Regulation of assembly and cell surface dynamics of caveolae

Jagan Mohan
Abstract
A typical mammalian cell plasma membrane displays a high level of plasticity counter balanced with stability. The plasma membrane show various kinds of invaginations to meet physiological demands of the cells such as nutrient uptake, receptor signaling etc. An example of one such invagination observed in many cell types is “Caveola”. Caveolae are bulb shaped invaginations of the plasma membrane enriched in sphingolipids and cholesterol. Each individual caveola is in equilibrium with multi caveolar assemblies and exhibits various dynamics ranging from stable association with cell surface to the “kiss and run” type and internalized caveolae called cavicle. The principle protein components of caveolae involve caveolin1, 2 and 3, cavin 1, 2, 3 and 4 along with EHD2, PACSIN2 and dynamin. Caveolin1 acts as the hallmark of caveolae, whereas caveolin3 and cavin4 are limited to muscle cells. Caveolae are appreciated as important plasma membrane structures in maintaining cellular homeostasis of many cell types. Its dysfunction is associated with several human diseases such as cancer, vascular diseases, lipid and muscular dystrophies.

This thesis aims to understand the assembly of caveolae and the molecular machineries involved in the regulation of caveolae dynamics. In particular, we have focused on the mechanism of cavin coat assembly, the influence of cavin3 in the regulation of caveolae dynamics, along with the mechanistic cycle of EHD2.

Cavins form characteristic striations around caveolae, and in this work we showed that the N-terminus of cavin3 interacts with the trimeric N-terminus of cavin1 in competition with the N-terminus of cavin2 to form cavin sub-complex. We also observed that cavin3 interacts with caveolin1 in a cholesterol dependent manner and cavin3 may promote scission by acting as a positive regulator of caveoloae dynamics, opposite to the cellular function of EHD2. The stringent roles of cavin3 and EHD2 control the equilibrium between stably cell surface associated caveolae and the “kiss and run” type of caveolae, undergoing rounds of fission and fusion. Our results demonstrated the molecular composition of the caveolae coat at a domain level with the stoichiometry of cavin sub-complexes. We also showed the function of cavin3 in the regulation of caveolae dynamics at the plasma membrane. Previous work from our lab showed that EHD2 is a dimeric ATPase localised to the caveolae neck and confines caveolae to the cell surface. In the present study, we showed that EHD2 oligomerized in an open conformation stabilized by ATP and in a G-domain loop dependent manner. The oligomerization of EHD2 in cells is finely tuned by the N-terminal region and the C-terminal EH domain, where both of these regions act as negative regulators of membrane binding. Our results showed the stringent regulation of EHD2 oligomerization and its importance with respect to various statuses and/or function of caveolae.

In summary, the current study provides a novel insight into the assembly of cavin coat and protein machineries involved in the regulation of caveolae dynamics. In addition, it also contributes to the understanding of the molecular mechanism of ATPase activity of EHD2.

Keywords
caveolae, caveolin1, cavin, EHD2, cavin coat, oligomerization, caveolae dynamics, mechanistic cycle