3. REVIEW OF LITERATURE

3.1. Calcium

Calcium plays a number of critically essential roles in physiology and pathology, in addition to its most widely renowned function as a critical structural component of bone. Many cells have calcium-sensing receptors, with confirmation that the concentrations of calcium ions in the extracellular fluid directly regulate cell function (e.g., parathyroid, renal tubule, and many more). Extracellular calcium concentration directly impacts on cell membrane potentials, and so impacts on function of all excitable tissues, particularly the nervous system and the heart.[47] Calcium is a key messenger in the contraction of muscle, including the myocardium.[48] It is a co-factor for many enzymes and intimately implicated in blood coagulation indeed, calcium chelators are the most commonly used anticoagulants in blood collection. The role of calcium in vascular health is less clear-cut. There are calcium-sensing receptors on vascular smooth muscle cells and on platelets, calcium plays a role in smooth muscle contraction and its role in the electrophysiology of the heart and myocardial function have already been alluded to. Calcium deposition in the vasculature is a steady feature of vascular disease and is prognostic of adverse cardiovascular events.[49] Indeed, calcium deposition in other soft tissues (such as the kidneys or muscles) has adverse effects on tissue function, and to prevent this there is a complex system of mineralization inhibitors (such as pyrophosphate, fetuin-A, matrix GLA protein etc.). Obviously, tissue-specific targeting of mineralization regulation is critically important so that mineralization of bone is promoted but that of all other tissues is prevented. With ageing, the usefulness of these mechanisms appears to decline, particularly in the vascular system. Additionally, blood calcium levels appear to influence the enlargement of vascular disease.[50]

3.2. SOCE: Historical Development of the Concept

One earlier and essential concept is that Ca^{2+} signals can arise in two general ways: either by influx to the cytoplasm across the plasma membrane, or by release to the cytoplasm from storage depots within the cell. The finding that this same dual mechanism of Ca^{2+} utilization applied to other, non-excitable cell types led to the suggestion that it was a

universal property of Ca^{2+} signaling systems.[51] Normally both processes occur and they interrelate and control one another in a variety of techniques. One perfect example is the heart where the Ca^{2+} influx is amplified several fold through a process of Ca^{2+}-induced Ca^{2+} release through ryanodine receptors in the sarcoplasmic reticulum.[52] However, the more common mechanism operates in the reverse mode: release of intracellular Ca^{2+} activates Ca^{2+} influx channels in the plasma membrane, which is the process of Store Operated Calcium Entry (SOCE).

In 1983 came, the discovery of the Ca^{2+} mobilizing second messenger, inositol 1,4,5-trisphosphate (IP_3).[53] The early experiments elaborate demonstration of IP_3-induced release from intracellular stores, primarily by of permeable cell models but also with microsomal fractions.[54] Nevertheless, when plasma membrane segments were separated fromendo plasmic reticulum, it seemed that IP_3 did not trigger release from the plasma membrane vesicles. Putney proposed the process of Ca^{2+} entry as a constant refilling of the stores and constant emptying through the IP receptor; thus the resemblance with electrical circuitry whereby current flows by a resistor and capacitor in series. Consequent findings confirmed the simple idea of store Ca^{2+} content modifiable influx, but the route of entry was not as planned by either Putney or Casteels and Droogmans. This was evidently validated in two consequent experiments that delivered the initial proofs for the concept of store-operate Ca^{2+} entry.

The first was a report in the Biochemical Journal in 1989 in which transient increases in [Ca^{2+}] were observed during refilling of intracellular stores, independently of receptor activation. In 1992 a major improvement occurred when Hoth and Penner published the first recordings of whole-cell current activated by Ca^{2+} store depletion.[55] They called the current ICRAC for Ca^{2+} release-activated Ca^{2+} current. The current was analogous to other selective Ca^{2+} current in being extremely selective for Ca^{2+}, and thus powerfully inwardly rectifying with a positive reversal potential. From 1992 to 2005 research on store-operated Ca^{2+} entry followed three general lines: investigations into the properties and regulatory mechanisms for ICRAC and store-operated Ca^{2+} entry, for example, the multifaceted regulation by intracellular and extracellular Ca^{2+} ; endeavors to describe the mechanism by which exhausted Ca^{2+} stores signaled to plasma membrane Ca^{2+} channels; tries to identify the store operated channels.[56]

In 2005 the modern, molecular era of store-operated Ca^{2+} entry started with the early identification of the endoplasmic reticulum Ca^{2+} sensors, STIM1 and STIM2[57] followed shortly thereafter by the Ca^{2+} channel proteins, Orai1, 2 and 3.[58]

3.3. The Physiological Function of SOC Channels

At the level of the organism, it is clear that this process is very important as mutations in the key players, whether arising by chance in humans, or by design in animal models, in all cases creates severe phenotypes considered by general immune deficiencies as well as difficulties in musculo-skeletal development and hitches with ectodermally derived tissues and functions.[59] But what is the origin for these effects at the cellular level? The basic hypothesis has been that these channels assist to refill and conserve endoplasmic reticulum Ca^{2+} stores, which are essential for Ca^{2+} signaling as well as for proper protein synthesis and protein folding. In both excitable cells and non-excitable cells, Ca^{2+} signals utmost normally occur as one or a series of regenerative all-or-none bursts of cytoplasmic Ca^{2+} sometimes termed Ca^{2+} alternations.[60] In excitable cells these often result from the excitable performance of plasma membrane Na^{+} and Ca^{2+} channels. In non-excitable cells, they usually denote episodic releases of stored Ca^{2+}. In the vast majority of cases it is clear that they represent occasional release of stored Ca^{2+} by IP_3; however, opinions differ as to whether it is the IP_3 level which is wavering or whether complex feed forward and feedback mechanisms at the level of the IP_3 receptor can produce alternations in Ca^{2+} release at a constant IP_3 level.[61]

If it is in fact the Ca^{2+} incoming through the plasma membrane SOC channels that creates the critical Ca^{2+} signal, then how does the assumed ordinal nature of the Ca^{2+} oscillations fit with this idea? This may be due to the affiliation between the SOC channel signaling protein STIM1 and store depletion through fluctuations.[62] The all-or-none mechanism for producing Ca^{2+} undulations promises, firstly, that irrespective of the oscillation frequency, each Ca^{2+} spike will lower endoplasmic reticulum Ca^{2+} sufficiently to create SOC entry and some degree of downstream signaling, and secondly, it declares that minor fluctuations in endoplasmic Ca^{2+} will have no influence on the downstream pathways.

3.4. Structural insights into calcium sensing by Stromal interaction molecules

The calcium ion is a universal messenger which switches a vast number of cellular processes such as the extensive regulation of transcription, cell division and apoptosis, as well as more short-lived secretion and contraction.[63] Agonist induced stimulation of G-proteincoupled or tyrosine kinase receptors triggers phospholipase C β or γ2, respectively, leading to the hydrolysis of membrane-associated phosphoinositide 4,5-bisphosphate, yielding inositol 1,4,5-trisphosphate (IP_3). IP is a small diffusible second messenger which binds to the IP_3 receptor (IP_3R) on the ER membrane. Binding of IP_3 allosterically opens this Ca^{2+} release channel and Ca^{2+} moves down the concentration gradient from the lumen into the cytoplasm. The endoplasmic reticulum (ER) lumen can only transiently source the cytosol with Ca^{2+} before it is rapidly depleted. After diminishment of ER/SR stored Ca^{2+}, highly Ca^{2+} selective and permeable store-operated channels on the plasma membrane (PM) open, providing continuous Ca^{2+} influx into the cytosol from the essentially inexhaustible extracellular Ca^{2+} supply; eventually, the cytosolic influx of Ca^{2+} reloads the luminal stores via the SR/ER Ca^{2+} ATPase pump. This specific communicative interchange of Ca^{2+} between the ER/SR lumen, cytosol and extracellular space is termed store-operated Ca^{2+} entry (SOCE). Although the model for SOCE was proposed over two decades ago by Putney, the major molecular players have only been identified and characterized in recent years with the stromal interaction molecules functioning as the ER/SR Ca^{2+} sensors and activators of PM SOCs[57] and the Orai proteins serving as the major PM channel components.[64] Orai1-composed SOCs are termed Ca^{2+} release activated Ca^{2+} (CRAC) channels due to the voltage-independent, highly Ca^{2+} selective and inward-rectifying currents generated during activity, distinct from other SOCs.

3.5. Stromal Interaction Molecule Domain Organization

Human STIM1 is a type I transmembrane protein of 685 amino acids confined on ER/SR membranes or on the PM following post-translational glycosylation of Asn131 and Asn171.[65] Humans encode a second isoform, STIM2, with specific homologous districts to STIM1 inside both the luminal and cytosolic domains. Unlike STIM1, STIM2 (i.e.

open reading frame of 833 amino acids) does not appear to localize on the PM, despite conservation of the STIM1 Asn131 residue. The utmost homologous provinces between STIM1 and STIM2 embrace an EF-hand pair, sterile a motif (SAM) domain as well as two cysteines within the luminal domains and three putative coiled-coil domains, a Lys-rich and a Pro/Ser-rich fragment within the cytosolic region of the protein.

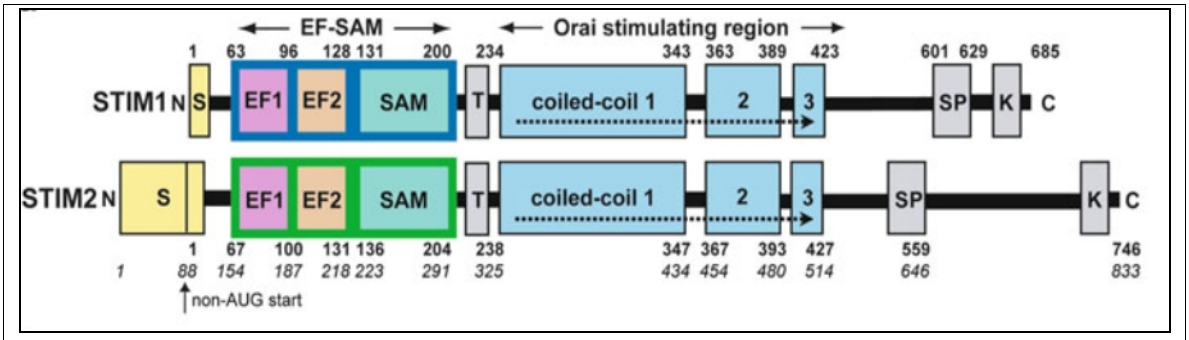

Figure 3.1Primary sequence and domain architecture of STIM1 and STIM2.

Conserved domains in human STIM1 and STIM2. Upstream of the signal peptides, S (yellow), the luminal domains include the canonical EF-hand, EF1 (violet), the non-canonical EF-hand, EF2 (beige), and the SAM domain (green). A single transmembrane pass, T, separates the cytosolic portion that include three putative coiled-coil domains (blue), a serine/proline-rich region, SP, and a lysinerich region, K. Residue boundaries are indicated above and below STIM1 and STIM2, respectively. STIM2 contains a second set of numbering (italics) corresponding to the entire STIM2 open reading frame.

ER localization of STIM1 is signaled through the first 22 amino acids. The process of CRAC activation by ER/SR-residing STIM1 is a multi-step procedure: first, STIM1 oligomerization on the ER membrane occurs in response to ER/SR luminal Ca^{2+} diminution; second, STIM1 homotypic oligomers translocate to ER/SR-PM junctions; third, CRAC channels are enlisted and open at these junctions. The cytosolic regions of STIMs play a role in oligomerization of Ca^{2+} -depleted STIM1, in pointing the molecule to ER/SR-PM junctions and in dealings with Orai1 pore subunits. STIM1 probably interacts with both the N- and C-terminal domains of Orai1 in the foundation and stimulation of CRAC channels. The crucial role of STIM1 in the initiation of CRAC channels is apparent from inhibiting RNA studies which determine a significant decrease in CRAC entry after STIM1 knockdown[66] and from STIM1/Orai1 co-overexpression data which indicate very large escalations in ER Ca^{2+} -depletion dependent SOCE.[67]

3.6. Identification of Orai

Orai was associated to store-operated Ca^{2+} entry through three RNAi screens in Drosophila S2 cells.[68] One screen[64] counted nuclear localization of the transcription factor NFAT, visualized as a human NFAT-GFP fusion protein, to report on persistent Ca^{2+} influx in response to ER Ca^{2+} store diminution. RNAi treatment identified a handful of Drosophila genes whose exhaustion averts nuclear consequence of NFAT-GFP, including a gene annotated at the time as olf186-F, now renamed Drosophila Orai.[64] This finding meshed with the genetic mapping of severe combined immune deficiency (SCID) characteristic in a human family to a region of human chromosome 12 comprising Orai1, a human homolog of Drosophila Orai.[64] Orai is a plasma membrane protein of mass ~33 kDa with four trans membrane helices. The channel is thought to assemble as a tetramer or to reorder into a tetramer upon initiation.[69] Following store depletion, Orai1 reallocates to discrete sites on the cell surface, parallel with the STIM1 "puncta" that have been shown to mark sites of Ca^{2+} influx.[70] Over expressed Orai cooperates with over expressed STIM to create large CRAC currents, beyond native CRAC currents in some cases by two orders of magnitude.[71]

3.7. Channel Gating and Inactivation

The TM1 helices give the principal Ca^{2+} binding site and line the pore of the Orai channel. Taking account of the additional findings that an N-terminal segment of Orai1 between residues 73 and 91 is required for inauguration of the channel by STIM,[72] and that STIM1 binds to the peptides Orai1(68–91) and Orai1 (65–87),[73] it is evenhanded to expect that TM1 movement is part of channel gating. STIM binding to the N-terminal cytoplasmic segment of Orai might, for example, initiate a rearrangement of residues at the selectivity filter or cause flexible sections of the TM1 helices to move apart.[74]

Fast Ca^{2+}-dependent inactivation is the decline in whole-cell CRAC current happening within 100–200 ms of initiating a large inward Ca^{2+}current.[75] Fast Ca^{2+} dependent inactivation is triggered by the prominent local Ca^{2+} near the intracellular mouth of the channel affected, rather than by elevated average Ca^{2+} in the cell, and sets limits on local Ca^{2+} influx.[76] The inactivation is not a simple reversal of gating, since it depends on STIM1 (475–483) and on calmodulin binding to Orai,[77] neither of which is obligatory for

channel opening. It is also exaggerated by the relative expression levels of Orai1 and STIM1.[78] The channel selectivity filter, protein-protein interactions of the N and C termini of Orai, and the intracellular TM2-TM3 loop of Orai have all been concerned in the process by mutational analysis.[79] The sensitivity to changes during the protein indicates that inactivation reflects a rigorous, though not necessarily large, rearrangement of the Orai channel.

3.8. Essential Structures within STIM1 that Mediate Interaction with and Activation of Orai

The luminal N-terminus of STIM1 contains a canonical and a hidden EF-hand as well as a sterile-alpha motif (SAM).[80] The initial trigger for STIM1 oligomerization is represented by its N-terminal EF-hand. Upon store-depletion the EF-hand looses bound Ca^{2+} and consequently allows for aggregation of STIM1 proteins.[70] A luminal Ca^{2+} drop initiates STIM1 oligomerization via deterioration of the entire EF-SAM entity. In line, a STIM1 deletion mutant lacking the whole C-terminus also oligomerizes, yet its aggregates are unstable.[81] The cytosolic C-terminus includes three coiled-coil domains, a serine/prolineand a lysine-rich region.[82]

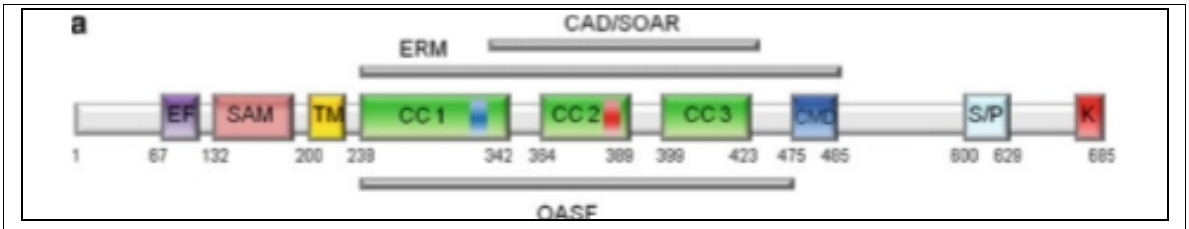

Figure 3.2 Predicted domains within human STIM1.

CAD (CRAC Activating Domain), CC (Coiled Coil domain), CMD (CRAC Modulatory Domain), EF (EF-hand motif), ERM (Ezrin Radixin Moesin like domain), K (polybasic cluster), OASF (Orai Activating Small Fragment), SAM (Sterile Alpha Motif), SOAR (Stim Orai Activating Region), S/P (Serine/Proline rich region), TM (transmembrane domain).

Within the cytosolic part of STIM1 the first coiled-coil domain supports formations of resting STIM1 oligomers.[81] These STIM1 C-terminal deletion mutants shortened after the first coiled-coil domain do not promote oligomerize upon store depletion as assessed by Fluorescence Resonance Energy Transfer (FRET) experiments.[81] A short C-terminal STIM1 fragments (233–420) including all three coiled-coil domains is unable to form elevated order aggregates.[83] Stable store-dependent STIM1 oligomers require all three

coiled-coil domains together with an comprehensive stretch (423–448). C-terminal fragments that contain this oligomerizaton domain are capable to interact and activate Orai1 channels.[84] The minimum domain named CAD (aa 342–448) or SOAR (aa 344–442)[85] that constitutively activates Orai1 channels includes the second (aa 364–389) and third (aa 399–423) coiled-coil domains together with an extended stretch (aa424–442). Shorter STIM1 fragments with deletions to amino acid 420 fail to interact with as well as activate Orai. A molecular interpretation has been provided by Muik in suggesting that aa 421–474 comprise a cytosolic STIM1 C-terminal homomerization domain (SHD). This segment is important for at least dimer creation which is required for both interaction with and activation of Orai channels. Its absence preferentially results in monomeric STIM1 C-terminal fragments.[83] STIM1 mutant with a deletion from residue 440 on still forms puncta and co-clusters with Orai1, yet fails to activate Orai1 currents.[73] Hence, aa 421–440 might characterize the minimum portion of SHD within the cytosolic part of STIM1 that fundamentally contributes to homomerization.

3.9. Structures Within Orai that Mediate Interaction with STIM1

The activation of Orai1 channels by C-terminal STIM1 fragments suggests a direct coupling. Yet such an interaction would require full-length STIM1 to span the distance between the plasma and ER membranes. Varnai et al. have anticipated a space of 11–14 nm between the two membranes. In line, electron microscopy techniques[86] have imaged a tetrameric Orai channel with an stretched out teardrop-shape that reaches 10 nm into the cytosol. In isolated vesicles of yeast, purified STIM1 cytosolic fragments and Orai1 proteins are sufficient for CRAC channel activation, without the prerequisite of other supplementary protein.[74] Hence, there is clear evidence for a direct binding of STIM1 to Orai1 that result in Ca^{2+} entry. Additional components within the CRAC channel signaling complex might rather function in a modulatory manner on the STIM1-Orai1 signaling machinery. Mutations of further hydrophobic amino acids in the C-terminal part of Orai1 have also resulted in an impaired interaction with STIM1.[87] Not only hydrophobic but also six nearby negatively charged residues within Orai1 C-terminus are crucial for coupling to STIM1.[88] Neutralization of a basic cluster (aa 382–387) within the second coiled-coil domain of STIM1 also disrupts coupling to and activation of Orai1

channels. Hence the overall communication between STIM1 and Orai channels is impaired by mutations within either the putative coiled-coil or electrostatic interaction domains in their C-termini. These data point to a functional, hetero-meric STIM1/Orai interaction that provides the coupling machinery with the flexibility for a dynamic assemble and disassemble upon store de- and repletion, respectively.[89] The flexible coupling of STIM1 to Orai channels involves both putative coiled-coil/electrostatic domains located in their C-termini.

3.10. Conformational Rearrangement of STIM1 Accompanies Orai1 Activation

A hetero-meric coiled-coil/electrostatic coupling of the C termini of STIM1 and Orai1 is essential for the activation of Ca^{2+} entry. Such an interaction is preserved with the CAD/SOAR domain of STIM1, signifying that this fragment contains all the requirements for interaction with and activation of Orai1. Hence, further STIM1 C-terminal domains are required that oppose this coupling in store-repleted cells besides the ER-luminal portion of STIM1.[90] This STIM1 sensor assumed a tight conformation in the absence of Orai1, and an extended conformation upon coupling to the channel Disruption of the Orai1 coiled-coil region by the L273S mutation also abolished the coupling to OASF and keeps the sensor in a tight conformation. Hence the extended STIM1 conformation may reflect an intra molecular transition which exposes the CAD/SOAR domain and promotes interaction of full-length STIM1 with Orai1. A similar comprehensive conformation has been engineered via mutations of hydrophobic residues in the first or third putative coiled-coil domain. In addition, the long first coiled-coil segment comprises two sites that apparently control a conformational switch from a tight into an extended conformation. One is in the N-terminal region around the hydrophobic leucine 251 located in the first putative coiled-coil domain.

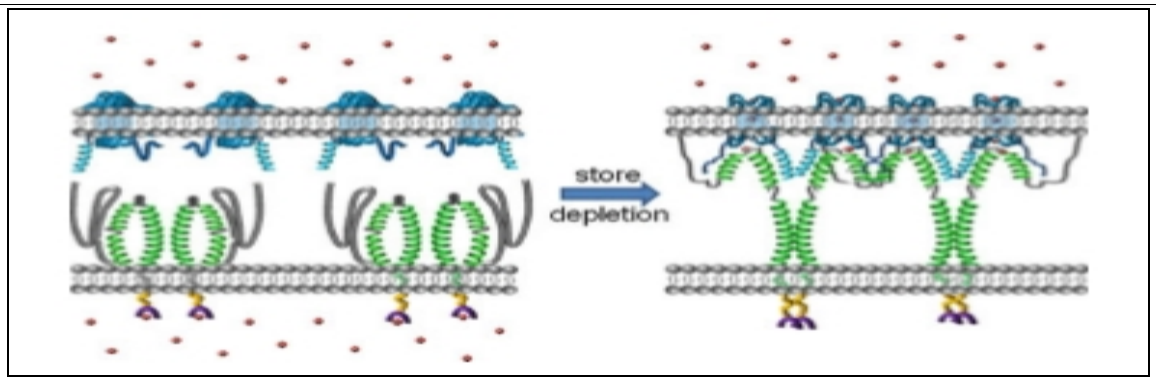

Figure 3.3 Model for STIM1-Orai coupling.

Ca^{2+}-store depletion results in oligomerization of STIM1 mediated by its luminal EF hand and SAM together with cytosolic STIM1 domains. This triggers a conformational switch that exposes the CAD/SOAR domain for interaction with Orai1 C- and N-terminus culminating in the Orai/CRAC activation.

The second site has been recently reported as an acidic cluster toward the C-terminal end of the first coiled-coil, which exhibits a partial homology with an acidic domain in the C-terminal coiled-coil region of Orai1.[91] This acidic STIM1 site has been recommended to form an electrostatic interface with a basic cluster in the second coiled-coil segment of STIM1.

3.11. Gating of Orai Channels

The subsequent steps after the C-terminal interaction of STIM1 and Orai1 resulting in Ca^{2+} influx are starting to emerge. The CAD/SOAR domain also binds to the conserved N-terminal region within Orai,[73] yet weaker than to the C-terminus. Within the N terminus of Orai1, a single-point mutation close to the plasma-membrane (K85E) abrogates establishment of store-operated currents.[92] Nonetheless, binding of a corresponding N-terminal Orai1 peptide including the K85E mutation to CAD is still retained, although possibly a little weaker than that of the wild-type N-terminal peptide. Hence, these experiments propose a role of this lysine in Orai gating. A second residue involved in gating is arginine 91 located in the interface between trans membrane segment 1 (TM1) and the N terminus.A possible mechanism to gate the channel involves a movement of TM1 either through a displacement of the lower portion of TM1 and/or through a reorientation of E106 in the selectivity filter.[64] Two serines (S89 S90) that are directly located between the conserved N-terminal cluster and the TM1 segment, may

also contribute by their flexibility to the movement of TM1 to open the gate. Their substitution to more flexible glycines enhances Orai1 currents, while insertion of rigid prolines at this site abolishes Ca^{2+} currents.[77] Hence, Orai1 gating into the open state may be triggered by interaction of STIM1 with the N-terminus of Orai1 which induces a movement of TM1, supported by the flexible serine and the charged lysine residues.

3.12. Fast Inactivation of Orai Channels by a STIM1 Domain

Ca^{2+}influx through the Orai pore consequences in increased cytoplasmic Ca^{2+} levels close (3–4 nm) to the channel's pore-mouth. As Ca^{2+} overload may lead to apoptosis, an intrinsic negative feedback mechanism termed Ca^{2+}-dependent inactivation (CDI) limits Ca^{2+} inward currents.[93] A cooperative interplay of cytosolic domains within Orai proteins together with calmodulin binding to the Orai N-terminus enables finetuning of fast inactivation of these channels.[79] In addition, fast inactivation is also regulated by a CRAC modulatory domain (CMD) within STIM1. The CMD domain (aa 474–485) includes a cluster of negative amino acids C-terminal to SOAR/CAD/ OASF.[83] This cluster seems to attenuate the affinity of STIM1 C-terminal fragments for Orai and CRAC channels. Beside the CMD, the drug 2-aminoethoxydiphenyl borate (2-APB) modulates coupling of the STIM1 C-terminus to Orai1.[84] A STIM1 fragment (aa 235–505) that is somewhat localized both to plasma membrane Orai1 and the cytosol quickly redistributes to the plasma-membrane upon application of 2-APB and activates Orai1 currents rapidly.[84]

3.13. STIM1, Communicating the Depleted State of the ER to the PM

STIM1 could be considered one of the most significant components of store-operated Ca^{2+} influx complex (SOCIC). The key role of STIM1 in SOCE was found by means of RNAi screenings in S2 Drosophila cells.[66] STIM1 has two determinant functions in SOCE, the first one is to sense the ER Ca^{2+} depletion and the other is to activate the store-operated channels. Both functions suggest the depleted state of the ER to the PM. The STIM1 N-terminal is facing the ER lumen and contains classical and hidden Ca^{2+} binding EF- hands (67–96) and a sterile a-motif (SAM, 132–200). The classical EF-hand binds Ca^{2+} with low affinity (200–600 nM), therefore when the ER is full (near 1 mM) Ca^{2+} is bounded to STIM1 and the protein is in its inactive state. Ca^{2+} unbinding causes a

conformational change in STIM1 resulting in the oligomerization of this protein, and initiates a process that leads to SOCE activation.[94]

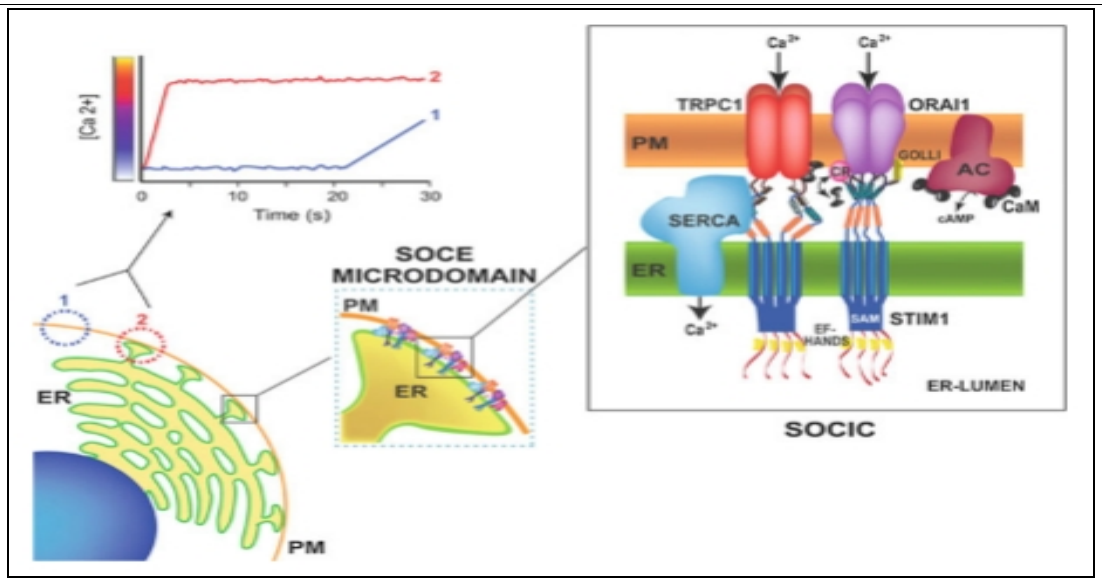

Figure 3.4 A model of SOCE microdomain organization.

Inside the cell, endoplasmic reticulum is organized in such a way that some regions are in close proximity to the plasma membrane. Under ER depletion, these regions function as SOCE microdomains where all molecules underlying Ca2+ entry converge. Inside microdomains these molecules are arranged as many copies of a store-operated Ca2+ influx complex. The inset shows a model of SOCIC organization taking into account the possible components identified so far. This complex include the Ca2+-sensor and channel activator STIM1, the PM-channels TRPC1 and Orai1, the regulator proteins calmodulin, CRACR2A and golli and the proteins related with SOCE physiological functions, such as SERCA and adenylyl cyclase. Restriction of SOCE machinery to microdomains causes localized Ca2+elevations (top-left), thus inside micro domains, Ca2+concentration increases faster than outside. This SOCIC localization may favor also the generation of gradients for Ca2+, cAMP and possibly other second messengers.

The hidden EF-hand and SAM domain interact to facilitate STIM1 clustering STIM1 C-terminal includes a serine/proline-rich domain (600–629) possibly involved in cytoskeleton interactions and a lysine-rich domain (672–685) mediating STIM1 anchoring to the PM and TRPC gating.[95] These polybasic domains are also important for STIM1 translocation to the ER-PM junction. In addition, there is an ERM domain (251–535) encompassing three adjacent alpha-helical coiled-coils. Inside this large domain there is the region accountable for Orai activation (known as SOAR or CAD) and a region related with Ca^{2+} -dependent inactivation of Orai (470–491).[79]

3.14. SERCA, Refilling the ER

One of the most important functions of SOCE is to refill the depleted ER. It has been seen that SOCE activation occurs at ER-PM junctions where Ca^{2+} deliberation transiently increases, avoiding a global Ca^{2+} elevation.[96] This spatial restriction relies in the efficient ER Ca^{2+} recapture mediated by SERCA. Indeed, it has been recently demonstrated that SERCA is a SOCIC component recruited to ER-PM junctions after store depletion.[97] SERCA might be an imperative component of the complex since ER refilling is necessary to maintain cell response to repetitive stimulation by agonists. SERCA transports Ca^{2+} ions to the lumen of the ER in order to keep low Ca^{2+} concentration at the cytoplasm and to concentrate Ca^{2+} inside the ER. SERCA is composed of one trans membrane domain consisting of 10 helices wherein Ca^{2+} binds, and three cytoplasmic domains classified in an actuator domain, a phosphorylation domain and a nucleotide-binding domain.[98] There are three known isoforms of SERCA. Concerning to interaction of SERCA with other SOCIC components, it has been shown that SERCA2 and SERCA3 associate with STIM1 probably by an interaction with its C-terminus.[99]

3.15. Adenylyl Cyclase, SOCE Downstream

Although ER-refilling is one of the most important functions of SOCE, Ca^{2+} elevation in micro domains could activate different Ca^{2+}-dependent signaling pathways, giving SOCE the capacity to generate a diversity of downstream effects. That is the case for cAMP-signaling, which under convinced conditions is specifically stimulated by SOCE.[100] SOCE may increase cAMP synthesis by means of recruiting adenylyl cyclase 8 (AC8) into the SOCIC.[101] Ca^{2+} entry through SOC then stimulates AC8 and potentiates cAMP production. As Ca^{2+} and cAMP are two fundamental second messengers of G-protein-coupled receptor signaling, regulation of cAMP by SOCE could be a way to govern a vast array of cellular functions. Stimulation of AC8 causes an increment in cAMP production and activation of PKA being the most essential link between SOCE and diverse signaling pathways downstream SOCE.

3.16. STIM1/Orai1-Mediated Store-operated Ca^{2+} Entry in Cardiovascular disease

Cardiovascular diseases are the number one killer in the western world secretarial for more than 830,000 deaths yearly in the United States. According to the American Heart Association, cardiovascular diseases claim approximately 1 out of every 3 lives in the developed world. Cardiovascular diseases include hypertension, cardiac hypertrophy, myocardial infarction and vascular occlusive diseases such as atherosclerosis, thrombosis, and restenosis. In most cardiovascular diseases, the initiation occurs in the vascular bed, which supplies blood to the heart; these vascular beds are occluded due to accumulating deposits on their lumen and the vascular wall. For instance, narrowing of the coronary artery lumen results in coronary heart diseases such as angina pectoris and myocardial infarction. Although significant improvement in the treatment and health care management has condensed the number of deaths resulting from cardiovascular diseases, they still remain the largest health issue in developed societies. Hence, there is constant need for discovery, understanding and targeting of potential proteins and mechanisms that contribute to the development of these diseases. The role of Ca^{2+} in mediating cardiovascular diseases is well established.[102, 103] One of the most ubiquitous regulated means of Ca^{2+} influx into cells is the store-operated Ca^{2+} entry pathway.[104] Strong evidence supports the idea that deregulation of the SOCE pathway is an important contributor to the development of cardiovascular diseases.[105]

3.17. SOCE and STIM1/Orai1 Proteins

Under physiological conditions, when Gq/11 receptors or receptor tyrosine kinase that are coupled to phospholipase C (PLC) are activated by their specific agonists, PLC breaks down phosphatidylinositol 4,5 bisphosphate (PIP_2)[106] and generates two second messengers: diacylglycerol (DAG) and inositol 1,4,5 trisphosphate (IP_3).[107] DAG in turn can either directly activate ion channels on the plasma membrane or can generate other second messengers such as arachidonic acid, known to activate the store-independent Arachidonate-Regulated Ca^{2+} (ARC) channels.[108] On the other hand, IP_3 binds to IP receptor nearby on the endoplasmic reticulum resulting in release of Ca^{2+} from the ER into the cytoplasm. This release of Ca^{2+} from the ER results in depletion of intracellular

Ca^{2+} stores causing the activation of SOCE. Hence, SOCE is defined as the Ca^{2+} influx route into the cell via plasma membrane Ca^{2+}-permeable channels activated as a direct outcome of intracellular (primarily ER) Ca^{2+} store depletion. SOCE is a ubiquitous pathway that is conserved during the course of development from lower unicellular eukaryotes to complex species such as Man.

3.18. STIM/Orai1 Proteins and Cardiovascular Diseases

3.18.1. Thrombosis

One of the most important steps in thrombus formation is platelet activation upon stimulation with agonists like thrombin, ADP and thromboxane A. Interestingly, activation of platelets via all these agonist's results in elevation of intracellular Ca^{2+} levels.[109] This increase in cytosolic Ca^{2+} concentration is required for platelet aggregation at the site of thrombus formation.[110] Strong confirmation supports a role for STIM1 and Orai1 proteins in mediating the increase in platelet Ca^{2+} concentration and thrombosis. Grosse et al. established that STIM1 is required for platelet activation. They generated a mouse cell line expressing constitutively active STIM1 by mutating an acidic amino acid (aspartate) to a neutral one (glycine) at position 84 (D84G) located in its EF-hand, using the chemical mutagen N-ethyl-N-nitrosourea. The platelets with EF-hand-mutated STIM1 were shown to have 3-fold higher basal level of intracellular Ca^{2+} in comparison to wild-type platelets.[111]

3.18.2. Atherosclerosis

The role of STIM1 and Orai1 in atherosclerosis is only starting to emerge. It has been reported that in apoE $^{-/-}$ mice, SOCE is increased in vascular smooth muscle cells before development of atherosclerotic plaques.[112] Likewise, it has been demonstrated that both SOCE and expression of STIM1 is significantly higher in coronary smooth muscle cells of pigs fed apro-atherosclerotic high calorie diet in comparison to pigs raised on standard chow diet.[113] In addition, coronary atherosclerosis observed in pigs fed a high calorie diet was attenuated when these pigs were subjected to exercise. These experiments suggest that high fat western diet increases SOCE and STIM1 expression in coronary smooth cells, which correlates with increased atherosclerotic lesions. This increase in SOCE, STIM1 expression and coronary atherosclerosis can be attenuated by exercise.[113] Orai1

mediates SOCE in neutrophils and is involved in neutrophils arrest and recruitment under shear flow.[114]

3.18.3. Hypertension

Hypertension is a chronic pathological condition of the systemic circulation. It has been known for several years as one of the major risk factors for several diseases, including heart failure, vascular occlusive diseases, myocardial infarction, renal failure, stroke, dementia and Alzheimer's disease.[115] Hypertension and increased vascular tone have been associated with elevated cytosolic Ca^{2+} levels and abnormal expression of Ca handling proteins.[116] An increase in the intracellular Ca^{2+} stores has been reported in hypertensive rats in comparison to nor-motensive rats. Involvement of STIM1 and Orai1 in hypertension has just begun to emerge. Giachini et al. observed an increased expression of STIM1 and Orai1 in hypertensive rats in comparison to wild type rats.[117] This group further hypothesized that STIM1 and Orai1 can be a vital player involved in sexual differences associated with hypertension severity and expansion. These gender-dependent differences in hypertension incidence are anticipated, at least partially, to be due to differences in Ca^{2+} handling protein expression, activation and molecular mechanisms involved in cellular Ca^{2+} handling.[118] Expression of STIM1 and Orai1 was reported to be higher in hypertensive male aorta in comparison to hypertensive female and normal aorta. These studies suggest that STIM1 and Orai1-mediated SOCE could contribute to the gender differences observed in the severity of hypertension.[119]

3.18.4. Cardiac Hypertrophy

Cardiac hypertrophy is a chronic disease condition resulting from enlargement of the cardiac tissue primarily due to increase in the size of cardiac myocytes. It leads to weakening of cardiac muscle, decrease in cardiac muscle contractility, relaxation and eventually affecting cardiac pumping. Cardiac hypertrophy is one of the most important risk factors for heart failure and sudden death. It is well recognized that Ca^{2+} plays an crucial role in physiological cardiac myocytes contractility and growth as well as pathological cardiac hypertrophy.[119] Ca^{2+} contributes to cardiac hypertrophy at least partially via Ca^{2+}/calmodulin-dependent kinase (CamK) activation. It has been shown that CamK activity, which regulates a fetal gene program, increases in hypertrophic hearts.[120] The first report indicating contribution of STIM1 in the cardiac hypertrophy

was published by Ohba et al. This group showed that STIM1 is articulated in rat cardiomyocytes; knockdown of STIM1 resulted in abrogation of SOCE mediated by thapsigargin as well as by endothelin-1. These authors further showed that STIM1 knock down results in a robust decrease in NFAT activation caused by endothelin-1 treatment. STIM1 knockdown inhibited the expression of BNP (Brain natriuretic peptide), a cardiac fetal gene and an conventional marker for cardiac hypertrophy. Moreover, STIM1 knockdown significantly reduced the increase in surface area of cardiac myocytes induced by hypertrophic stimuli, suggesting STIM1 as an central player in the activation of cardiac fetal gene program and the agonist-mediated increase in cell size of cardiac myocytes. In addition, STIM1 and Orai1 knockdown resulted in a significant decrease in spontaneous Ca^{2+} transients in cardiomyocytes. However, only STIM1 knockdown reduced the caffeine-mediated Ca^{2+} release from the sarco-plasmic reticulum while Orai1 knockdown had no significant effect, suggesting that STIM1 might be involved in maintaining sarco-plasmic reticulum Ca^{2+} stores.[121]