulator; however recent reports indicate that STIM2 is primarily involved in maintaining basal Ca^{2+}, rather than initiating SOCE [37]. In addition, STIM1 has been shown to interact with TRPCs as well as Orai proteins and regulate SOCE [12,20,26,28,29,31,39,40]. Further, interaction of STIM1-TRPC1 has been shown to be lipid raft associated and dynamic in nature, which is regulated by the status of the ER Ca^{2+} [20]. Although, expression of GFP-STIM1 has been shown to form clusters, the significance of these clusters is still not clear since deletion of the region necessary for STIM1-Orai1 interaction prevents activation of Orai1 but not its co-clustering [41]. These results are fascinating and provide valuable clues on the regulation of SOCE. However, still many critical questions remain to be answered. For example it is not clear, if STIM1 needs to migrate to the plasma membrane upon store depletion or whether clustering of STIM1 in the ER regions is absolutely sufficient to communicate with PM – SOCE channels? Is there a requirement of cross-talk between ER – STIM1 and PM – STIM1 for efficient SOCE. Do SOCE channels need to be scaffold at specific domains or they migrate to these domains after store depletion, if so, what is the trigger for these channels to migrate to particular domains/locations upon store depletion. Are there other proteins that are required to coordinate STIM1 – SOCE channel interactions? The answers to many of these questions depends on our understanding of the precise PM – microdomains/platforms where STIM1 and SOCE channel come together to coordinate Ca^{2+} signaling.

3. Membrane rafts as centers of signal transduction

The initial concept of lipid rafts was that they represents small, but discrete platforms within the plane of the membrane, in which they function as signaling organizers [6,15,42–44]. Membrane rafts have now been suggested to play a significant role in many biological processes, including signal transduction pathways, apoptosis, viral infections, cell adhesion and migration, synaptic transmission, organization of the cytoskeleton, and plasma membrane protein sorting during both exocytosis and endocytosis [5,14,45]. Many of the above physiological process are also know to require Ca^{2+} and hence it was postulated that lipid rafts will regulate these processes by manipulating Ca^{2+} signaling.

Mammalian PM is highly heterogeneous in nature, comprising a variety of lipids and proteins that are not evenly distributed. Regions of the PM that are enriched in cholesterol, sphingolipids and a defined set of proteins constitute the PM 'lipid rafts' [5,46,47]. According to a recent revised consensus on the definition – membrane rafts are defined as small (10–200 nm), heterogeneous, highly dynamic, cholesterol and sphingolipids containing microdomains that compartmentalize cellular processes [48]. Under stimulating conditions, these smaller microdomains display a striking ability to coalesce and give rise to larger and stable membrane domains. The stability of the larger domains is conferred by protein-protein and/or protein-lipid interactions. In fact there are multiple ways as to how lipids can regulate Ca^{2+} signaling. i) They can either directly activate Ca^{2+} channels [45,49,50]. ii) can serve as signal transducer by generating intracellular signals, for example PtdIns(4,5)P_2 functions as substrates for receptor-regulated PLC activation thereby generating DAG and IP_3. iii) Plasma membrane lipids can also regulate trafficking of key proteins critical for Ca^{2+} signaling [49,51]. iv) In specific combinations, the lipids can assemble to form lipid rafts microdomains and thus coordinate cellular signaling [6,14,43,44].

The physical make-up of lipid rafts is quite unique with a highly ordered liquid state that is distinct from the surrounding disordered phospholipid bilayer [45,46,52]. This unique property exhibited by lipid rafts is primarily due to the enrichment of specialized lipid molecules, which makes these microdomains resistant to solubilization in non-ionic detergent such as Triton X-100, especially at low temperature (4°C). This characteristic feature of detergent insolubility gives an advantage for biochemical isolation of raft domains from rest of the cellular components. In spite of the ease in employing such protocols for isolating lipid rafts the use of detergents raises many questions regarding the true physiological existence of rafts. Therefore, detergent free methods for lipid raft isolation have been adopted to study their biochemical nature [53–55]. It has been a great challenge to define lipid rafts in their native state, since they cannot be readily visualized and distinguished from rest of the membranes either by light or electron microscopy. Unlike planer membrane rafts, 'Caveolae' are a subset of raft characterized by flask/omega shaped membrane invaginations. Caveolae are widely expressed in a variety of cell types and are known to compartmentalize numerous signaling processes [5,14,15,44,46]. Identification of this distinct morphological feature of caveolae has greatly improved our understanding of the physiological existence of raft microdomains. Biochemically, the definition of lipid raft largely suits for caveolae as well, however, caveolar microdomains contain caveolins as their major structural element that makes them unique. There are three known isoforms of Caveolin proteins, Caveolin 1 (Cav1), Caveolin 2 (Cav2), and Caveolin 3 (Cav3) [56], which forms higher order oligomers that line the membranes and structurally stabilize the inverted omega shaped membrane invaginations. More recently however, by proteomic analysis of detergent resistant membranes (DRM), Cavin (also known as PTRF – polymerase I transcript releasing factor or Cav-p60) was identified as a novel and integral component for caveolae biogenesis [44,57,58]. The significance of Caveolins and cavin in forming caveolae is underscored by the fact that their genetic disruption (with the exception to Cav2) results in a loss of caveolae formation [57,59,60]. Additionally, *de novo* caveolae biogenesis has been shown by the expression of Cav1 in immune cells [61].

Caveolae have been shown to be about 50–100 nM invaginations of the plasma membrane [5,62]. Such invaginations have the advantage of being able to facilitate interactions between proteins that are localized in separate organelles (e.g. - ER and mitochondria) [63], thereby mediating a communication between separate membrane compartments (e.g.- PM with ER), that would otherwise be several microns apart. Although caveolins are relatively small proteins (21–24 kDa), they have a distinct scaffolding region (conserved caveolin-interacting domains aa 82–101) that is essential in binding to many signaling proteins [44,59]. Caveolins are also known to bind and transport cholesterol to PM [5,14]. Membrane rafts have been shown to be efficient in organization of cell signaling machinery, including T and B cell receptor activation,

G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTK) pathways [13,64–67]. For example in cardiac myocytes, GPCRs and G-proteins co-localize with Cav3 and regulate Ca^{2+} influx (via L-type Ca^{2+} channels), which is essential for receptor mediated regulation of cardiac cells [68]. Similarly, The RTK pathways initiated by EGF, IGF and PDGF etc are also localized in caveolae and regulate cell proliferation and differentiation. Regulation of kinase cascades such as AKT and MAPK pathway is shown to be dependent on raft/caveolae integrity [14].

Figure 2 outlines a few ways to study membrane rafts. Staining of endogenous caveolins and visualizing with modest optical resolution is the most simplistic approach to identify the raft domains (Figure 2A). High resolution TEM is perhaps the best way to morphologically identify caveolar microdomains (Figure 2B and C gives an example). However, immunolabeling is required to demonstrate caveolar association of any protein of interest. Figure 2D illustrates a typical caveola and the various components that it can possibly contain. Many key signaling proteins along with phospholipids are known to be localized in lipid rafts. To study their functional importance, biochemical isolation of lipid rafts can be performed by ultra-centrifugal separation of the cellular components on flotation gradients such as -sucrose or OptiPrep gradients. Figure 2E provides examples of lipid raft/caveolae associated proteins resolved in discontinuous sucrose gradients. A high ratio of lipid to protein in membrane rafts confers it sufficient buoyancy so as to migrate to top fractions in the gradient (fraction 3–5) and thus separating it from the bulk PM.

4. Lipid rafts/caveolae and their importance in Ca^{2+} signaling

Ca^{2+} signals are generated across wide spatial and temporal ranges through various channels that can last from microsecond to several minutes [3,4]. This broad range of Ca^{2+} signals can be efficiently coordinated through organization of specific Ca^{2+} channels, pumps, buffers, exchangers and protein scaffolds into common microdomains. Membrane rafts provide such a microdomain wherein highly specific signaling events can be efficiently executed.

Involvement of lipid rafts/caveolae in Ca^{2+} regulation was suggested more than 30 years ago [69,70], but direct evidence was only shown recently [18,71]. The first evidence that suggested that the involvement of caveolae in Ca^{2+} homeostasis was observed in muscle cells where the SR was localized immediately underneath the plasma membrane and was in close proximity to caveolae [69]. Soon after, other investigators identified that caveolae can effectively increase intracellular Ca^{2+}, which may activate the contractile apparatus to produce a sustained vasoconstriction [72,73]. Histochemical methods further confirmed that Ca^{2+} was found in the lumina of caveolae, suggesting the importance of caveolae in Ca^{2+} signaling [74]. Further, X-ray spectral analysis confirmed that Ca^{2+} -peak (corresponding to increases in $[Ca^{2+}]_i$) can be found within two different cellular compartments: in small invaginations of the sarcolemma, which is caveolae, and in the intrafibrillar sarcoplasmic reticulum [75]. Additionally, PMCA pumps as well as IP_3-regulated Ca^{2+} channels were shown to be localized in caveolae [76–78]. Although these initial studies performed in mid 1990 provided clues that caveolae are important for Ca^{2+} signaling, not much research was performed to understand the mechanism or role of caveolae and caveolins in Ca^{2+} influx *per se*. It was Anderson's group that initially showed that agonist-stimulated Ca^{2+} signal originated in specific areas of the plasma membrane that were enriched in Cav1 [79,80]. Interestingly, not only G-protein coupled receptors but also $G_{q/11}$, phospholipase C, IP_3R, and SOCE channels, are now known to be present in lipid raft domains (Table 1 lists membrane raft associated proteins involved in Ca^{2+} signaling).

The ability to concentrate most proteins of the SOCE cascade in a single microdomain suggests that proteins are grouped together to effectively coordinate Ca^{2+} signaling. Importantly, direct interaction between TRPC1 and Cav1 had been shown to have a role in regulating Ca^{2+} influx

[17,51], which has now been confirmed using Cav1$^{-/-}$mice [69]. Similarly, voltage-gated Ca^{2+} channels have been also shown to be concentrated in cholesterol-rich lipid rafts microdomains [81,82], however, a direct association with Cav1 remains to be established. Nevertheless, alteration of Cav1 expression or sequestration of membrane cholesterol has been shown to affect the function of voltage-gated Ca^{2+} channels [81,82]. Similarly, Ca^{2+} influx-regulated processes, such as the generation of cAMP and the activation of nitric oxide synthase (NOS) have been also associated with caveolae [44,83,84]. Thus caveolae can provide a platform for the assembly of diverse Ca^{2+} signaling complexes, that are critical for spatial-temporal regulation of Ca^{2+} signals [5,51,66,85].

5. Lipid raft microdomains as organizers of TRPC signalplex

Lipid rafts microdomains are essential as they can provide a platform for direct protein-protein interactions, which can orchestrate Ca^{2+} signaling. These microdomains are also essential in defining the importance of mediators involved in Ca^{2+} signaling, mainly with regard to the understanding of their trafficking, assembly, maintenance, and turnover. Caveolins have been shown to either promote signaling via enhanced receptor-effector coupling, or inhibit the activity of signaling proteins [86]. Cholesterol depletion disrupts lipid rafts, but enhanced CaV2.1/alpha2delta-2/beta4 currents, suggesting that these domains influence the channels functionality [81]. In contrast, loss of lipid rafts in non excitable cells has been shown to decrease SOCE [18,21,40,87,88]. Thus, it indicates that these domains can explicitly regulate several Ca^{2+} channels, thereby differentially regulating Ca^{2+} entry.

TRPC channels protein needs to be associated with other regulatory signaling molecules [17]. In *Drosophila*, the TRP channels are associated with INAD signalplex, which includes TRPL, calmodulin, PLC, G-protein, and PKC [11]. Similarly, mammalian TRPC channels associate with multiple proteins that influence channel function (Figure 3A). However, in mammals a scaffold similar to INAD has not yet been identified. Although, numerous TRPC interacting proteins have been identified, it is unlikely that all the identified proteins remain attached with TRPC channels as a result of direct interactions. Rather, a stimulus dependant and dynamic association appear to be realistic. Nonetheless, since most TRPC interacting proteins are shown to be localized in lipid rafts, they together can provide a unique platform/scaffold to coordinate Ca^{2+} entry. Lipid rafts are also dynamic entities, where small rafts merge into bigger platforms [52,89], thereby increasing the possibility of protein-protein interactions. Thus, lipid raft microdomains can, in a dynamic, spatiotemporal fashion, facilitate direct physical, or functional, coupling between molecular components that are critical in the activation or inactivation of Ca^{2+} entry channels.

In addition to its role of clustering related signaling molecules, Cav1 proteins can also regulate trafficking of various receptors/mediators to the membrane [90]. It is now evident that TRPC channels are assembled into lipid raft/caveolar microdomains, where they interact with Cav1 (Table 1). This interaction is not only critical for channel assembly but also for the function and retention channels at the plasma membrane [18–21,40]. Importantly, all functional mammalian TRPC proteins have putative Cav1 binding domains at both their N and C terminus (Figure 3B). Moreover, isolation of lipid rafts indicate that all of the TRPC proteins are indeed associated with lipid rafts domains (Figure 3C). Interestingly, the N-terminus domain of TRPC1 which interacts with Cav1 has been shown to be critical for its plasma membrane retention. However, the significance of the putative C-terminus binding domains identified in figure 3B remains to be explored. Expression of a mutant Cav1 (lacking its protein scaffolding and membrane anchoring domains) or *cav1* gene knockout has been shown to disrupt the plasma membrane localization of TRPC1 leading to a significant decrease in Ca^{2+} influx upon store depletion [18,71]. Additionally, cholesterol-depletion and re-introduction has been shown to have a profound effect on the TRPC1-dependent, endothelin-evoked arterial

contraction, implying TRPC1 regulation by cholesterol enriched membrane rafts is critical in vascular pathology [19]. TRPC1 has been shown to be partitioned in both raft and non raft fractions and upon stimulation (store depletion) a significant increase in TRPC1 presence in lipid raft fractions have been shown recently [20]. Intriguingly, since, other TRPCs are also partitioned in both raft and non-raft fractions (Figure 3C), it is plausible to speculate that upon stimulation these proteins can also associate preferentially in lipid raft fraction in a way which is similar to TRPC1. Interestingly, it has also been shown that STIM1, a key activator of SOCE, is scaffolded by lipid raft domains especially upon stimulation [20,86]. Moreover, disruption of these lipid raft domains inhibits STIM1 movement into lipid rafts and its association with TRPC1, thereby decreasing TRPC1 dependent SOCE [20]. Overall, it can be speculated that lipid raft/caveolar microdomains can bring about an assembly of TRPC signalplex that would include Cav1, STIM1, GPCR, G-proteins among other key regulators of TRPC channels.

6. Membrane rafts host the interplay of critical Ca^{2+} signaling components

Two critical, questions regarding SOCE have plagued the field for long; (i) what are the molecular mechanism(s) whereby the status of the internal ER Ca^{2+} store is communicated to the plasma membrane that initiate Ca^{2+} influx? and (ii) What are the molecule(s) that mediates Ca^{2+} influx upon store depletion? Although, a number of mechanisms for the activation of SOCE has been proposed [91], the molecular mechanism involved in the channel opening upon store depletion *per se* has been a long-lasting unresolved issue. Identification of STIM1 as a regulator for SOCE channels, has at least in part answered the first question. Importantly, it has been shown that STIM1 is distributed throughout the ER in unstimulated cells and following depletion of ER Ca^{2+} stores, STIM1 redistributes into clusters or puncta at ER-PM junctional sites [34]. Although, the issue of whether STIM1 physically translocate into the PM remains controversial, increasing amount of evidence suggests that ER associated STIM1 clusters upon store depletion and translocates to specific sub-plasma membrane regions, if not functionally inserted into the PM. Interestingly, since caveolar lipid rafts form flask like invaginations of 50–100 nM deep in the cell, they might in principle facilitate SOCE channel interaction with ER associated STIM1 puncta, by literally bridging the spatial distance between the two membranes i.e. PM and ER. Although, at present there are no data available to suggest which domains of STIM1 are involved in its interaction with lipid rafts, the poly lysine rich region at the C-terminus has been shown to be critical for membrane anchoring and might facilitate its association with lipid rafts. The other possibility can be that STIM1 does not directly associate with lipid rafts or Cav1, but is indirectly associated via the SOCE channels present in lipid rafts.

Although, the molecular identity of the SOCE channels is still not completely resolved, several reports have shown that both TRPCs and Orai function as SOCE channels [10,22,28,33]. Also, assembly of a ternary TRPC-STIM1-Orai1 complex has been previously demonstrated [26] and the cytosolic C-terminus of STIM1 was shown as a common factor in activation of TRPC1 or Orai1 channels [26,28]. Interestingly disruption of membrane cholesterol has also been shown to decrease Orai1 association with TRPC1 and STIM1 [40]. Similarly, disruption of lipid rafts in T and B cells decreases SOCE, which is known to be mediated by Orai1 channels [12,92]. It is interesting to note that STIM1 and Orai1 have been shown to be associated with immunological synapse and cap-like membrane structures [93,94], which although they do not satisfy the definition of membrane rafts are biochemically akin to lipid rich membrane domains. The indirect lines of evidences suggest the possibility that Orai1, in addition to STIM1 and TRPC1, can also associate with membrane rafts.

Store depletion also prompted movement of TRPC1 as well as STIM1 into lipid rafts and knockdown of TRPC1 in some cell types has been shown to attenuate SOCE [20,26]. Importantly, two conserved acidic amino acids (DD) in the C-terminus of TRPCs have been

identified as essential for STIM1 mediated gating of the channels [28], which are localized within the putative C-terminal Cav1 binding domain in TRPC1-6 (Figure 3B). Thus, this junctional zone/domain can account for TRPCs function by regulating dynamic association among its regulators. Importantly, Cav1 has been shown to function as a negative regulator for many signaling proteins [14,44]. Overexpression of Cav-1ΔCSD construct which decreases TRPC1-Cav1 interaction, actually showed an increase in SOCE [95]. However, since Cav1 interaction is critical for TRPC1 targeting/retention [18,71], it is possible that it may have a dual role. Presumably as an initial step - Cav1 may target TRPC1 to the plasma membrane, but holds it inactive as observed in Cav1 mediated regulation of eNOS [44]. This is interesting since C-terminus of TRPC1 has both Cav1 binding domains and STIM1 gating domain that overlap (Figure 3B). Thus, it is possible that STIM1 can functionally regulate TRPC1 channel activity by modulating its interaction with Cav1 preferentially in lipid rafts. In aggregate, SOCE channel are perhaps more complexly regulated and major proteins (TRPC1, Orai1, STIM1, and Cav1) are localized into specialized lipid raft compartments wherein their dynamic assembly and molecular rearrangements underlie one of the fundamental aspects of cellular Ca^{2+} signaling.

7. Conclusion

In conclusion, Ca^{2+} channels are present within specialized membrane raft microdomains. Interaction of SOCE channel with its modulators in these domains facilitate Ca^{2+} entry, which can generate the spatiotemporal changes in $[Ca^{2+}]_i$ necessary for cellular functions. Further, lipid raft domains and Cav1 largely determine trafficking of SOCE (TRPC1-STIM1) channels to the plasma membrane. Store depletion promotes targeting of SOCE channels and its modulators to specific lipid raft domains thereby enhancing protein-protein interactions necessary for Ca^{2+} influx. Although, several studies have unveiled the importance of these microdomains in Ca^{2+} signaling, certain key steps still need to be defined. For example, do TRPC and Orai channels traffic to lipid raft/caveolar microdomains in response to their activation? If so what is the trigger/mechanism that specifies the channel targeting to these discrete membrane microdomains? How is STIM1 targeted to lipid rafts upon store depletion and is there any role of ER Ca^{2+} release and Cav1 in this process? Do Caveolins influence the functioning of Orai proteins? Do Caveolins control the turnover of the SOCE components? Endocannabinoids, which are associated with lipid rafts [96], regulate the functions of other TRP channels such as TRPVs and TRPMs. So, is it possible that these channels also localize to caveolae/lipid rafts domains? These and many other questions need to be addressed as we proceed to explore the relationship between membrane rafts and Ca^{2+} signaling. However, with the current understanding of the field, it is evident that caveolae/lipid rafts do regulate Ca^{2+} signaling pathways. By providing a stable yet dynamic platform for SOCE channel trafficking and assembly, these unique membrane microdomains greatly modulate Ca^{2+} responsive cellular processes.

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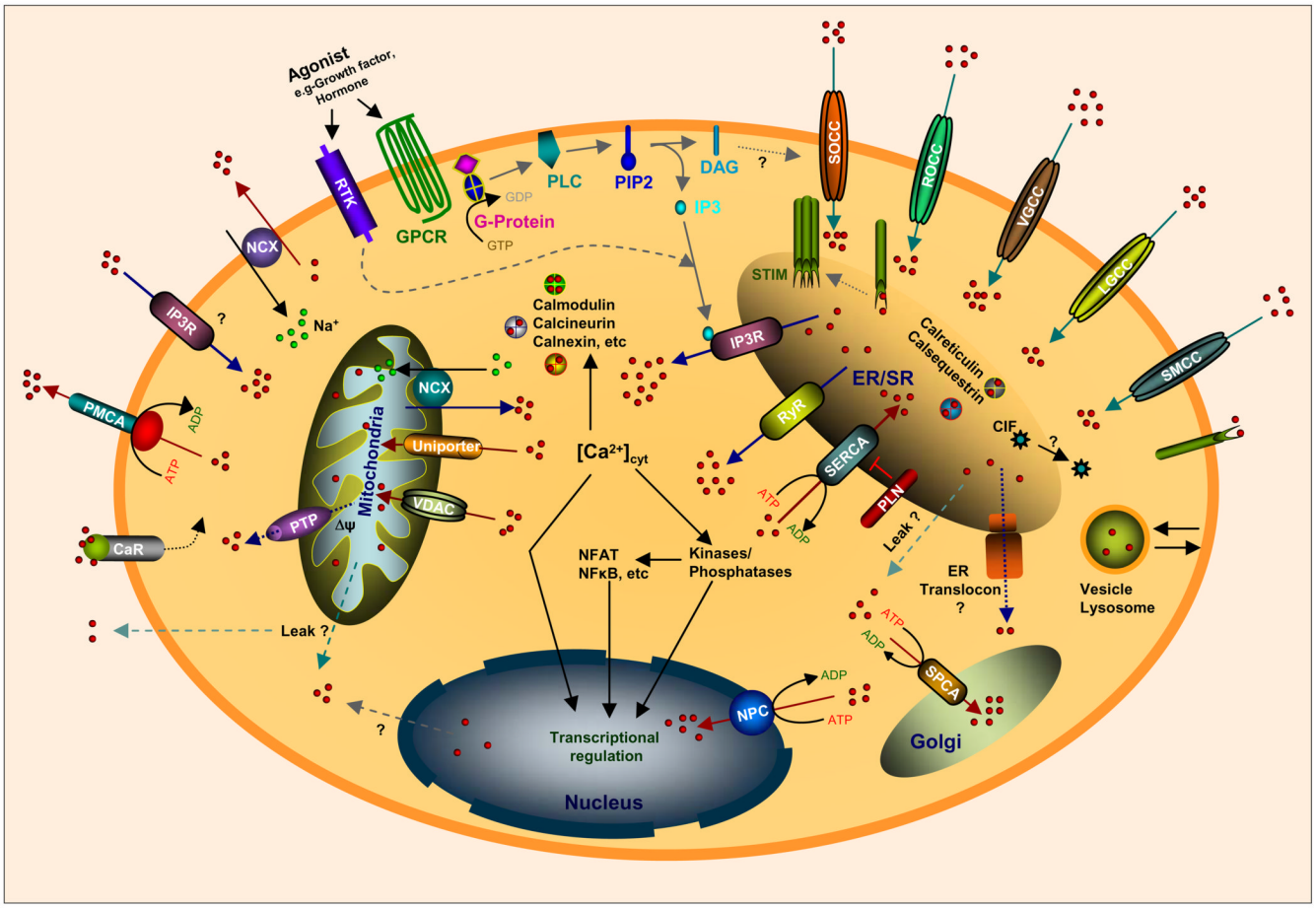

Figure 1. Model depicting major components of cellular Ca^{2+} homeostasis

A prime signaling cascade that mediates changes in $[Ca^{2+}]_{cyt}$ (cytosolic) initiates with the activation of plasma membrane (PM) receptors (ex-GPCR, G-protein coupled receptor). This leads to generation of the diffusible messenger - IP_3 (inositol 1,4,5-trisphosphate) which releases the $[Ca^{2+}]_{ER}$ by activating IP3R (IP3 receptor). Depletion of the ER/SR(endoplasmic/sarcoplasmic reticulum) stores induces clustering of STIM proteins (stromal interaction molecule1 and 2) and the STIM1 clusters/puncta then facilitate Ca^{2+} influx via the store operated Ca^{2+} channels. Besides this other significant Ca^{2+} influx routes such as, receptor operated (ROCC), voltage gated (VGCC), ligand gated (LGCC), and second messenger regulated (SMCC) calcium channels also contribute to increase in $[Ca^{2+}]_{cyt}$. Other components that are also known to regulate $[Ca^{2+}]_{cyt}$ include - undefined (?) source such as Ca^{2+} leaks/diffusion, ER translocon and debatable components such as CIF (Ca^{2+} influx factor) and PM IP3R. In addition to Ca^{2+} buffering by proteins such as calmodulin, calreticulin etc., the steady-state levels of $[Ca^{2+}]_{cyt}$ is achieved by its extrusion into cells exterior and/or by sequestration into organelles. The major components that bring about this homeostasis of Ca^{2+} includes Ca^{2+} ATPases of the PM (PMCA), ER (SERCA pump), golgi (SPCA – secretory pathway Ca^{2+} ATPase), nucleus (NPC - nuclear pore complex), NCX (sodium Ca^{2+} exchangers), VDAC (voltage dependant anion channel) and the mitochondrial uniporter. The PM Ca^{2+} receptor (CaR) is known to act via sensing the extracellular $[Ca^{2+}]$ whereas the mitochondrial permeability transition pore complex (PTP) regulates the change in mitochondrial membrane potential ($\Delta\psi$) in response to mitochondrial $[Ca^{2+}]$. In aggregate, the resultant increase in $[Ca^{2+}]_{cyt,}$ via various PM Ca^{2+} channels, would engage (to list a few) a variety of short-term

(ex-phosphorylation, secretion) and long-term (ex-gene regulation, proliferation, death) cellular functions thereby translating extracellular cues into observable physiological changes. Other abbreviations: RTK – receptor tyrosine kinase, PLC – phospholipase C, PIP2 – phosphatidylinositol 4,5-bisphosphate, DAG – diacylglycerol, PLN –phospholamban, NFAT (nuclear factor of activated T cells), NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells).

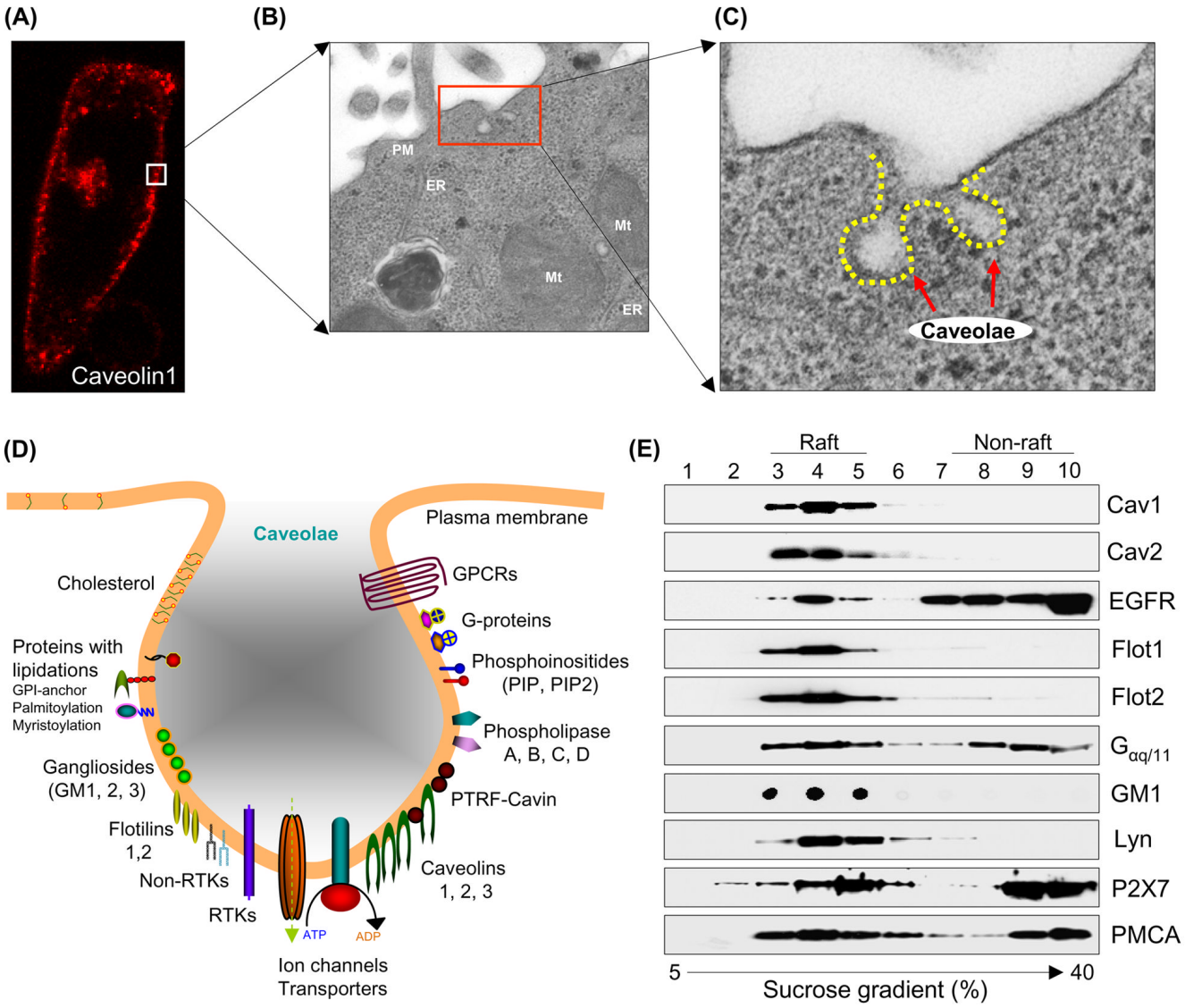

Figure 2. Lipid rafts/caveolae in salivary epithelial cells

(A) Confocal image of human submandibular gland (HSG) cells stained for endogenous caveolin1. (B) Transmission electron micrograph (TEM) of HSG cells indicating caveolar microdomains and (C) enlarged section from (B) showing caveolae (omega 'Ω' shaped membrane invaginations). (D) A model indicating various components of caveolae. (E) Representative blots of caveolae/lipid raft associated proteins, obtained from HSG cells by floatation gradients as described in [20]. Abbreviations: Mt – mitochondria, PTRF (polymerase I transcript releasing factor), Cav (Caveolin 1, 2), Flot (flotilin 1, 2)

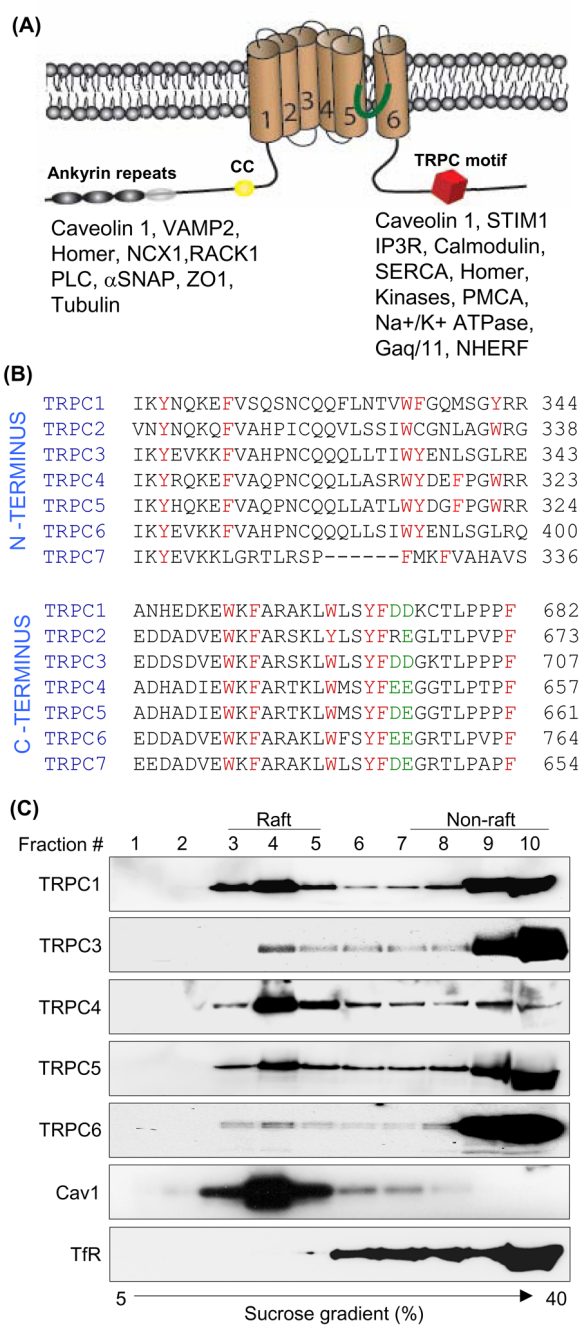

Figure 3. TRPC – topology, Cav1 binding motif and lipid raft association

(A) Model showing the topology of TRPC channels with N- and C-termini regulatory motifs and examples of proteins that are known to interact with TRPC channels is also listed. (B) Partial N- and C-termini sequence alignment of TRPCs indicating putative Cav1 binding motif. Amino acids shown in red are the aromatic residues that are predicted be involved in TRPC-Caveolin interactions. Amino acids shown in green are critical for STIM1 gating of the TRPC channel [28] and are shown here to overlap with the C-terminus Cav1 binding sequence. (C) Presence of TRPC channels in raft/non raft fractions isolated from HSG cells. Lipid rafts were isolated as described in [20] from HSG cells expressing HA/FLAG tagged TRPC cDNAs. Western blotting of the fractionated samples was performed with anti-tag antibodies.

Endogenous Cav1 was used as marker for raft fraction, whereas transferrin receptor (TfR) was used as a non-raft marker.

Table 1

Calcium signaling proteins localized in membrane raft

Proteins	Lipid raft/caveolae	Reference
Receptors		
-Muscarinic	Lipid rafts	[97]
-Bradykinin B2	Lipid rafts	[13]
-Cholescystokinin	Caveolae	[98]
-α, β-adrenergic	Lipid rafts/Caveolae	[99]
-P_2X and P_2Y	Lipid rafts	[100]
-Angiotensin II	Lipid rafts/Caveolae	[101]
-Ca^{2+} sensing receptor	Caveolae	[102]
-Chemokine	Lipid rafts	[103]
-Metabotrophic glutamate	Caveolae	[104]
-Serotonin	Caveolae/Lipid rafts	[105]
-Oxytocin	Caveolae/Lipid rafts	[106]
-EGFR	Caveolae/Lipid raft	[107]
-PDGF	Lipid raft	[108]
Channels/pumps/exchangers		
-Ca(v)1.2 Ca^{2+} Channel	Lipid rafts/Caveolae	[68,109]
-Ca(v)2.1 Ca^{2+} Channel	Lipid rafts	[81]
-Ca(v)2.2 Ca^{2+} Channel	Lipid rafts/Caveolae	[109]
-Voltage-gated K^+ Channel	Lipid rafts/Caveolae	[110]
-Ca^{2+} activated K^+ Channel	Lipid rafts	[111]
-ATP-sensitive K^+ channels	Caveolae	[112]
-TRPC1	Lipid rafts/Caveolae	[18,21,71,87]
-TRPC4	Caveolae	[71,87]
-TRPC6	Lipid rafts	[113]
-ORAI1	Lipid rafts	[40]
-PMCA	Lipid rafts	[114]
-NCX	Lipid rafts/Caveolae	[115]
-IP3R	Caveolae	[20,116]
-RyR2	Caveolae	[97]
Effectors of Ca^{2+} signaling		
-STIM1	Lipid rafts	[20,31]
-Homer	Caveolae	[97]
-Gα subunits	Lipid rafts/Caveolae	[105]
-Gβγ subunits	Lipid rafts	[117]
-eNOS	Caveolae	[118]
-Ca^{2+}-sensitive adenylate cyclase	Lipid rafts	[119]
-CAMK II	Lipid rafts	[120]
-PKC	Lipid rafts/Caveolae	[121]
-PLC	Lipid rafts/Caveolae	[122]
-Annexin	Lipid rafts	[123]