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Nucleoside diphosphate kinase B is required for the formation of heterotrimeric G protein containing caveolae.

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Résumé en anglais	Caveolae are flask-shaped invaginations in the plasma membrane that serve to compartmentalize and organize signal transduction processes, including signals mediated by G protein-coupled receptors and heterotrimeric G proteins. Herein we report evidence for a close association of the nucleoside diphosphate kinase B (NDPK B) and caveolin proteins which is required for G protein scaffolding and caveolae formation. A concomitant loss of the proteins NDPK B, caveolin isoforms 1 (Cav1) and 3, and heterotrimeric G proteins occurred when one of these proteins was specifically depleted in zebrafish embryos. Co-immunoprecipitation of Cav1 with the G protein Gβ-subunit and NDPK B from zebrafish lysates corroborated the direct association of these proteins. Similarly, in embryonic fibroblasts from the respective knockout (KO) mice, the membrane content of the Cav1, Gβ, and NDPK B was found to be mutually dependent on one another. A redistribution of Cav1 and Gβ from the caveolae containing fractions of lower density to other membrane compartments with higher density could be detected by means of density gradient fractionation of membranes derived from NDPK A/B KO mouse embryonic fibroblasts (MEFs) and after shRNA-mediated NDPK B knockdown in H10 cardiomyocytes. This redistribution could be visualized by confocal microscopy analysis showing a decrease in the plasma membrane bound Cav1 in NDPK A/B KO cells and vice versa and a decrease in the plasma membrane pool of NDPK B in Cav1 KO cells. Consequently, ultrastructural analysis revealed a reduction of surface caveolae in the NDPK A/B KO cells. To prove that the disturbed subcellular localization of Cav1 in NDPK A/B KO MEFs as well as NDPK B in Cav1 KO MEFs is a result of the loss of NDPK B and Cav1, respectively, we performed rescue experiments. The adenoviral re-expression of NDPK B in NDPK A/B KO MEFs rescued the protein content and the plasma membrane localization of Cav1. The expression of an EGFP-Cav1 fusion protein in Cav1-KO cells induced a restoration of NDPK B expression levels and its appearance at the plasma membrane. We conclude from these findings that NDPK B, heterotrimeric G proteins, and caveolins are mutually dependent on each other for stabile localization and caveolae formation at the plasma membrane. The data point to a disturbed transport of caveolin/G protein/NDPK B complexes from intracellular membrane compartments if one of the components is missing.
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