Influence of caveolin-3 upon membrane raft lipids and its implications for trafficking in muscle

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Lipid rafts are subdomains of the plasma membrane that contain high concentrations of cholesterol and glycosphingolipids. Some members of the transient receptor potential canonical (TRPC) channel family have been shown to be present in lipid rafts. Caveolae are a subtype of lipid rafts present in some cells expressing proteins of the caveolin family. We recently reported that the muscle caveolae-forming protein, caveolin-3, binds to TRPC1 facilitating plasma membrane targeting and channel activity (Gervásio *et al*., 2008). By what mechanism might caveolin-3 influence TRPC1 targeting and function? Since caveolins are associated with cholesterol/sphingolipid-enriched membrane microdomains, we investigated the effect of caveolin-3 expression upon the mobility and density of lipid rafts using Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Resonance Energy Transfer (FRET) methods respectively. Cholera toxin subunit B (CT-B) was used to label the raft domain-associated ganglioside GM1, on C2 myoblasts.

FRAP measurements confirmed that in the absence of transfected caveolin-3, the raft marker was highly mobile on the plasma membrane (mobile fraction 0.77). However, when caveolin-3-YFP was transfected into the cells, the mobility of the lipid raft marker was decreased compared to non-transfected control cells, particularly in regions of the plasma membrane rich in caveolin-3-YFP (mobile fraction 0.27; $p < 0.05$). Next we developed a new FRET method to measure the assembly of the raft marker into molecular lattices (using CT-B-Alexa555 and CT-B-Alexa647 as FRET donor and acceptor respectively). Interestingly, cavaeolin-YFP-rich regions of the plasma membrane showed the tightest CT-B packing density (highest FRET) ($p < 0.05$) compared to non-transfected cells. We propose that the convex shape of the caveolae may contribute to higher FRET efficiency in those microdomains, as a result of closer proximity of neighboring CT-Bs. Together this suggests that cavaeolin-3 drives the packing and immobilization of raft marker GM1 into stable membrane domains (presumably clusters of cavaeolae). Treatment of cells with cytochalasin D, an agent that disrupts microfilaments, was able to rescue the mobility of lipid rafts in caveolin-3-rich regions of the plasma membrane (mobile fraction 0.74; $p < 0.05$). Double labeling revealed a sub-compartment of caveolin-3 under the plasma membrane, which stained positively for the raft marker GM1. Using 4 dimensional laser scanning confocal microscopy analysis (Z stack plus time), we observed that mechanical stretch was able to induce trafficking of caveolin-3 to the plasma membrane in some cells. The ability of mechanical stretch to induce remodelling of such microdomains suggests a mechanism whereby TRPC1 channel, and other caveolin/lipid rafts-associated proteins, might be shuttled to the plasma membrane in response to stretch.

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