MEC-6, a protein needed for degenerin channel activity, is expressed at the cell surface

Dattananda Chelur, Marty Chalfie

Department of Biological Sciences, Columbia University, New York, NY 10027

The response to gentle touch in Caenorhabditis elegans requires the activity of at least twelve touch function genes. Among them, mec-4 and mec-10 are likely to encode subunits of a mechanosensitive channel; they belong to the DEG/ENaC ion-channel superfamily, and dominant mutations in them cause degeneration of the touch cells. Since mec-4 and mec-10-induced degenerations are completely suppressed by mutations in mec-6, wild type MEC-6 protein is essential for this degenerin channel activity. Also, mutations in mec-6 suppress the degenerations caused by deg-1, unc-8 and partially suppress the hypercontracted phenotype of unc-105 dominant mutation. Thus, mec-6 is more generally required for degenerin channel function.

We have cloned mec-6 gene and had earlier reported that it encodes a predicted protein of 377 amino acids with limited sequence similarity to the mammalian enzyme paraoxonase. mec-6 is widely expressed in many cell types including the touch receptor neurons consistent with its requirement for functioning of different degenerins. MEC-6 is a type II transmembrane protein with a single N-terminal transmembrane domain and a large extracellular C-terminal tail. MEC-6 is glycosylated when synthesized in vitro in presence of microsomes.

We have now confirmed the predicted topology and subcellular localization of MEC-6 by expressing it in cultured cells. MEC-6 was tagged with HA epitope at the C-terminal end and was transiently transfected into CHO cells. Immunostaining of the non-permeabilized cells with anti-HA antibodies revealed surface expression of MEC-6, which appears as discrete dots that are reminiscent of lipid rafts/caveolae. Punctate pattern of expression was also seen in C. elegans when the rescuing mec-6::gfp or the full-length mec-4::gfp constructs were injected. In case of mec-6::gfp, expression in neuronal processes is weak and hence only a few dots could be discerned, but the punctate expression was very clear in body wall muscle cells. Using the two color variants of GFP, we have shown that MEC-6 and MEC-4 are colocalized; coinjection of full-length mec-4::yfp with P_mec-4 cfp into wild type worms resulted in uniform expression of CFP and punctate expression of YFP along the entire length of all the touch cell processes. However, when the two constructs were introduced into mec-6 mutants, the punctate expression of YFP was totally abolished whereas the CFP expression from mec-4 promoter was unaffected. Thus, MEC-6 affects the stability and/or localization of MEC-4 protein, but not the transcription of the mec-4 gene. Since u3 allele of mec-6 also partially suppresses the hypercontracted phenotype of dominant mutation in unc-105 (a muscle-specific degenerin), we are testing to see if MEC-6 colocalizes with UNC-105.
We have expressed epitope tagged MEC-4 (with FLAG), MEC-6 (with HA) and MEC-10 (with MYC) in CHO cells to study the interactions among these proteins. Immunoprecipitation of MEC-6 pulls down both MEC-4 and MEC-10. Similarly, immunoprecipitation of MEC-10 coprecipitates both MEC-4 and MEC-6 thereby demonstrating that all the three proteins physically interact with one another. In addition, MEC-4 and MEC-10 form amiloride-sensitive Na⁺ channels in *Xenopus* oocytes only when coexpressed with either MEC-6 or MEC-2, a stomatin-like protein (see the abstract by Goodman et al.). These results taken together suggest that MEC-6 either function as a channel subunit and/or is required for clustering of the channel complexes into discrete microdomains of the membrane.