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Cholera Toxin B Subunit Crosslinks Rafts Causing Confinement by Cortical Actin

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Lipid rafts are assemblies of proteins and lipids that regulate membrane trafficking and cell signaling events. Current models suggest that lipid rafts are small, dynamic structures rather than stable platforms. However, certain proteins are thought to facilitate the formation of longer-lived rafts, through a process of crosslinking and stabilizing these otherwise nanoscopic domains.

One of the best-studied examples of a protein with the capacity to build stabilized lipid rafts is cholera toxin, a bacterial toxin that binds to raft-associated glycolipids via its homopentameric B subunit (CTXB) and enters cells by raft-mediated endocytic pathways. In model membranes, CTXB has the ability to induce global membrane reorganization [1, 2], suggesting that CTXB either crosslinks small domains or stabilizes transient domains. Interestingly, compared to other raft proteins, CTXB behaves as if it is very strongly confined in terms of its lateral diffusion, a read-out of how molecules interact with membrane domains [3]. This suggests that CTXB may induce the formation of stabilized rafts that in turn are diffusionally trapped by interactions with other types of membrane domains such as the actin cytoskeleton. To test this hypothesis, in the current study, we investigated the mechanisms that control the lateral diffusion of CTXB compared to other "raft" and "non-raft" proteins and lipids in living cells, using confocal fluorescence recovery after photobleaching (FRAP).

We first asked whether there is any evidence that CTXB crosslinks lipid rafts in intact cells. A prediction of this model is that stabilized rafts form in a concentration-dependent manner. To test this, we measured the diffusional mobility of CTXB in COS-7 cells as a function of surface density of bound toxin. We found that CTXB diffusion decreases with increasing levels of bound toxin, consistent with the possibility that it actively forms stabilized, toxin-induced domains. To rule out a possible contribution of crowding to this effect [4], we measured the diffusional mobility of other proteins in the presence of bound CTXB, and found that they were identical in the presence and absence of CTXB. This indicates that the concentration-dependent changes in CTXB diffusion are not the result of increased global protein density at the cell surface, but rather are the specific result of a change in the way that CTXB interacts with the membrane.

Next, we examined the mechanisms that trap CTXB-induced domains, leading to its slow diffusion on the cell surface. We focused on the role of the actin cytoskeleton and caveolae, two classes of membrane domains with which CTXB has been postulated to specifically interact [5, 6]. Disruption of actin by Latrunculin A treatment led to faster diffusion of CTXB, an effect not seen for other raft proteins. In contrast, the diffusion of CTXB was identical in caveolin-1 +/+ and caveolin-1 -/- mouse embryonic fibroblast cells. Thus, CTXB is slowed by interactions with the actin cytoskeleton, but not caveolae.

Based on these findings, we propose that in live cells, CTXB crosslinks small transient domains to create stabilized, toxin-induced rafts. These stabilized rafts diffuse slowly as the result of trapping by the actin cytoskeleton. Studies are underway to determine how these toxin-induced domains regulate the endocytosis of CTXB by raft-dependent pathways.

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