Subcellular Processes in Plants and Animal Cells

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Syndapin II interacts with caveolin 1 and participates in shape control of caveolae

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F-BAR domain proteins like syndapins are dimeric, lipid-binding, peripheral membrane proteins. They induce membrane curvature by partial membrane insertion and by imposing their curved shape onto the lipid bilayer [1]. Syndapin I is a predominantly neuronal protein, syndapin II is expressed ubiquitously [2]. Syndapin I and II were shown to play a promoting role in endocytosis and in vesicle formation processes [3, 4]. The direct observation of such membrane shaping processes by peripheral membrane proteins was hitherto hindered by the difficulty to combine cytochemistry with a high-resolution view of the membrane topology.

Using the new freeze-fracture replica immunolabeling technique (FRIL) we solved this limitation by achieving large and detailed ultrastructural views of cellular membranes in the transmission electron microscope combined with the immuno-localization of membrane components. In particular we established conditions for the efficient labeling of the peripheral membrane protein syndapin II at electron microscopic resolution.

When applying the FRIL technique chemically unfixed cellular samples are rapidly cryofixed in their natural environment. In a second step the samples are fractured, replicated and immobilized by a platinum/carbon evaporation followed by a careful SDS treatment. After the “SDS-digestion” [5] molecules like membrane proteins or lipids that are in direct contact with the platinum-carbon replica keep bound to the replica film and are accessible to immunolabeling [6].

In this work we show the localization of endogenous syndapin II at the plasma membrane of NIH-3T3 cells using the FRIL technique (Figure 1, 2).

Syndapin II was detected at flat and curved membrane domains at the protoplasmic fracture face of the plasma membrane (Figure 1). Co-localization studies at both the cellular and the ultrastructural level identified these sites as caveolin 1-positive areas and thus elucidated syndapin II as new component of caveolae (Figure 2). Biochemical analyses revealed that syndapin II binds caveolin 1 via its F-BAR domain and that over-expressed syndapin II F-BAR domain affects caveolin 1 distribution in NIH3T3 cells. Ultrastructural analyses showed that syndapin II loss-of-function by RNAi lead to a shift of the proportion of invaginated caveolae towards flat caveolin 1 positive areas suggesting that syndapin II is required for the invagination of caveolae at the plasma membrane. Mechanistic insights into syndapin II mediated caveolar shaping was revealed by tilt series analysis of immunolabeled freeze-fracture replicas showing syndapin II asymmetrically localized at the neck of caveolae.

In summary, the freeze-fracture replica immunolabeling technique showed that plasma membrane-localized syndapin II binds to both flat and curved membrane domains *in vivo* and that it plays an important role in caveolar shaping. Our data are an excellent example for studying peripheral membrane proteins in terms of localization and function at the ultrastructural level using FRIL.

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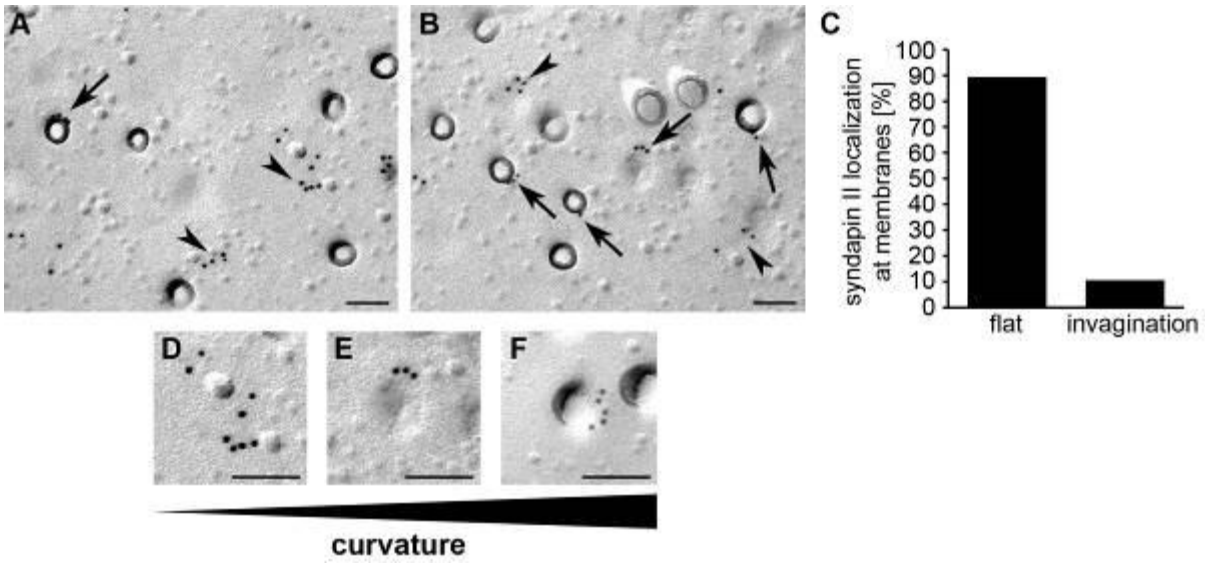

Figure 1 Syndapin II is localized to both flat and deeply invaginated plasma membrane of NIH 3T3 cells. NIH 3T3 cells were grown to confluence and subsequently processed for freeze-fracturing and immunolabeling. **A**, **B** Besides the presence of syndapin II at flat membranes (arrowheads), a substantial fraction of syndapin II signal was detected at circular deep membrane depressions with diameters of about 60 nm (arrows). **C** Quantification of immunolabeling revealed that a profound fraction of syndapin II was present at invaginated membranes. **D–F** Detail views of syndapin II at membranes with different curvatures (increasing from left to right). Scale bars 100 nm

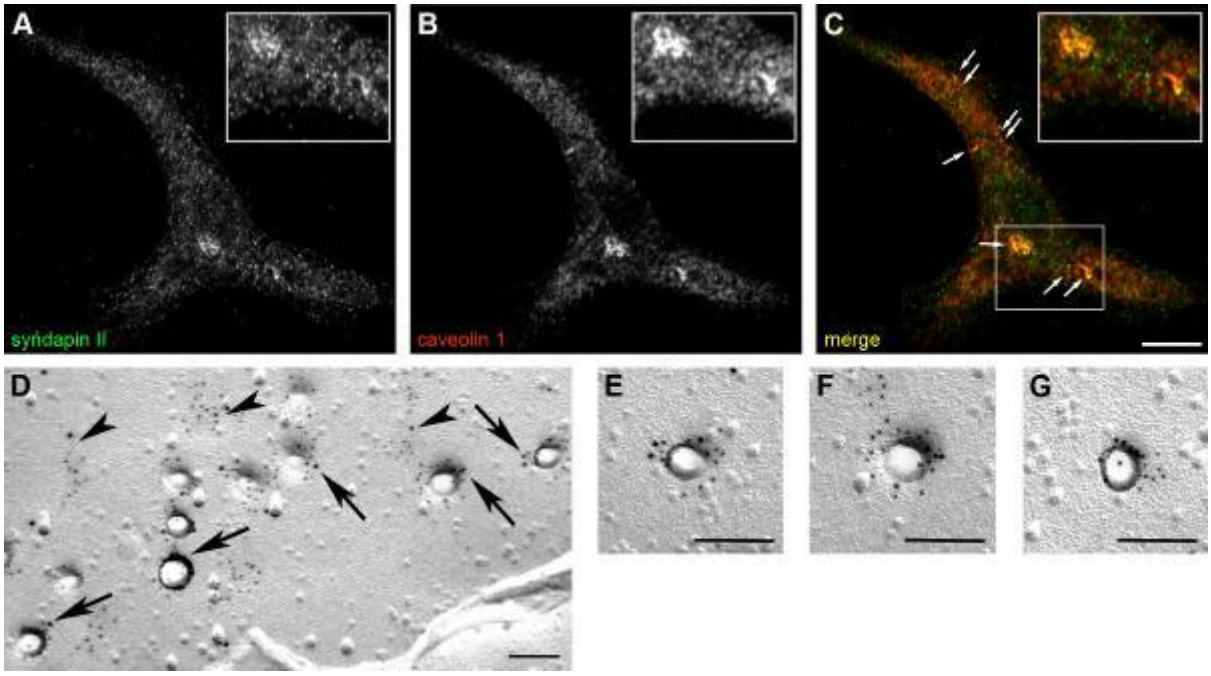

Figure 2 Syndapin II localizes to caveolin 1-positive structures in NIH 3T3 cells. Apotome image of methanol-fixed NIH 3T3 cells stained for endogenous syndapin II a) and caveolin 1 b) showed high degree of colocalization (c, merge). d–g Freeze-fracture electron micrographs of the plasma membrane of NIH 3T3 cells. Caveolin 1 (indicated by 5 nm gold particles) and syndapin II (indicated by 10 nm gold particles) colocalize at invaginated caveolae (arrows), but also at flat caveolin 1-positive spots (arrowheads) (d). Exemplary detail views of syndapin II (arrows) and caveolin 1 colocalizing at caveolae are shown in (e–g). Scale bars 10 µm (c) and 100 nm (d–g)