CHAPTER 1

LIPID RAFTS, CAVEOLAE AND GPI-LINKED PROTEINS

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Abstract: Lipid rafts and caveolae are specialized membrane microdomains enriched in sphingolipids and cholesterol. They function in a variety of cellular processes including but not limited to endocytosis, transcytosis, signal transduction and receptor recycling. Here, we outline the similarities and differences between lipid rafts and caveolae as well as discuss important components and functions of each.

INTRODUCTION: LIPID RAFTS

The Singer-Nicholson fluid mosaic model is the classic textbook example for describing the cell membrane and the lipid-protein interactions within the membrane, yet it does not accurately describe the organized microdomains found in the membrane. These domains have a definite physical state and composition that are different from the neighboring membrane.[1,2] Sphingolipids and cholesterol are packed in the outer leaflet of the membrane. The abundance of saturated hydrocarbon chains of phospholipids and sphingolipids and the exclusion of unsaturated lipoproteins makes these microdomains more densely packed than other regions of the plasma membrane. This organization of lipids is termed liquid-ordered domain and lipid rafts are one example of these membrane domains.[1,3-6] Cholesterol and sphingolipids can interact with each other, as well as other lipids and proteins on both the inner and outer leaflets of the cell membrane to form lipid raft domains.[7] Sphingolipids associate with each other through their head groups and cholesterol interlocates between the sphingolipids in the outer leaflet of the membrane.[6] Although this mixture of lipids is found in the outer leaflet of the plasma membrane, it is not the same mixture of lipids found on the inner leaflet suggesting additional

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components on the inner leaflet are required or that outer leaflet molecules penetrate into the inner leaflet to help form the raft domain.[8] Investigation into the organization of the microdomains inner leaflet found that scaffolding proteins and acylated proteins often clustered on the inner leaflet.[9,10]

Lipid rafts are small, highly organized but mobile groupings of cholesterol and sphingolipids in the exoplasmic leaflet of the cell membrane involved in signal transduction.[2,4,11] Although these small domains cannot be viewed by light microscopy, they can be indirectly assessed using fluorescent techniques.[12] Because of their size, lipid rafts are too small to function on their own; however, clustering of rafts promotes function. Some resident scaffolding proteins, such as flotillin, caveolins and annexins, are involved in anchoring the inner leaflet of lipid rafts together, while other lipid raft-binding proteins, such as glycosylphosphatidylinositol-linked proteins, connect the rafts on the extracellular side of the membrane. These clustered rafts can then function as platforms in signaling processes.[13]

A large number of proteins are involved in signal transduction and many of these proteins preferentially reside in lipid raft domains either in the outer or the inner leaflet of the cell membrane.[14] Resident lipid raft proteins such as GPI-linked proteins are found in greater numbers inside lipid raft regions than nonraft regions of the membrane.[15] GPI-linked proteins are found on the outer leaflet of the plasma membrane and are attached by their carboxyl lipid additions. Src-family tyrosine kinases, as well as cholesterol-binding proteins, G proteins and other phospholipid-binding proteins also mediate lipid raft function.[4,16-18] Flotillins and annexins reside in lipid rafts on the inner leaflet of the membrane.

CAVEOLAE

Caveolae are described as flask-shaped invaginations with an approximate diameter of 70-120 nm on the plasma membrane and in the cytoplasm.[19] Caveolae are a subset of lipid rafts based on size and lipid content. However, not all lipid rafts are caveolae. These two microdomains have similar constituents and both overlapping and unique functions. Caveolae were described on the plasma membrane as early as 1953 by Yamada in the gall bladder epithelium and again by Palade in 1961 in blood capillaries and have since been described in nearly all cell types as nonclathrin coated membrane invaginations.[20,21] Caveolae are found in most cell types and are found in great numbers in endothelial cells and adipocytes.

Many proteins are known to be associated with the outer leaflet of the caveolae membrane such as GPI-linked proteins and a variety of transmembrane protein receptors such as GPCRs, insulin receptor and beta adrenergic receptors (Fig. 1). However, G proteins, cholesterol-binding proteins, such as caveolins and the Src-family of tyrosine kinases are attached to the inner leaflet of the plasma membrane.[22-24] After transport to the plasma membrane, GPI-linked proteins are sequestered in cholesterol rich caveolae. However, this idea is debated. Some research has shown that GPI-linked proteins reside near, but not in, caveolae and only move into caveolae after cross-linking. On the other hand, some say GPI-linkage is important for connecting proteins on the exoplasmic membrane to interior membranes and/or organelles such as the ER or Golgi.[25]

Caveolae function in endocytosis, transcytosis and also serve as signaling platforms and signal transduction.[26-28] Caveolae endocytosis is dependent upon dynamin-II. Dynamin-II resides along the neck of the invaginations and promotes caveolae budding.[29]

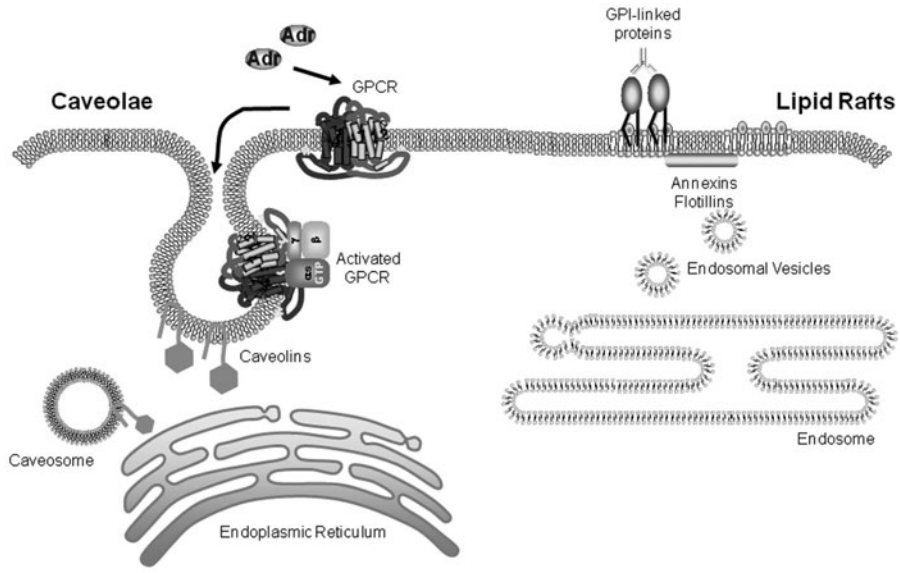

Figure 1. Membrane receptor regulation by caveolae and lipid rafts.

Research has shown that cross-linked GPI-anchored proteins move into caveolae and subsequently become endocytosed. Also, non-enveloped viruses, such as SV40, exploit the endocytocytic function of caveolae to gain entry into the cell. Recent research has shown that these internalized viruses are sent to endosome-like structures called caveosomes that are rich in caveolin-1, a cholesterol-binding scaffolding protein.[30] These caveosomes are different from the endosomes and are now recognized as distinct intracellular organelles. Caveosomes are thought to be regulators of receptor turnover (Fig. 1). Caveosomes fuse with caveolae vesicles that have pinched off from the membrane thereby regulating the downstream signaling pathways as well.[31] These vesicles usually contain receptors that can be recycled without using the endosome pathway. Many signaling molecules and/or their downstream targets move into or out of caveolae to initiate their activation, or to be held inactive.

Caveolae are enriched in important signaling lipids such as ceramide, phosphatidic acid, diglyceride and glycosphingolipids.[32,33] Ceramide is produced in caveolae in response to interleukin-1 beta. Caveolae and ceramide produced in caveolae may participate in blocking platelet derived growth factor stimulated DNA synthesis.[33] The enzymes required to make phosphatidic acid and diglyceride are found in caveolae. Both diglyceride and phosphatidic acid are important second messengers in many cellular signaling pathways including cytoskeletal arrangement and coordinated secretion.[34]

Endothelial cells are rich in caveolae and caveolae have a unique function in these cells. Caveolae are involved in mechanotransduction. Caveolae contain many receptors that modulate blood flow and vascular tone, such as VEGF and insulin, through downstream signaling cascades after ligand binds the receptor. However, endothelial cells with direct exposure to blood flow have been shown to activate the downstream signaling proteins without ligand binding to receptors. Therefore, endothelial cell caveolae have a distinct characteristic of sensing changes in blood flow and modulate vascular tone in

a cholesterol dependent manner by activating endothelial nitric oxide synthase and the MAPK pathways without ligand.[35]

Due to the organization of lipid rafts, the isolation of lipid rafts has proved challenging. The lipid composition of the rafts gives these domains a degree of insolubility in non-ionic detergents, a property that has been used to isolate lipid rafts from the rest of the plasma membrane.[36] Lipid rafts are resistant to cold Triton-X 100 solubilization however isolation using this method causes disruption of the native lipid raft states in the membrane.[37,38] Caveolae membrane fractions can be isolated from the plasma membrane by using Percoll and Optiprep gradient centrifugations instead of the detergent methods used to isolate lipid rafts.[39] This method preserves the resident proteins within caveolae allowing a comprehensive analysis of the role of caveolae and related caveolins in signal transduction.

CHOLESTEROL

The shape of caveolae is most often described as flask-shaped; however this is dependent on the cholesterol content of the cell. Cholesterol is located in the outer leaflet of caveolae and depletion of cholesterol causes the caveolins to move to the endoplasmic reticulum and/or the Golgi apparatus.[40] Cholesterol is a major component of caveolae and depletion of cellular cholesterol, either by extraction using methyl beta-cyclodextrin or depletion using cholesterol oxidase, reduces the number of invaginated caveolae.[41,42] Importantly, cholesterol levels are not static and cholesterol moves in and out of caveolae which suggests that caveolae have a role in cholesterol transport.[43] Caveolin-1, one of the coat proteins that make up caveolae, has been shown to move from the caveolae membrane and to intracellular compartments. Located in caveolae, the scavenger receptor class B Type I is a receptor for HDL.[44] Caveolin-1 directly binds the cholesterol esters in the HDL particle and moves them into the cell without disrupting the HDL particle.[44]

CAVEOLIN-1 ROLE AND FUNCTION

Further investigation into the structure of caveolae revealed a striated pattern seen on the cytoplasmic surface indicative of the presence of a resident protein.[45] The resident protein was determined not to be clathrin when treatment with high salt, which strips the membrane of clathrin, did not alter the morphology of the striated coats found in caveolae.[46,42] VIP21 was positively identified as a scaffolding protein contained within the caveolae striated coat in human fibroblasts and was later called caveolin.[42,47] Caveolins and flotillin are similar in that both are scaffolding proteins that are involved in managing lipid rafts; however caveolins are found in caveolae, while flotillin are found in noncaveolae lipid rafts. Following the positive identification of caveolin as a caveolae coat protein, the 22kDa protein was sequenced and determined to be 178 amino acids long with a unique hydrophobic end.[48] Caveolins have the ability to form high molecular weight homo- and hetero-oligomers and can directly interact with many signaling molecules. Caveolins also can interact directly with many of the proteins in the lipid rafts through a unique protein sequence called the caveolin scaffolding domain.[49] The caveolin scaffolding domain is a 20 amino acid domain on the amino terminus end of caveolin. Proteins bound to the caveolin scaffolding domain are held in an inactive state, for example, caveolin-1 bound to endothelial nitric oxide synthase (eNOS) keeps eNOS in an inactive conformation.[50]

Therefore, caveolins, particularly the caveolin scaffolding domain, are thought to have an inhibitory effect on signal transduction when bound to proteins and only the release from caveolin allows the protein to become active. However, data contradicting this concept have arisen. In insulin and Ras signaling pathways, caveolin-1 plays an activating role.[51,52]

Since the sequencing of caveolin, many researchers have investigated the role of caveolin within the cell. Caveolins have the capacity to bind cholesterol and glycosphingolipids and are required to form caveolae.[53-55] Caveolin-1 is a resident protein of caveolae and is capable of forming invaginated caveolae in the plasma membrane of most cell types. This is just one role of caveolin-1; caveolin-1 also exists outside of the caveolae membrane on vesicles such as insulin granules and liposomes and in caveosomes.

Caveolin-1 directly binds to cholesterol and fatty acids and is involved in the transport of fatty acids through the caveolae membrane.[56,57] Cholesterol binds caveolin-1 and phospholipid liposomes which are required to form caveolae.[58] When cholesterol is depleted from the cell, caveolin moves out of the caveolae and to the endoplasmic reticulum. Upon cholesterol replenishment, caveolin moves back to caveolae independent of Golgi trafficking suggesting a role in cholesterol trafficking. Cholesterol is directly bound and trafficked by caveolin in a lipoprotein chaperone complex.[59,60] This complex contains caveolin, cyclophilin A, cyclophilin 40 and HSP56 and is used to deliver cholesterol to caveolae from the ER. HSP56 provides the specific drive to transport cholesterol from the ER to caveolae by exploiting acylated sites on caveolin that bind cholesterol.[60] Additionally, a lipoprotein chaperone complex can also be used to take up cholesterol into caveolae. This complex is comprised of caveolin, cyclophilin A cyclophilin 40 and annexin II where annexin II provides the specificity to move cholesterol from caveolae to the ER.[44,59,61]

Caveolae are capable of making tunnel like projections through the cell in order to allow movement of material from the apical side of the membrane to the basolateral side of the membrane in a process called transcytosis. Insulin, albumin and LDL are all known to be transported using caveolae and the transcytosis pathway. Transcytosis can be either receptor-mediated or constitutive; however, in both cases, transcytosis through caveolae is a specialized process.

Caveolae and caveolin-1 have roles in insulin secretion, insulin receptor mediated signaling and potentially in diabetes. As stated previously, GPCRs are partially localized to caveolae and one of these receptors, GPR40 is involved in insulin secretion. GPR40 is an orphan G-protein coupled receptor that binds long-chain fatty acids and stimulates insulin secretion.[62] Some ion channels involved in insulin secretion are partially localized to caveolae such as the Kv2.1 channel.[63,64] Caveolin-1 mediates the ATP dependent-potassium channels in pancreatic beta cells thereby regulating glucose stimulated insulin secretion.[64] Caveolin-1 also acts as a guanine nucleotide dissociation inhibitor blocking the exchange of GDP for GTP and activating cdc42, a small GTPase on the surface of the insulin granules.[65,66] Additionally, the docking/fusion proteins, VAMP2 and SNAP, that are required for insulin granule fusion and insulin secretion are also localized to caveolae.[67] Insulin receptors are found in caveolae membranes.[68] Caveolae can transduce insulin signals and recycle insulin receptors. Furthermore, both caveolin-1 and caveolin-2 are expressed in pancreatic islets however; caveolin-1 is only expressed in the beta cells while caveolin-2 is expressed in beta cells and non-insulin secreting cells.[64] Caveolin-1 null mice are thought to be hyperphagic, hypertriglyceridemic and have elevated free fatty acid levels in serum yet have normal blood glucose and insulin levels.[69]

In addition to its role in insulin signaling, caveolin-1 appears to play an important role in cardiac function. Caveolin-1 null mice develop cardiac hypertrophy resulting in a reduced life-span by as much as 50% compared to wild-type mice.[70] Echocardiography in twelve month old caveolin-1 null mice revealed a reduction in left ventricular systolic function evidenced by a decrease in fractional shortening, defined as end diastolic dimension minus end systolic dimension divided by end diastolic dimension.[70] Further, caveolin-1 null mice had an increase in wall thickness, indicative of concentric left ventricular hypertrophy. Similar to these findings, another group found that caveolin-1 null animals had depressed cardiac function measured by echocardiography showing a decrease in fractional shortening.[71] In contrast they documented that caveolin-1 null mice had dilated right and left ventricular chambers (eccentric hypertrophy) and thin posterior walls and septum compared to littermate controls as shown by histological examination. The dramatic difference in morphology of caveolin-1 null hearts between these two groups may be due to the use of different background strains.

Caveolin-1 has been studied in cancer development and has been shown to have varied effects in the progression of tumors. In breast cancer, caveolin-1 is suggested to be a tumor suppressor because it is down regulated in certain oncogenic cells.[72] Research has shown that approximately 16% of all breast cancer patients have a mutation in caveolin-1.[73] Mice lacking caveolin-1 have an increase in mammary tumors. Estrogen receptor beta, also involved in breast tumorgenesis, is partially localized to caveolae.[74] Estrogen receptors have elevated expression under tumorigenic conditions and overexpression is found in the majority of human breast cancers.[75,76] When caveolin-1 gene is inactivated in mammary epithelial cells, estrogen receptor and cyclin D1 are up-regulated leading to tumorigenesis.[77] In contrast to breast cancer, caveolin-1 expression is increased in prostate cancer.[78] Knocking out caveolin-1 expression in a prostate cancer mouse model showed that the lack of caveolin-1 reduced tumorigenesis, suggesting that caveolin-1 promotes the development of prostate cancer.[78]

CAVEOLIN-2 ROLE AND FUNCTION

Another caveolin protein, caveolin-2, was discovered in murine adipocytes when caveolin-enriched membrane domains were isolated and probed for protein components which were then microsequenced. It was determined that a 20 kDa protein, similar to caveolin-1, was present but it differed from caveolin-1 at several residues. When compared to human caveolin-1, caveolin-2 was determined to be roughly 38% identical and 58% similar with a conserved region of eight identical amino acids.[79] Caveolin-2 is expressed in white adipose tissue and lung tissue as well as endothelial cells, smooth muscle cells, skeletal myoblasts and fibroblasts as well as pancreatic islets. Caveolin-2 colocalizes with caveolin-1 and, interestingly, is unable to directly bind cholesterol without caveolin-1 interaction.[79,80] Unlike caveolin-1, caveolin-2 cannot form homo-oligomeric complexes and requires caveolin-1 to form stable hetero-oligomers.[81] In rat thyroid tissue, which expresses caveolin-2 but not caveolin-1, it was shown that caveolin-2 is retained in the Golgi complex. However, if caveolin-1 is expressed by transfection or adenovirus-mediated transduction in these cells, caveolin-2 redistributes to the plasma membrane indicating that caveolin-1 is required for caveolin-2 localization to the plasma membrane.[82] These combined results suggested that caveolin-2 acts only as an accessory protein to caveolin-1. However in caveolin-2 null mice, a severe pulmonary dysfunction was present indicating that caveolin-2 may have a role in lung function independent of caveolin-1.[83]

CAVEOLIN-3 ROLE AND FUNCTION

The identification of caveolin-2 gave rise to the caveolin family of genes. First described in 1995, caveolin-3 is a muscle specific member of the caveolin family of proteins, though caveolin-1 is also expressed in muscle.[84,85] Caveolin-3 is roughly 64% identical to caveolin-1 and similarly can form homo-oligomeric complexes with itself and does not require caveolin-1 to drive caveolae formation.[86] Caveolin-3 null mice have muscle fiber necrosis and regeneration, a phenotype similarly observed in human patients with muscular dystrophy.[86] Additionally, caveolin-3 deficient mice develop a severe cardiac phenotype as early as four months of age resulting in severe cardiac hypertrophy and fibrosis resulting in reduced fractional shortening with an increased activation of the Ras-p42/44 MAPK pathway.[87]

Extensive research has determined many roles of caveolin-3 in cardiac function mainly by regulating G-protein coupled receptors (GPCRs) function at the level of the myocyte. β_1 adrenergic receptors are found predominantly in noncaveolae lipid rafts and a small number can be found in caveolae. Contrastingly, β_2 adrenergic receptors are enriched in the caveolae membrane.[88] It has been determined that β_2 adrenergic receptors and downstream targets are localized to caveolin-3 enriched fractions at rest and translocate out of the caveolin-3 enriched membrane after treatment with isoproterenol.[89,90] Interestingly, a study by Insel et al raised the possibility that GPCR signaling components have different localization patterns in adult cardiomyocytes compared to neonatal myocytes suggesting that there are multiple subcellular microdomains involved in GPCR signaling in cardiac myocytes.[91] In a recent study, Calaghan et al disrupted the caveolae membrane in isolated cardiac myocytes and recorded the lusitropic response (myocardial relaxation) to β_2 adrenergic receptor stimulation using video edge detection.[92] Disruption of the caveolae membrane caused a decrease in relaxation in myocytes as well as a significant increase in the phosphorylation of phospholamban and Troponin I suggesting that the cAMP-dependent signal is no longer confined to the sarcolemma.[92] This study confirms the spatial orientation of the β_2 adrenergic receptors to the caveolae compartment and regulation by caveolin-3.

PTRF

Polymerase I and transcript release factor (PTRF), also known as cavin, is also localized to the cytosolic face of caveolae membrane. PTRF, a 60kDa protein, has the same abundance in a variety of cell types as caveolin-1. In a recent paper by Liu et al, mice deficient in PTRF did not form caveolae in lung epithelium, intestinal smooth muscle or skeletal muscle suggesting that PTRF is required for the formation of caveolae in these tissue.[93] However, in adipocytes, PTRF has a functional role beyond structure. In adipocytes, PTRF functions in the release of the polymerase complex from the transcript during rRNA transcription by dissociating the elongating complexes from the transcript.[94] Interestingly, mice lacking PTRF had elevated serum triglycerides, free fatty acids and insulin as well as decreased leptin and adiponectin expression suggesting that PTRF may also be involved in cholesterol and fatty acid transport as well as insulin signaling. PTRF is suggested to play a role in insulin signaling. After insulin exposure, PTRF moves from the cytosol to the nucleus along with hormone sensitive lipase.[95]

CONCLUSION

Lipid rafts and caveolae have similar components and functions. Both lipid rafts and caveolae are microdomains of the plasma membrane rich in sphingolipids and cholesterol. Both are involved in endocytosis. Caveolae are unique in that they also form intracellular structures, caveosomes. Caveolae are important in cholesterol uptake and transport across the cell membrane. The scaffolding proteins associated with each microdomain are unique to that domain. Lipid rafts use flotillins while caveolae employ caveolins to organize the membrane domain. GPI-linked proteins are found in both lipid rafts and caveolae. Cross-linked GPI-anchored proteins move into caveolae, are then endocytosed and enter the caveosome without going through the endosome recycling process. In lipid rafts, GPI-linked proteins are internalized and moved to the endosome for recycling.

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