Is Piezo protein the mechanotransduction channel in spider *Cupiennius salei* mechanosensilla?

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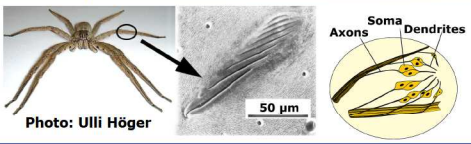
Introduction

Mechanotransduction is essential for detecting external mechanical signals, such as touch, vibration and sound, as well as for sensing internal forces including stretch in the pulmonary and cardiovascular systems. Mechanical stimuli create electrical signals by opening mechanosensitive ion channels. Recent evidence suggests that evolutionally conserved large pore forming Piezo proteins play crucial roles in mammalian and insect mechanosensory processes, including cell volume regulation and sensory transduction. The tropical wandering spider, *Cupiennius salei*, provides a model of mechanotransduction due to accessibility of its mechanosensory neurons for experimental manipulation. Our analysis of the spider transcriptome revealed one gene encoding a Piezo protein, *CsPiezo*. Here, we investigated the expression of this gene in the spider peripheral and central nervous systems using in situ hybridization with digoxigenin labeled RNA probes and immunocytochemistry with a custom-made antibody.

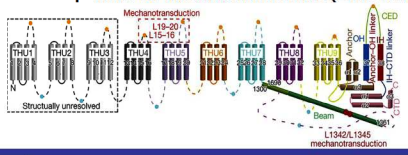
Cupiennius salei VS-3 Mechanosensilla

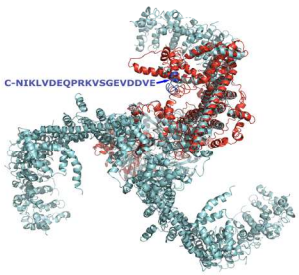


Piezo Protein

Piezos are evolutionarily conserved cation selective mechanosensitive ion channels. The cryo-EM structure of mouse Piezo1 (mPiezo1) has revealed that the channel is a triskelion with arms consisting of repeated arrays of transmembrane units surrounding the pore. Cartoon model of the trimeric mPiezo1 (PDB 5Z10, Zhao et al 2018) viewed from top is shown as ribbon diagram on the left (*blue*). The *C. salei* Piezo protein (CsPiezo) has 2571 residues and a molecular weight of 296 kDa. Homology model of the CsPiezo amino terminal end (residues 1265-2571) was created using the I-Tasser server and is shown in red aligned to one of the mPiezo1 subunits. Location of residues that were used for CsPiezo antibody production are indicated.

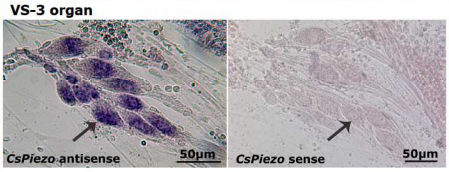
The mPiezo1 transmembrane topology model shows 38 transmembrane helices with designated components for mechanotransduction (Zhao et al 2018).



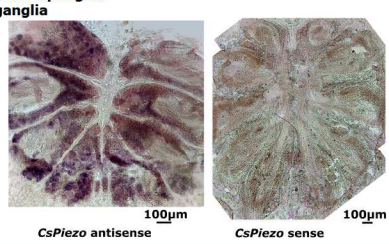


CsPiezo mRNA is expressed in mechanosensory neurons and in many CNS neurons

VS-3 organ



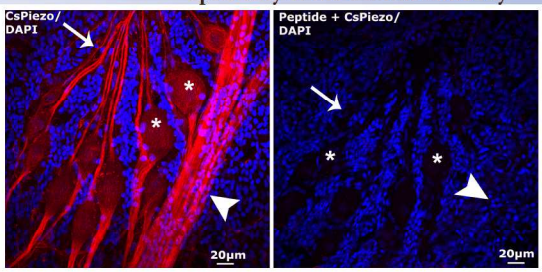
Subesophageal ganglia



In situ hybridization using digoxigenin labeled mRNA antisense probe for CsPiezo produced strong signals in all mechanosensory neurons in the whole-mount preparations of leg hypodermis. Labeling in VS-3 neurons is shown.

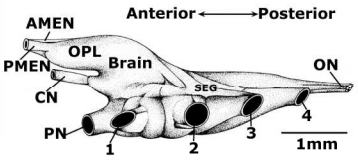
The same probe also produced strong signals in many neuronal somata on the ventral subesophageal ganglia and some labeled neurons were found in other parts of the CNS. Most labeled neurons were 20-100 µm diameter; smaller neurons were not labeled. The control (sense) probe did not produce any signal in the hypodermis or the CNS.

Specificity of the CsPiezo antibody



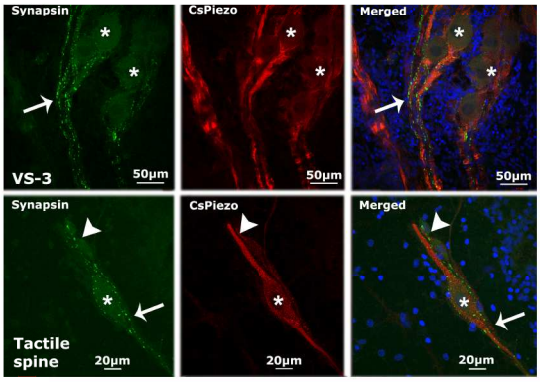
An antibody against the CsPiezo protein was produced by Biomatik in rabbits. This antibody was tested by immunocytochemistry in whole-mount preparations of the spider patellar hypodermis with CY 3 as the secondary antibody (Jackson ImmunoResearch Laboratories). Confocal images show strong red fluorescence at 1:2,000 concentration in VS-3 neuron dendrites (*arrow*), axons, around the cell body (*) and in the leg nerve (*arrowhead*). Dapi (4′,6-diamidino-2-phenylindole, 1µg/mL) was used as nuclear counterstain. Western blot of the spider brain homogenate detected a band close to the molecular weight of CsPiezo proteins (296kDa). When the CsPiezo antibody was incubated for 1 hour with 5-fold excess of the peptide used for immunization, there was no labeling in the hypodermis nor specific band in the Western blot.

Anatomy of the *C. salei* CNS



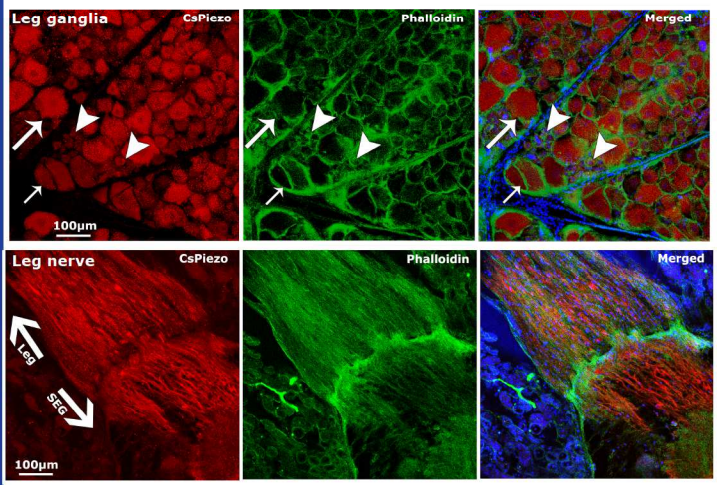
Lateral view of the prosomal ganglion complex. The supraoesophageal ganglion includes the optic lobes (OPL) and the brain. Optic nerves entering the optic lobes are the anterior and the posterior median eye nerves (AMEN and PMEN). The fused subesophageal ganglions (SOG) is innervated by the cheliceral nerves (CN), the pedipalpal nerves (PN), the leg nerves (1-4), and the opisthosomal nerve (ON) (Modified from (Babu and Barth 1984).

CsPiezo and synapsin double labeling

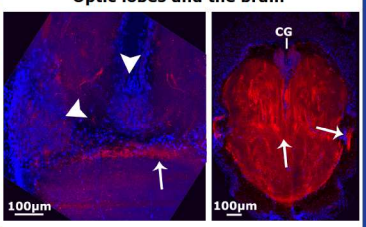


Spider peripheral mechanosensory neurons are innervated by efferent fibers. We performed double labeling with anti-CsPiezo and a monoclonal antibody for *Drosophila* synapsin (Klagges et al 1996). AlexaFluor 488 was the secondary antibody (Jackson). Synapsin labeling was detected in nerve fibers surrounding the axons (*arrows*), somata (*) and dendrites (*arrowheads*) of VS-3 and tactile spine neurons. Merged images show that the CsPiezo and synapsin immunoreactivity were not co-localized. Blue is nuclear counterstain by Dapi.

CsPiezo immunolabeling in the CNS



Optic lobes and the brain



Above: CsPiezo labeling in the supraesophageal ganglia was mainly found in nerve fibers in the optic neuropils (*arrow, left*) and in the central and lateral areas of the brain (*arrows, right*). Areas rich with cell bodies were not immunoreactive to CsPiezo (*arrowheads*). CG=Cheliceral Ganglia. Blue is the Dapi nuclear stain.

Above: CsPiezo antibody labeled many large (50-100µm, *arrow)* and medium sized (20-40µm) somata in the leg ganglia. The smallest neurons (*arrowheads*) were not labeled. Lower: Multiple nerve fibers projecting to the legs were strongly labeled by CsPiezo. Phalloidin (Cell Signaling technology, 1:40 dilution) labeled the F-actin and Dapi (*blue*) stained the nuclei.

References:
Babu KS, Barth FG (1984) Neuroanatomy of the central nervous system of the wandering spider, *Cupiennius salei* (Arachnida, Araneida). Zoomorphology 04:344-359.
Klagges BR, Heimbeck G, Godenschwege TA, Hofbauer A, Pflugfelder GO, Reifegerste R, Reisch D, Schaupp M, Buchner S, Buchner E (1996) Invertebrate synapsins: a single gene codes for several isoforms in *Drosophila*. J Neurosci 16:3154-3165.
Zhao Q, Zhou H, Chi S, Wang Y, Wang J, Geng J, Wu K, Liu W, Zhang T, Dong MQ, Wang J, Li X, Xiao B (2018) Structure and mechanogating mechanism of the Piezo1 channel. Nature 554:487-492.

Summary and Conclusions

- *C. salei* Piezo protein is homologous to the mouse Piezo1/2 and *Drosophila* Piezo proteins (~30%).
- *CsPiezo* mRNA is expressed in all mechanosensory neurons of the spider patella. It is also widely expressed in the SEG and in some regions of the brain and optic lobes.
- Immunoreactivity against a custom-made CsPiezo antibody was detected in the dendrites, around the cell bodies and in the axons of mechanosensory neurons as well as in the leg nerve. Double labeling with anti-synapsin indicates that CsPiezo is not in the efferents.
- Anti-CsPiezo labeling was widespread in neurons of the SEG, many nerve fibers projecting to the legs and it was also found within various regions of the brain and optic lobes.
- Labeling of mechanosensory dendrites may indicate that CsPiezo has a role in sensory mechanotransduction. However, the extensive expression in other parts of these neurons and in many neurons of the brain, suggests that this protein must have other important functions in *C. salei* CNS and PNS.