Tetraspanins Regulate the Protrusive Activities of Cell Membrane

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SUPPLEMENT

Supplemental Materials and Methods

Transmission Electron Microscopy (TEM)

After being harvested, cells were washed once with PBS buffer at 4^{o}C, resuspended in 2.5% glutaraldehyde for fixation at RT for 4 h, and then post-fixed in 1% osmium tetraoxide in PBS at RT for 4 h. After post-fixation, the cells were rinsed briefly in deionized water, and *en bloc* stained with 2% uranyl acetate in 0.85% sodium chloride at 4ºC overnight. The cell pellets were dehydrated in graded solutions of ethanol, from 30% through 100%, at 1 h each, infiltrated first with 50% Spurr in 100% ethanol overnight at RT, then with 100% Spurr over an 8-h period involving at least three changes of Spurr. The cells were cured at 60ºC for 2 days. One-micron sections were cut on a Reichert Ultracut E microtome and stained with toluidine blue. Areas of interest were selected, sectioned at approximately 75 nm, and poststained with uranyl acetate and lead citrate immediately.

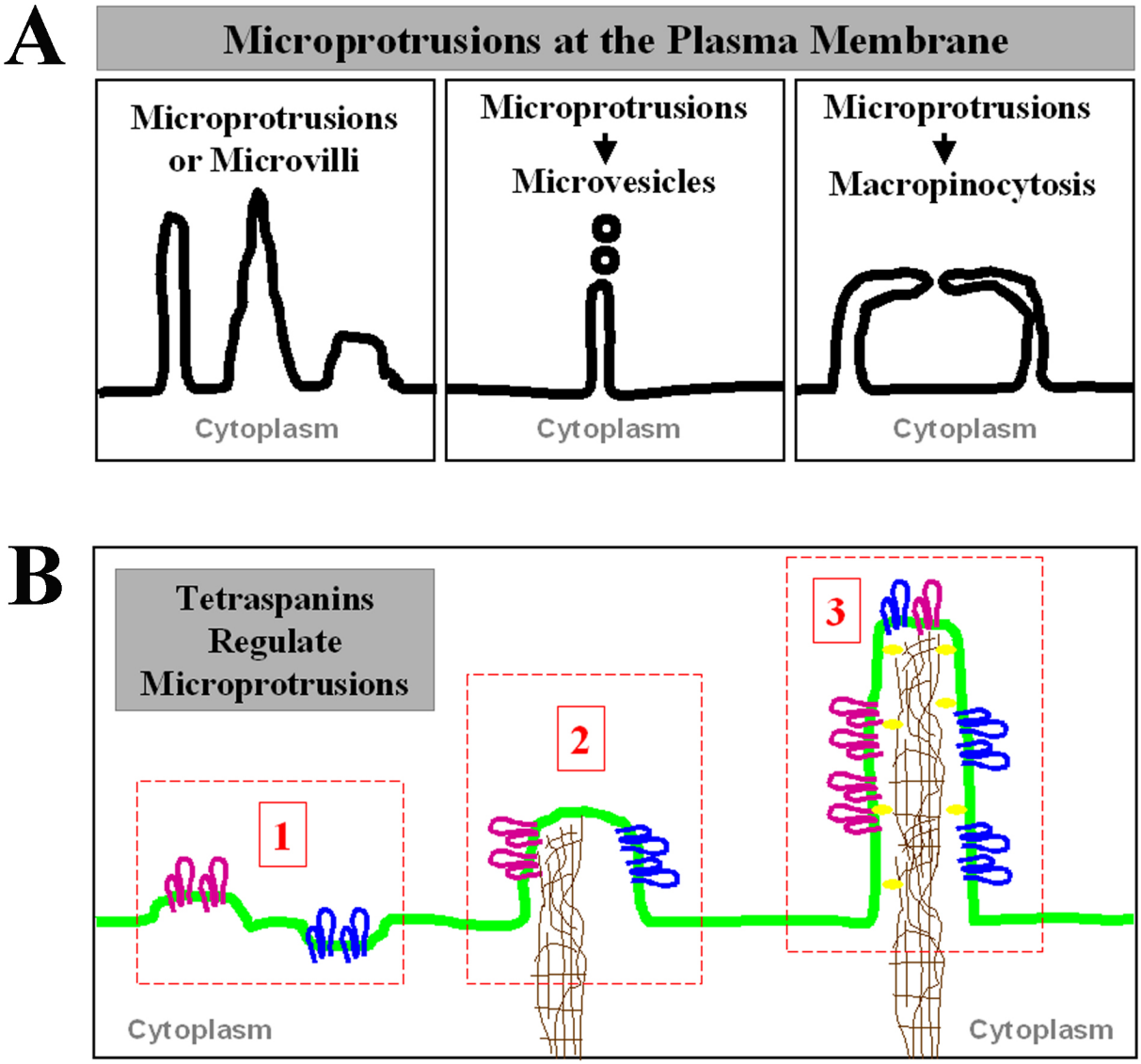
The cells were observed and photographed on a JEOL 2000EX transmission electron microscope at 60 kV.

Epifluorescent and Confocal fluorescent Microscopy

After being cultured on glass coverslips in complete medium for 2-4 days, cells were fixed with 3% paraformaldehyde, permeabilized with 0.1% Brij98, blocked with 20% goat serum in PBS for 1 h at RT, and incubated sequentially with primary mAb and fluorochrome-conjugated secondary Ab. For F-actin staining, cells were incubated with fluorochrome-conjugated α-phalloidin. The cells were analyzed using either a Zeiss Axiophot epifluorescent microscope or a Zeiss 510 confocal fluorescent microscope.

Supplemental Figure

Schematic representations of the membrane microprotrusions and the regulation of microprotrusion morphogenesis by tetraspanins. A. Microprotrusions at the plasma membrane (left panel). Microvilli can release microvesicles from the tips to extracellular environments (middle panel). Two adjacent microvilli may become enclosing, possibly, to engulf extracellular solutes, a process called macropinocytosis (right panel). **B**. Tetraspanins regulate the morphogenesis of microprotrusions. Tetraspanins regulate the formation of microprotrusions possibly by altering membrane curvature (box 1) and/or the interaction between plasma membrane and cortical actin network (box 2). Tetraspanins regulate the extension of microprotrusions possibly by altering the cytoskeletal dynamics within microprotrusions (box 3). The pink tetraspanins promote while the blue ones inhibit the morphogenesis of microprotrusions.



Supplemental Figure