TRPC1 BINDING OF CAVEOLIN-1 SCAFFOLDING DOMAIN REGULATES STORE-OPERATED CA2+ INFLUX IN ENDOTHELIAL CELLS

BY

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I. INTRODUCTION

The endothelium is a monolayer of cells that line the inside of all the blood vessels in the entire circulation. The primary function of the endothelium is to maintain a selectively permeable barrier between the circulating blood and the tissues. Septic shock, hypertension, hypercholesterolaemia, diabetes, atherosclerosis, and inflammation all contain endothelium dysfunction within their pathologies. During endothelium dysfunction, there is increased barrier permeability due to endothelial contraction and gap formation between the endothelial cells. Thrombin, histamine, and reactive oxygen species are pro-inflammatory mediators that increase vascular permeability, in part by activating calcium sensitive signaling pathways (Tiruppathi et al., 2003). Resting endothelial intracellular calcium concentration ($[Ca^{2+}]_i$) is 30-100 nM, whereas after thrombin stimulation this concentration is raised five to ten times higher of which about seventy-five percent is contributed by store-operated calcium entry (SOCE) (Tiruppathi et al., 2002). SOCE is activated by thrombin stimulation of endothelial cell surface protease-activated receptor-1 (PAR-1), which causes a rapid and transient increase in ($[Ca^{2+}]_i$) due to the release of calcium (Ca^{2+}) stored in the endoplasmic reticulum (ER) and subsequent Ca^{2+} entry induced by ER depletion (Sandoval et al., 2001a). The Ca^{2+} entry through plasma membrane cation channels activated by Ca^{2+} store depletion is a critical determinant of increased endothelial permeability (Sandoval et al., 2001a; Sandoval et al., 2001b; Tiruppathi et al., 2002). In endothelial cells, the plasma membrane cation channels activated by Ca^{2+} store depletion are known as store-operated cation channels (SOCCs) and contribute to the entry of Ca^{2+} into cells (Nilius and Droogmans, 2001). Transient Receptor Potential Channel 1 (TRPC1) is the primary isoform in human endothelial cells that operate as a SOCC.

TRPC1 has been shown to be localized within cholesterol-rich invaginations of the cell membrane called caveolae (Brazer et al., 2003). Caveolae are coated with a 22 kDa protein, caveolin-1 (Cav-1). Studies have shown that Ca^{2+} influx occurs via caveolae in response to ER store Ca^{2+} depletion in endothelial cells (Isshiki et al., 2002). Further, Cav-1 appears to be necessary for anchoring TRPC1 to caveolae (Lockwich et al., 2000), but it is unclear how this interaction regulates TRPC1 function in endothelial cells. The Cav-1 scaffolding domain (CSD), located between residues 82 and 101 of Cav-1, binds many signaling molecules including endothelial nitric oxide synthase (eNOS), *Src*-like kinases, Ha-Ras, and heterotrimeric G-proteins (Schlegel and Listanti, 2001). Binding of these proteins to the CSD in many cases negatively regulates their function (Drab et al., 2001). For example, binding of eNOS to the CSD, holds eNOS in an inactive state. Bucci et al. (2000) observed inhibition of acetylcholine-induced nitric oxide (NO) production and vasodilation in bovine aortic endothelial cells by a cell membrane permeable CSD-peptide. Moreover, systemic administration of the cell-permeable CSD-peptide prevented acute vascular inflammation in mice. Another study demonstrated that CSD-peptide administration markedly reduced platelet-activating factor-induced increase in microvessel permeability in rats (Zhu et al, 2004). Recently, Bernatchez et al. (2005) have shown that the CSD sequence containing the residues 89-95 is sufficient to inhibit eNOS activity and NO release from endothelial cells.

Ca^{2+} signaling in the endothelium is critical in regulating endothelial permeability. The study of the regulation of TRPC1, a significant component of SOCE, by Cav-1 will help elucidate the complex system that regulates endothelial cell barrier function. Developing an inhibitor of increased endothelial cell permeability will help treat many diseases such as septic shock and acute lung injury.

II. LITERATURE REVIEW

A. Acute Lung Injury

1. Definition

Rubenfeld et al. (2005) estimate there are 190,600 cases of acute lung injury in the United States per year, with 74,500 deaths. Acute lung injury and the more severe form, acute respiratory distress syndrome, are characterized by inflammation and a passage of water, protein, and polymorphonuclear leukocytes into the alveolar space which inhibits the movement of oxygen into the blood stream and leads to hypoxemia and respiratory failure (Kazemi, 1984). Several causes include pneumonia, sepsis, pulmonary contusion, and blood transfusions, among others (Atabai and Matthay, 2002; Silliman et al., 2005).

2. Thrombin Induced Endothelial Barrier Dysfunction

The primary event in acute lung injury is the disruption of the endothelial-capillary boundary. This is the first of three phases called the exudative phase (Figure 1). During this phase, there is a disruption in the endothelium, capillary and alveolar basement membranes, and the alveolar epithelium that allows water, protein, and inflammatory cells into the interstitium and alveolar space. This occurs between 24 and 72 hours after the initial injury (Udobi et al., 2003). The next phase is called the proliferative phase in which there is type II alveolar epithelial cell and fibroblastic proliferation. Some patients go on to the final fibrotic stage in which there is fibrosis of the interstitium. In addition to its role in fibrogenesis, thrombin has been shown to induce endothelial barrier dysfunction and thus contribute to the exudative phase of the acute lung injury pathology (Bogatkevich et al., 2005; Malik and Horgan, 1987; Dudek and Garcia, 2001; Patterson et al., 2001; Siflinger-Birnboim and Johnson, 2003).

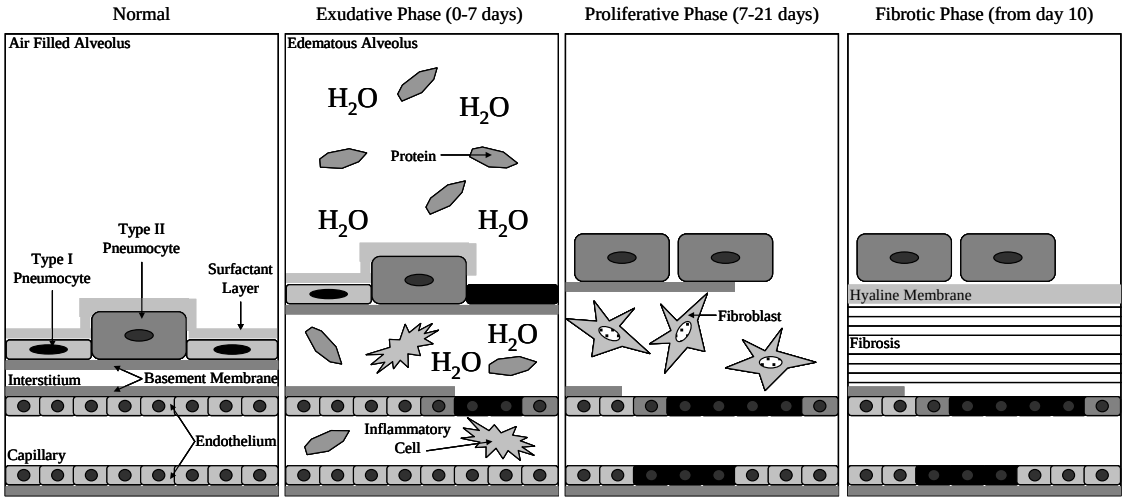

Figure 1: Phases of Acute Lung Injury. Panel 1 shows intact barriers between the alveolus and capillary. After initial injury, panel 2 shows disruption of the barriers and influx of water, protein and inflammatory cells into the interstitium and alveolus. Panel 3 shows proliferation of Type II pneumocytes and fibroblasts. The final panel shows fibrosis of the interstitum and hyaline membrane development.

a. **Endothelium**

i. **Function**

By responding to physical and chemical stimuli, endothelial cells participate in the maintenance of blood pressure, blood clotting, angiogenesis, edema, and atherosclerosis (Esper et al., 2006). The primary function of the endothelium is to maintain a selectively permeable barrier between the circulating blood and the tissues. By carefully regulating the movement of plasma proteins out of the bloodstream, the endothelium allows molecules to exit into the tissues while retaining water in the blood (Levick, 2000). The endothelium also regulates blood flow locally by synthesizing and secreting NO, prostacyclin, and endothelin to the vascular smooth muscle (Luscher et al., 1995; Rubanyi, 1991). In addition to acting on vascular smooth muscle,

the secretion of NO and prostacyclin also inhibits the aggregation of platelets (Radomski et al., 1990; Smith et al., 1980). On the other hand, by secreting von Willebrand factor, the endothelium provides an important component of the clotting cascade (Perutelli and Mori, 1997). The endothelium also recruits circulating white blood cells and immunoglobulins into pathogen infected and inflamed tissue (Wagner and Roth, 2000). Finally, endothelial cells initiate the creation of new blood vessels (Risau, 1997, Klagsbrun and D'Amore, 1991).

ii. **Structure**

The endothelium is a thin, flat monolayer of cells with a "cobblestone" appearance that line the inside of blood vessels (Ryan and Ryan, 1980). Three types of contractile protein structures, the cortical web, the junction-associated actin filament system, and stress fibers play a role in the determination of cell shape and barrier permeability (Figure 2) (Garcia and Schaphorst, 1995). The cortical web, a thin layer of actin filaments, is anchored to the cell membrane on both the luminal and abluminal surfaces. It is thought to act as a scaffold to immoblize some membrane proteins (Drenckhahn and Ness, 1997). The junction –associated actin filament system is a ring of actin filaments around the cell perimeter that attaches to intercellular junctions (Schnittler et al., 2001). Transmembrane adhesive proteins that form three types of junctions: tight junctions, adherens junctions, and gap junctions, allow endothelial cells to maintain normal barrier function (Figure 2) (Bazzoni and Dejana, 2004). The expression and the arrangement of these junctions depend on the vessel the endothelial cells line as well as the organ that is being perfused by the vessel. Tight junctions form a diffusion barrier that modulates the passing of ions, water, and macromolecules through the intercellular space (Balda and Matter, 1998). Leukocyte paracellular permeability, contact inhibition of endothelial cell growth, angiogenesis regulation, and apoptosis all involve adherens junctions (Bazzoni and

Dejana, 2004). Gap junctions mediate cell to cell communication by passing small molecular weight solutes between communicating cells (Hirschi, 2003). The last type of contractile protein structure is stress fibers. They are a combination of actin and myosin filaments that attach to the abluminal cell membrane at focal contacts and align themselves along the direction of blood flow (Gotlieb, 1990). Their purpose is to resist the force the blood flow exerts on the cell.

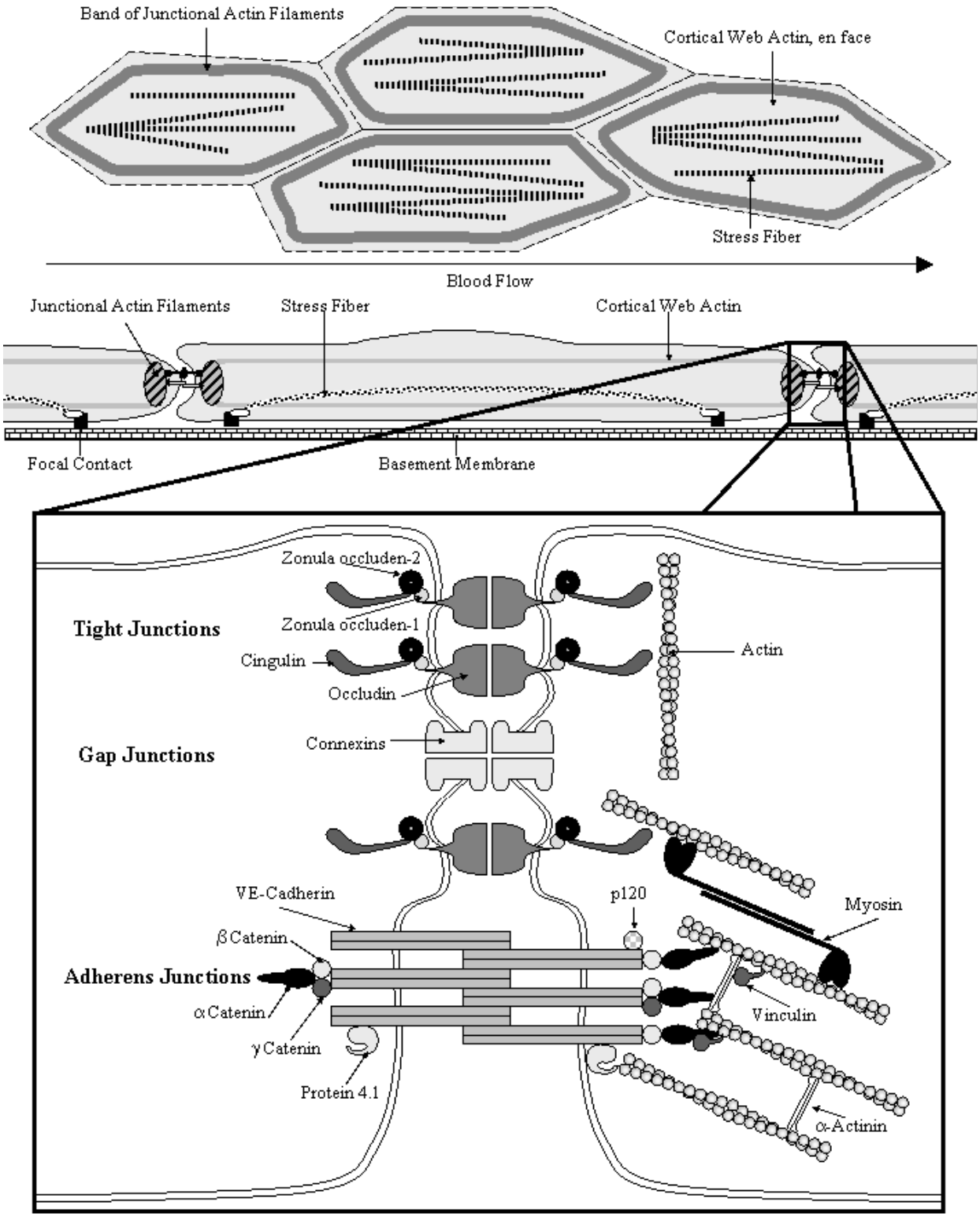

Figure 2: Endothelial Contractile and Junctional Protein Structures. The top panel shows endothelial contractile proteins en face. The bottom panel shows a magnified view of three types of endothelial junctions, tight junctions, gap junctions, and adherens junctions.

b. **Thrombin**

i. **Activation**

After the initial injury of acute lung injury, tissue factor is released and starts the coagulation cascade in which a clotting factor (inactive enzyme precursor) undergoes proteolysis and becomes an active protease, which in turn proteolyzes the next clotting factor precursor (Figure 3) (Hambleton, 2001; Laterre, 2003). Ultimately, activated factor X_a, in the presence of Factor V_a, Ca^{2+}, and membrane phospholipids, cleaves the prothrombin bound by Ca^{2+} to the platelet phospholipids surface (Mann et al., 1990). The cleavage of prothrombin releases thrombin, a serine protease that circulates in the bloodstream and is responsible for converting fibrinogen into fibrin during clot formation and perpetuating the pathophysiology of acute lung injury (Grand et al., 1996; Idell, 2003; Miller et al., 2002).

ii. **Structure**

The first computer generated molecular models of thrombin were developed based on its homology with another serine protease, trypsin (Furie et al., 1982). After the cleavage of prothrombin, the active enzyme consists of a 36-residue A chain and a 259-residue B chain covalently linked by a disulfide bridge (Figure 3) (Bode et al., 1992). The active site containing the catalytic triad of H57, D102, and S195 is located on the B chain (Di Cera et al., 1997). Along the rim of the active site there are two insertion loops called the 60-loop and the γ-loop (Huntington, 2005). The rigid, hydrophobic 60-loop provides a cap over the active site. The more flexible, hydrophilic γ-loop can interact with the substrate. Thrombin also contains two anion-binding exosites, sites outside the active site that contain a sequence of basic residues, important in interacting with negatively charged areas on substrates. Anion-binding exosite 1

has been shown to be particularly important in the binding and cleavage of proteinase activated receptor-1 (Myles et al., 2001).

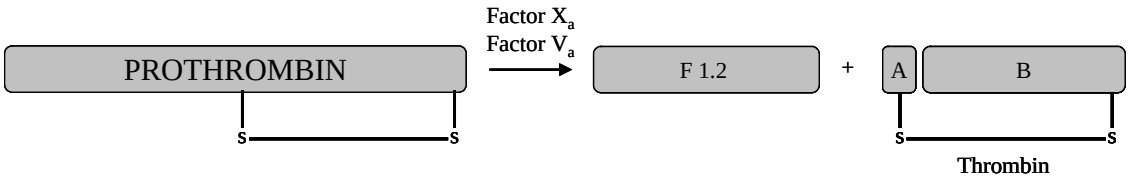

Figure 3: Thrombin Cleavage and Structure. Prothrombin is cleaved by Factor X_a, in the presence of Factor V_a, into three fragments. These fragments include fragment F1.2 and fragment A and B, which are connected by a disulfide bridge.

iii. **Function**

In addition to the conversion of fibrinogen into fibrin, thrombin also induces an increase in endothelium permeability directly by promoting cell contraction and the formation of endothelial gaps (Malik and Horgan, 1987; Lum and Malik, 1996, Kawkitinarong et al. 2004, Siflinger-Birnboim and Johnson, 2003). Increases in endothelial permeability are regulated by increases in $[Ca^{2+}]_i$ (Figure 4). When the $[Ca^{2+}]_i$ rises, Ca^{2+}/calmodulin activates the enzyme myosin light chain kinase, which is responsible for the phosphorylation of the 20 kd myosin light chain (Garcia et al., 1995). When myosin light chain is phosphorylated, it increases the actin-myosin interaction which contracts the cell (Dudek et al., 2001). An alternative pathway involves the binding of Ca^{2+} to protein kinase C alpha (PKCα) and the subsequent binding of diacylglyerol (DAG), which activates PKCα to directly phosphorylate myosin light chain

(Bolsover et al., 2003). PKCα also indirectly phosphorylates myosin light chain by activating Rho kinase, which then phosphorylates myosin light chain phosphatase, inhibiting the enzyme (Amano, 1997). Activation of PKCα also allows the phosphorylation of β-catenin of the vascular endothelial cadherin complex, which leads to the disruption of adherens junctions and thus an increase in permeability (Yuan, 2002).

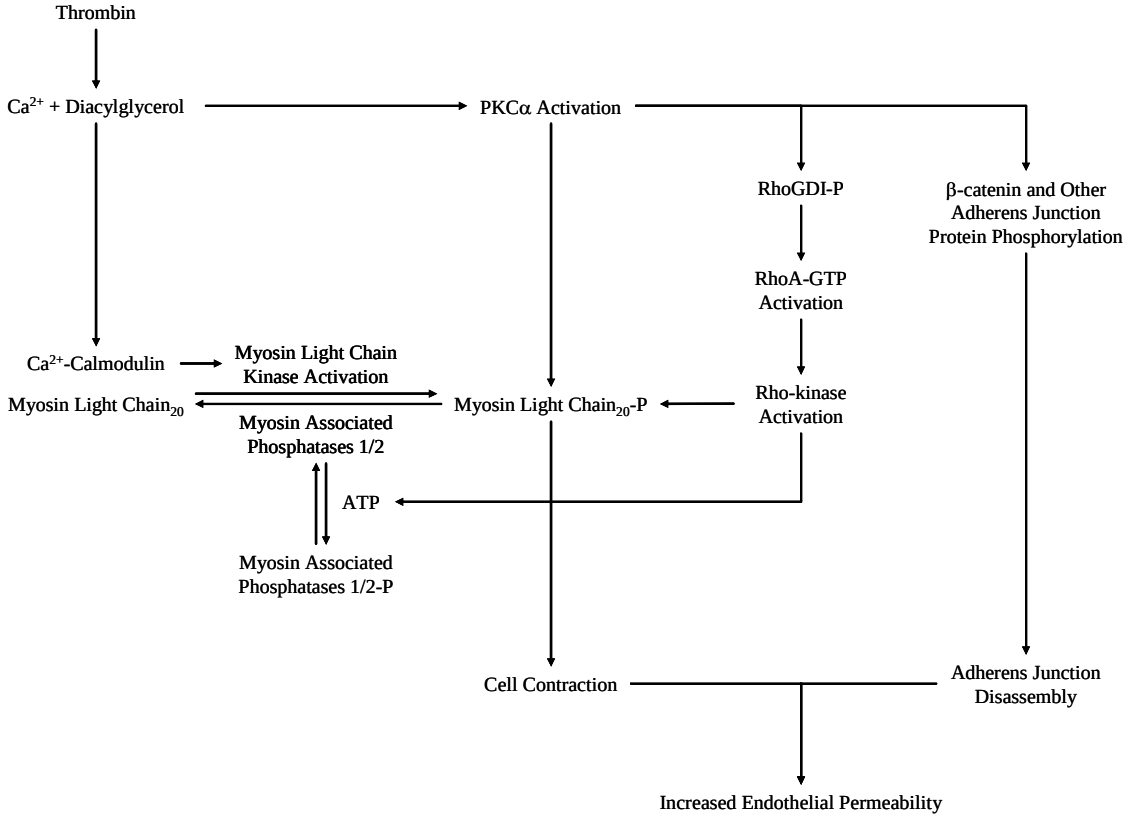

Figure 4: Mechanism of Thrombin Induced Increase in Endothelial Permeability. Thrombin induced increases in $[Ca^{2+}]_i$ increases the Ca^{2+}/Calmodulin concentration and activates myosin light chain kinase, which phosphorylates myosin light chain and leads to cell contraction. Increased $[Ca^{2+}]_i$ also activates PKC_α, which leads to myosin light chain phosphorylation directly or through a Rho dependent pathway and causes cell contraction. PKC_α also phosphorylates β-catenin and other adherens junction proteins to cause adherens junction disassembly. Cell contraction and adherens junction disassembly leads to increased endothelial permeability.

B. **Store-Operated Calcium Entry**

Our lab has shown that Ca^{2+} entry from outside the cell is as important as the release of ER Ca^{2+} in regulating endothelial permeability (Sandoval, 2001b; Tiruppathi, 2003). The mechanism of increasing cytoplasmic Ca^{2+} that our lab is interested in is thrombin stimulated SOCE (Figure 5).

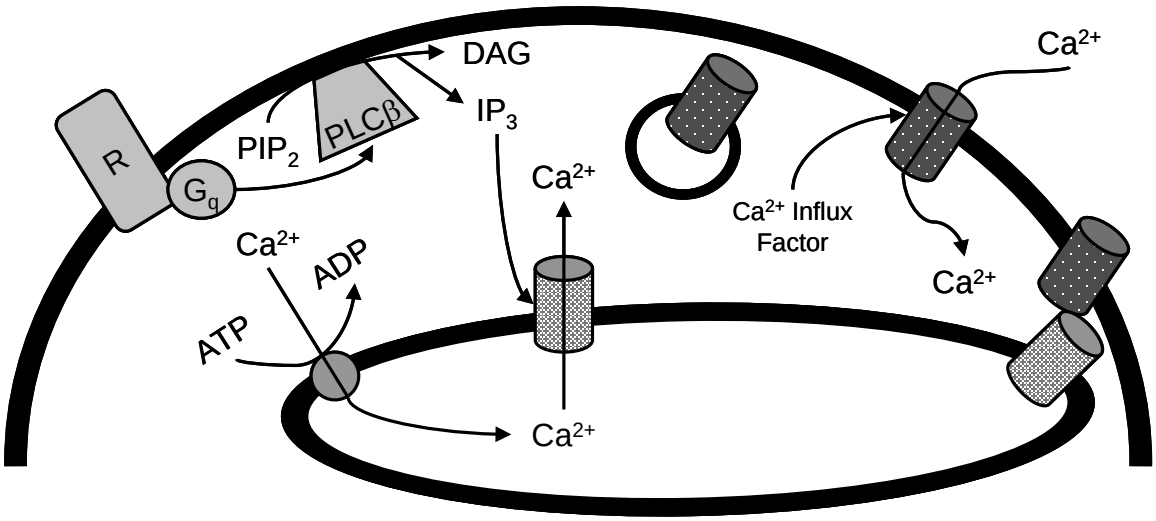

Figure 5: Store-operated Calcium Entry. When a receptor such as PAR-1 is activated by a pro-inflammatory mediator, like thrombin, $G_{\alpha q}$ is released and activates PLC_{β}. PLC_{β} produces IP_3, which stimulates the IP_3 receptor on the ER to release Ca^{2+} from the store. Three hypothetical mechanisms exist for the activation of Ca^{2+} influx after Ca^{2+} release from the ER. First, there is exocytosis of TRPC1 containing vesicles to the plasma membrane. Second, a Ca^{2+} influx factor activates the channel on the membrane. Third, TRPC1 interacts directly with the IP_3 receptor. Half circle is the plasma membrane, while the oval symbolizes the ER inside the cell. Dark cylinders indicate TRPC1. Light cylinders indicate IP_3 receptor. Rectangle labeled R indicates PAR-1. The circle on the ER membrane is a Ca^{2+} ATPase pump.

1. **Protease-Activated Receptor-1**

a. **Structure**

Thrombin stimulates SOCE by activating the PAR-1 receptor. PAR's are expressed in various cell types including platelets, skeletal muscle, vascular smooth muscle, fibroblasts, epithelial cells, and endothelial cells among others (Hirano and Kanaide, 2003). There have been four types of proteinase-activated receptors cloned to date. All four PAR's have seven transmembrane domains, functionally couple to heterotrimeric G proteins, and are activated by cleavage of the receptor (Barnes et al., 2004). PAR-1, PAR-3, and PAR-4 are all activated by thrombin cleavage, whereas PAR-2 is activated by trypsin or tryptase cleavage (Coughlin, 1999). In the vasculature, PAR-1 and PAR-2 are located on endothelial cells, while PAR-3 and PAR-4 are located on platelets (Hirano and Kanaide, 2003). Studies using PAR-1 knockout mice show that PAR-1 is critical in mediating thrombin induced permeability in pulmonary microvessels (Vogel et al., 2000).

b. **Activation**

Through its protease activity, thrombin activates proteinase activated receptor-1 (PAR-1) on the endothelial plasma membrane to stimulate SOCE (Bogatcheva et al., 2002). Thrombin activates PAR-1 by cleaving the extracellular N terminus between residues Arg-41 and Ser-42, it creates a tethered ligand with an amino acid sequence of SFLLRN (Figure 6) (Vu et al., 1991). A peptide of this sequence has been shown to activate PAR-1 as well. This tethered ligand binds PAR-1 on the second extracellular loop and activates the receptor to release the G proteins $G_{12/13}$, $G_{i/o}$, and G_q (McLaughlin et al., 2005; Ellis et al., 1999a; Vanhauwe et al., 2002). G_q is

responsible for activating the next step in SOCE. After PAR-1's release of its G-proteins, within thirty minutes the C-terminus tail can be phosphorylated and signaled for internalization where a large portion moves into lysosomes to be degraded (Macfarlane et al., 2001; Ellis et al., 1999b). PAR-1 from a preformed intracellular pool surfaces after ninety minutes, while it takes eighteen hours for newly synthesized PAR-1 to reach the membrane (Ellis et al., 1999b).

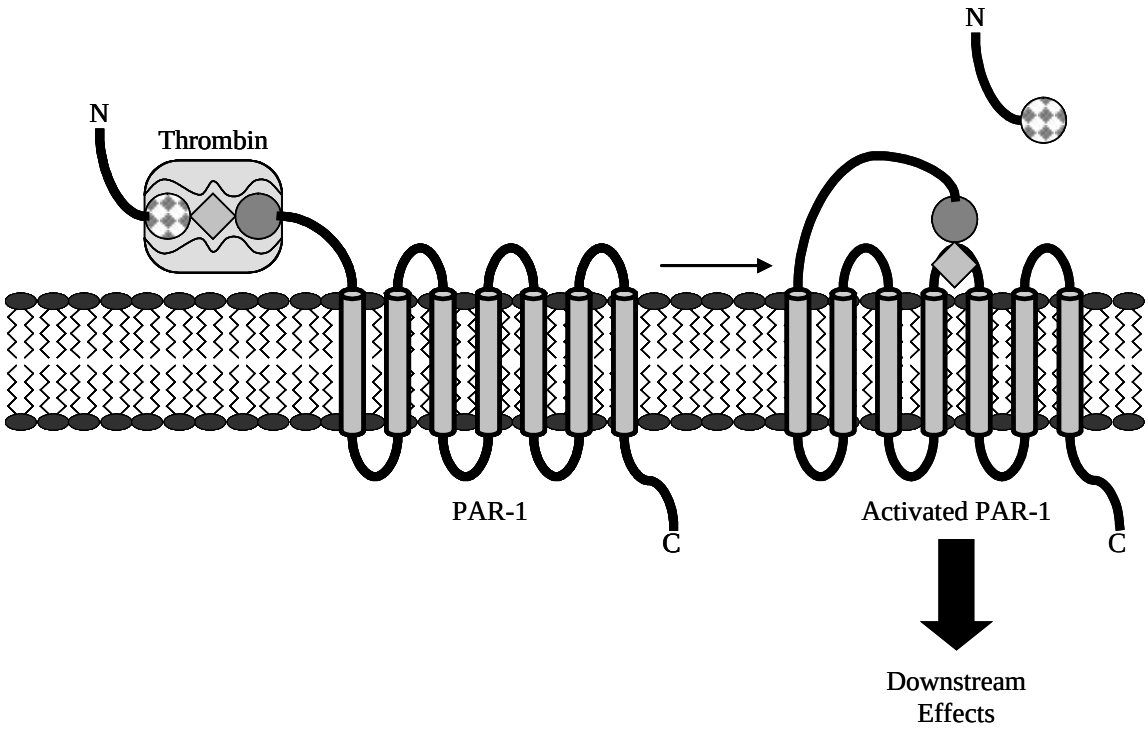

Figure 6: Thrombin Activation of the Proteinase Activated Receptor-1. Thrombin cleaves the N-terminus of PAR-1 to create a tethered ligand that activates the receptor. The dark circle and the circle with diamonds represent the sites thrombin recognizes amino terminal and carboxy terminal to the cleavage site. The diamond represents the tethered ligand sequence, SFLLRN, created after thrombin cleavage.

2. **Inositol-1,4,5-trisphosphate**

Inositol-1,4,5-trisphosphate (IP_3) is a critical component in G-protein mediated SOCE. It is generated when G_q stimulates PLC_β to hydrolyze phophatidylinositol-4,5-bisphophate to produce 1,2-DAG and inositol-1,4,5-trisphosphate (IP_3) (Sternweis and Smrcka, 1992). IP_3 diffuses through the cytosol to bind the IP_3 receptor on the ER membrane. (Freichel et al., 1999). The IP_3 receptor consists of a tetramer of proteins with six transmembrane domains and cytosolic amino and carboxy termini that form a channel that has a high Ca^{2+} conductance (Moore et al., 1998). This allows Ca^{2+} to exit through the IP3 receptor into the cytosol. The Ca^{2+} from the cytosol then stimulates Ca^{2+} to enter from outside the cell through one of three ways. One, the Ca^{2+} released from the ER causes a Ca^{2+} influx factor to stimulate Ca^{2+} entry through a channel on the plasma membrane (Randriamampita and Tsien, 1993). Two, the initial Ca^{2+} release stimulates the exocytosis of the channel to the plasma membrane and thus allows Ca^{2+} to enter the cell (Fasolato et al., 1993; Yao et al., 1999). Three, the IP3 receptor directly binds the channel and stimulates Ca^{2+} entry (Putney, 1999; Boulay et al., 1999).

3. **Transient Receptor Potential Channel 1**

Previous studies have identified the channels that allow Ca^{2+} entry following ER depletion of Ca^{2+} as the mammalian homologues of TRP gene family of channels (Nilius and Droogmans, 2001; Tiruppathi et al., 2003). The TRP genes encode a superfamily of proteins with 6 transmembrane helices (Figure 7). This superfamily is divided into 7 subfamilies: TRPC (Canonical or Classical), TRPV (Vanilloid), TRPM (Melastatin), TRPA (Ankyrin), TRPML (Mucolipin), TRPP (Polycystin), and the TRPN (no mechanoreceptor potential C [NOMPC]) (Pedersen et al., 2005; Montell et al., 2002). The TRPC subfamily contains 700 to 1000 amino

acids and 7 isoforms (TRPC1 to 7) that are expressed in mammalian cells. TRPCs are grouped into 4 subfamilies. i) One group consists of TRPC4 and TRPC5. Their activation is dependent on Ca^{2+}-store depletion and they have high Ca^{2+} selectivity as assessed by their sensitivity to La^{3+} (Nilius and Droogmans, 2001). TRPC4 and TRPC5 are activated by G protein-coupled receptors and receptor tyrosine kinases coupled to PLC. ii) TRPC1 is closely related to TRPC4 and TRPC5; although it forms SOCCs, it is a less selective Ca^{2+} channel. iii) TRPC3, TRPC6, and TRPC7 form store-independent non-selective cation channels activated by DAG (Dietrich et al, 2003); however, a store-dependent activation mechanism has been described for human TRPC3 (Liu et al., 2000). iv) TRPC2 function is unclear and it is believed to be a pseudogene in humans (Montell et al., 2002).

TRPC1 is the channel that is responsible for the SOCE in human endothelial cells (Brough et al., 2001; Moore et al., 1998). On the other hand, TRPC4 is the predominant isoform in mouse endothelial cells (Tiruppathi et al., 2002). Like other TRPC's, TRPC1 is composed of six transmembrane helices with a pore-forming loop between the fifth and sixth helix (Nilius and Droogmans, 2001). The N and C terminus are both cytosolic, with several Ankyrin domains on the N-terminus. A TRP domain, proline rich domain, and Calmodulin binding domain are all located on the C-terminus (Nilius et al., 2003).

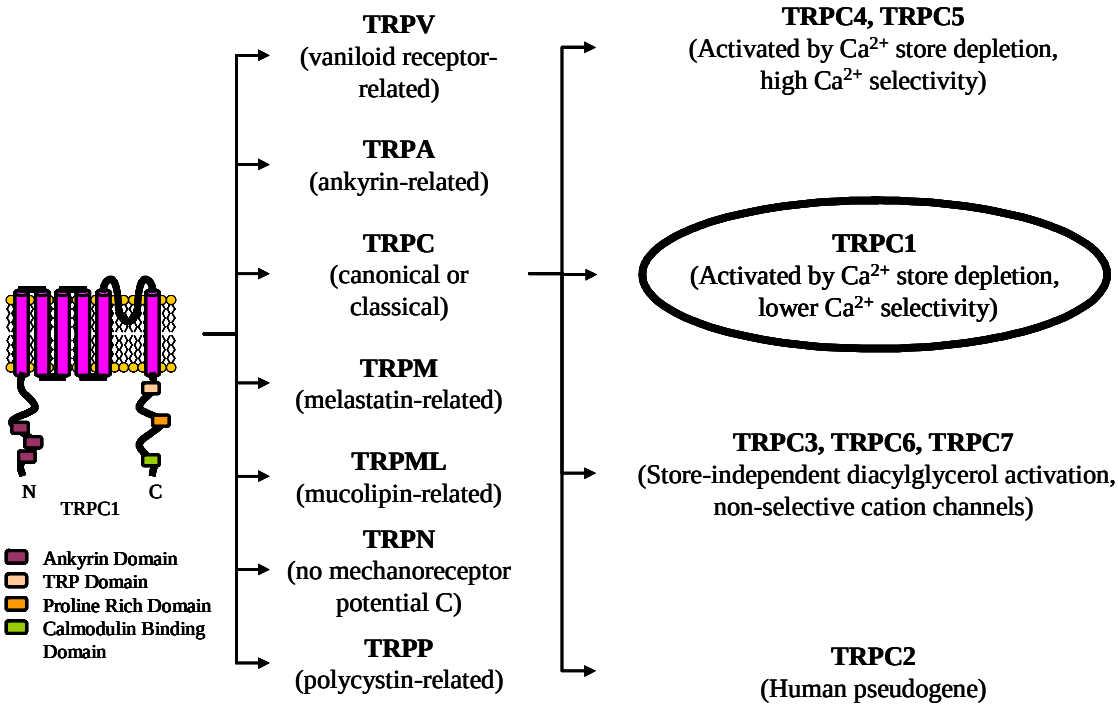

Figure 7: Transient Receptor Potential Superfamily. All TRP channels contain six transmembrane domains and cytosolic N and C termini. They also contain a pore between the fifth and sixth transmembrane domain.

C. **Caveolae**

1. **Structure and Function**

Caveolae are detergent insoluble, sphingolipid and cholesterol rich plasma membrane domains (Anderson, 1993). Due to the high cholesterol content, caveolae are sensitive to cyclodextrin treatment. Cyclodextrin sequesters cholesterol from the plasma membrane, which then flattens caveolae (Rothberg et al., 1992). Caveolae can function as vesicles that participate in endocytosis and transcytosis (Gratton et al., 2004). More importantly, in the case of SOCE, they function as signal transduction domains. With the help of Cav-1, caveolae's coat protein, signaling proteins, enzymes, and channels are assembled into a signalplex (Montell, 2005).

2. **Role of Caveolae in Store-Operated Calcium Entry**

Several studies have shown that caveolae play an integral part in SOCE. Isshiki et al. (1998) originally observed that Ca^{2+} waves originated along Cav-1 rich cell edges. In another study, Isshiki et al. (2002) engineered the Ca^{2+} sensor yellow cameleon to target either the plasma membrane, caveolae, or the cytoplasm. The sensor targeted to the caveolae detected transiently higher Ca^{2+} when the ER is depleted of Ca^{2+}. Brazer et al. (2003) studied the importance of Cav-1 in SOCE. In human submandibular gland cells they co-immunoprecipitated and co-localized HA tagged TRPC1 and YFP tagged Cav-1 using confocal microscopy. They also found that the Cav-1 binding domain in the N-terminus of TRPC1 is important to localize the channel to the membrane. Finally, Bergdahl et al. (2003) also found that cav-1 was key in SOCE. In caudal artery sections they colocalized TRPC1 and cav-1 using immunoflourescene and after methyl beta cyclodextrin treatment found a decreased amount of co-localization.

3. **Caveolin-1**

As mentioned earlier, caveolae are coated on the cytoplasmic side of their invaginations by a 22-24 kDa protein, cav-1 (Rothberg et al., 1992). Cav-1 has a hydrophobic domain and cytoplasmic N and C termini (Figure 8). High molecular mass oligomers containing 14-16 individual molecules are formed by cav-1, which serves as a scaffold for many proteins and channels (Schlegel and Lisanti, 2001). These oligomers have also been found to bind G_q and concentrate it in caveolae (Oh and Schnitzer, 2001). In addition, cav-1 travels between the ER and caveolae as a cytosolic complex containing chaperone proteins and cholesterol (Gratton et al., 2004). Cav-1 knockout mice showed an absence of caveolae. Measurements made in the vascular smooth muscle cells of these mice show an increase in NO production and a decrease in Ca^{2+} signaling (Drab et al., 2001). Cav-1 is also a substrate for the *Src* family kinases (Minshall

et al., 2003). *Src* kinase phosphorylates cav-1 on tyrosine residue 14. This phosphorylation may result in signal vesicle formation and trafficking (Minshall et al., 2000).

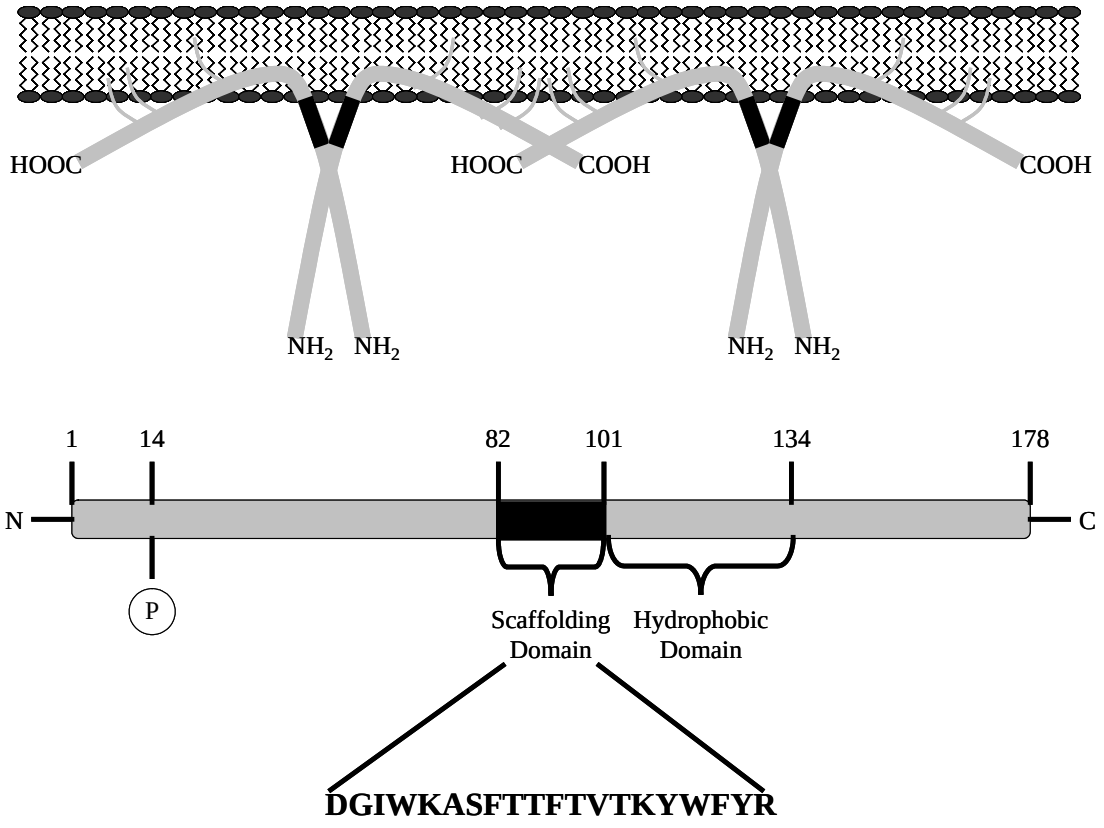

Figure 8: Caveolin-1 Structure. Cav-1 has cytosolic N and C termini with a hydrophobic domain. The scaffolding domain is located N terminal to the hydrophobic domain at amino acids 82-101. Cav-1 is also phosphorylated at tyrosine 14.

4. **Caveolin-1 Scaffolding Domain**

The scaffolding domain of cav-1 binds many molecules including receptor tyrosine kinases and their downstream targets, non-receptor tyrosine kinases, G-protein coupled receptors and their downstream signaling molecules, and regulated enzymes (Schlegel and Lisanti, 2000). Cav-1's scaffolding domain is located between amino acids 82 and 101 and is on the N-terminal side of the hydrophobic domain (Figure 8). Bucci et al. (2000) was one of the first to create a peptide of the scaffolding domain and attached a positively charged antennapedia sequence to it, with a biotin label to study its importance in signaling. They originally used this peptide to study its effect on eNOS activation. They incubated cross sections of aortic rings for 20 hrs with 100 μM of the peptide and then stained with an avidin-horseradish peroxidase complex. The peptide entered the endothelium and the adventitia. They also found that the peptide inhibited vascular relaxation in response to acetylcholine. Zhu et al. (2004) found that the CSD peptide inhibited the increase in hydraulic conductivity in response to platelet activating factor. Platelet activating factor generates NO which increases the hydraulic conductivity. They also used L-NMMA, a NOS inhibitor, to show that the inhibition was similar to the CSD peptide.

Increasing $[Ca^{2+}]_i$ via SOCE is important in regulating endothelial permeability (Sandoval et al., 2001b; Tiruppathi et al., 2003). TRPC1 is the channel identified as the SOCC in human endothelial cells (Nilius and Droogmans, 2001). Others have shown that SOCE and TRPC1 are localized in caveolae (Isshiki et al., 1998; Brazer et al., 2003; Bergdahl et al., 2003). Cav-1, caveolae's coat protein is known to bind and regulate many proteins and enzymes. Taking all that into account the focus of my studies is to elucidate how the CSD interacts with TRPC1 and alters SOCE.