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

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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 ectopically expressing *mec-4::cfp* fusion construct under the control of *myo-3* promoter along with *mec-6::yfp* resulted in overlapping punctate expression in body wall muscle. Furthermore, 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.

