



Институт физиологии им. А.А. Богомольца НАНУ
Международный центр молекулярной физиологии НАНУ



Новый взгляд на молекулярную природу ощущений

Я.М. Шуба

Time

16:33:35

h : min : s

IX Школа-семинар "Биофизические методы исследования"
Киев, 20-22 апреля 2016 г.

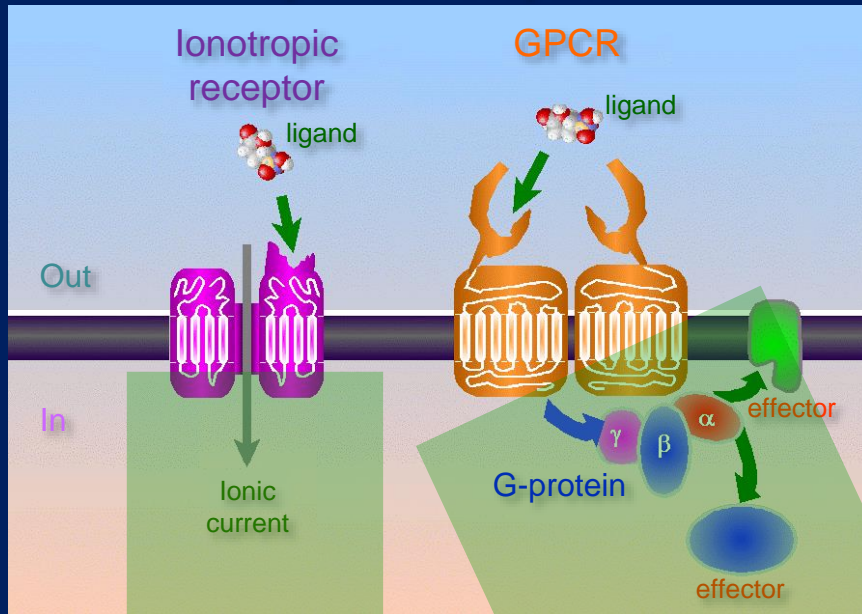
Slide

1 / 15

Lecture plan

- 1) Temperature sensitivity (slides 4-7);
- 2) Mechanosensitivity of invertebrates (8-19);
- 3) Mechanosensitivity of mammalian tissues (slides 12-14).

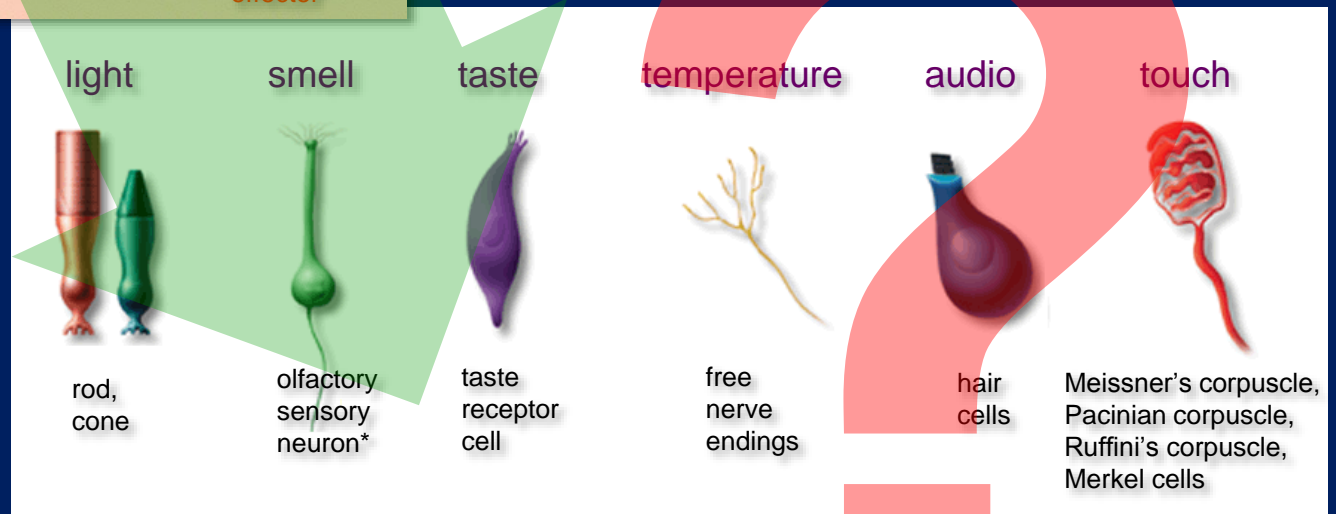
Classical molecular types of ligand-operated receptors



Receptor types

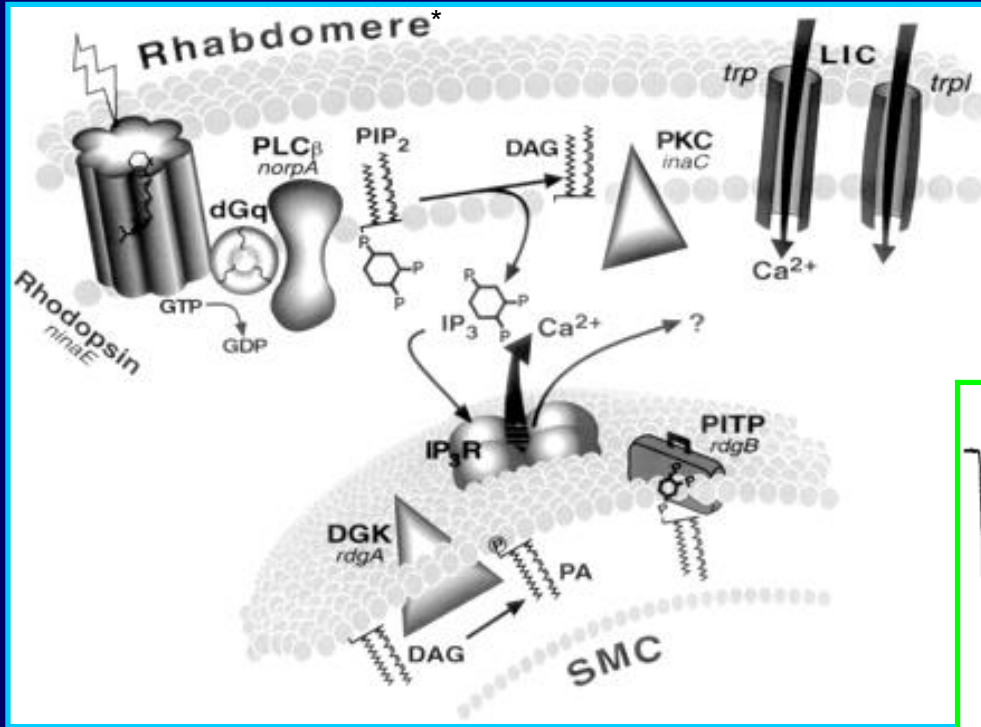
Cellular receptor types

Molecular nature

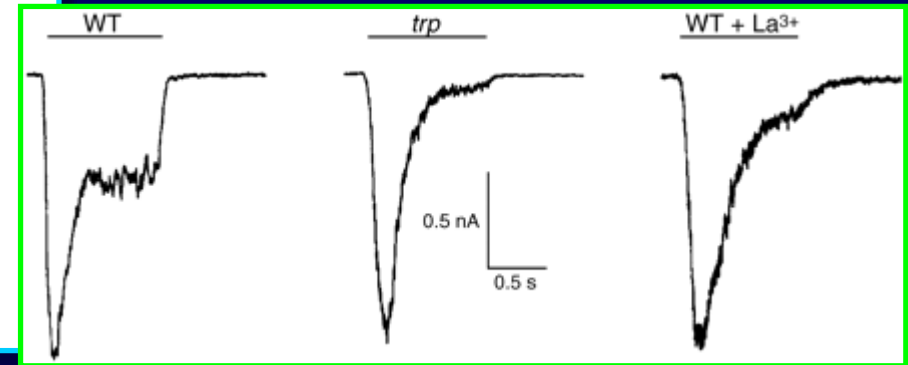


Transient Receptor Potential (TRP) channel in *Drosophila* vision

Phototransduction in the compound eye of arthropods



A spontaneous *Drosophila* phototransduction mutant identified in 1977 (Minke, 1977) displayed transient receptor potentials (trp) in response to continuous light. Identification of the gene product underlying that mutation and recognition of its function as an ion channel (Montel and Rubin, 1989) gave rise to the awareness of a new class of cation channels that differed significantly from the canonical voltage-dependent channels.

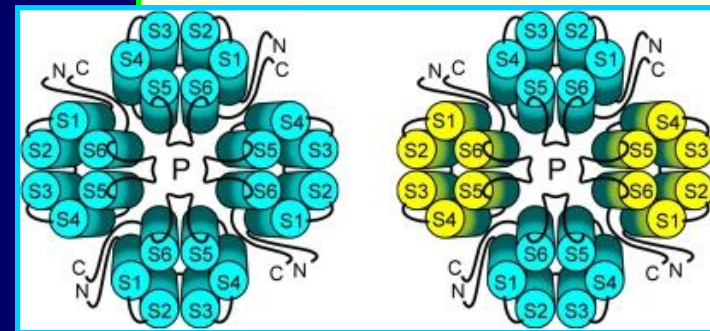
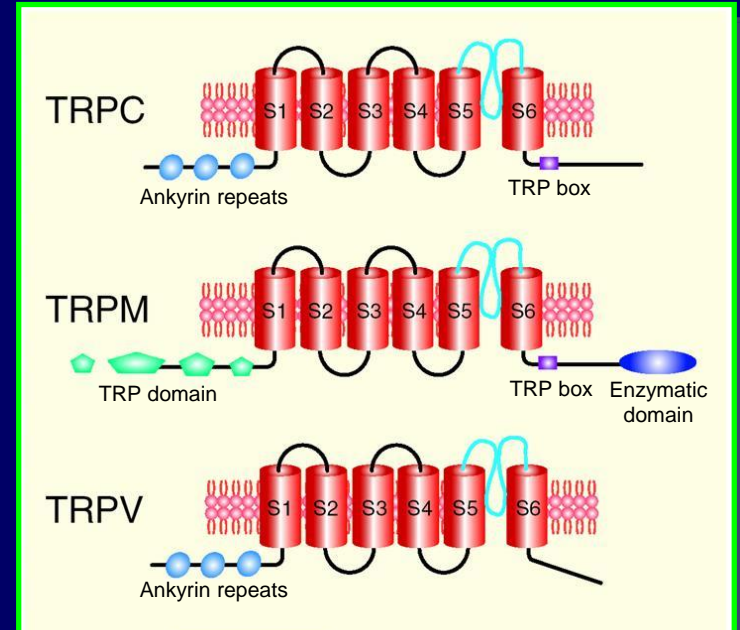
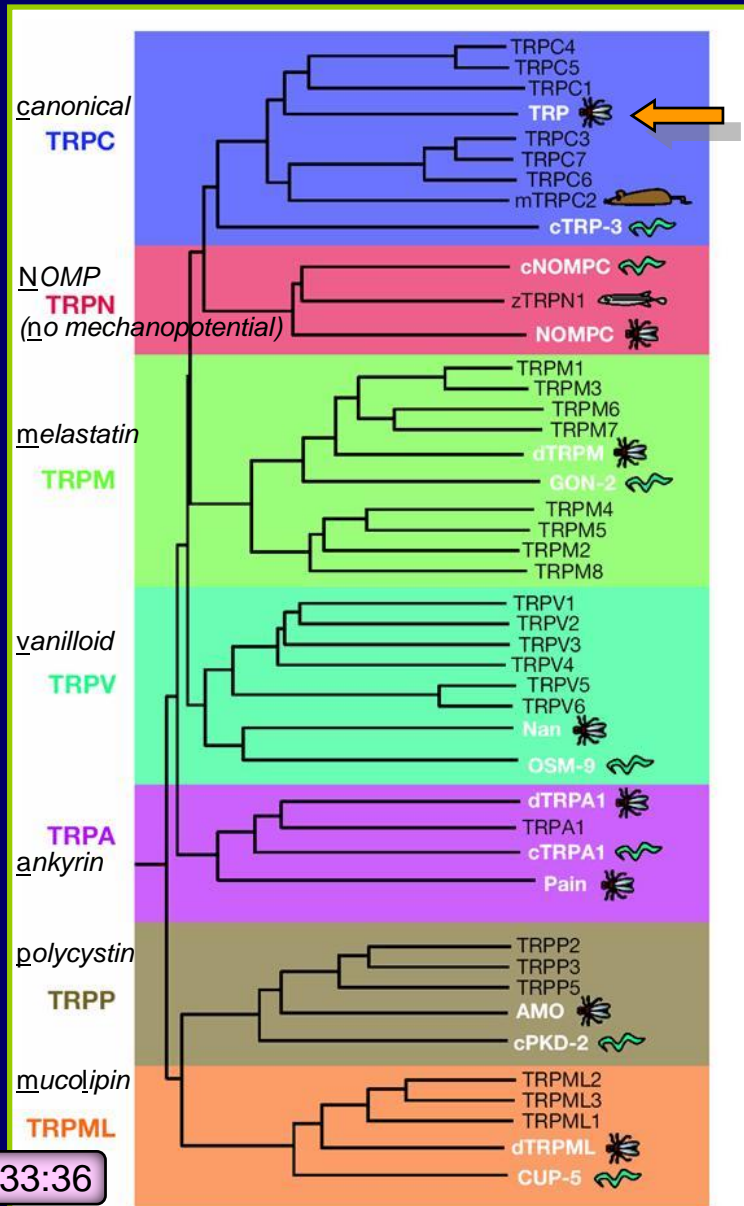


The phosphoinositide cascade of insect's vision. Upon absorption of light, rhodopsin (*ninaE* gene) is converted to the active metarhodopsin state, which activates a heterotrimeric G protein (dGq). This leads to activation of phospholipase C (PLC, *norpA* gene) and subsequent opening of two classes of light-sensitive channels encoded at least in part by *trp* and *trpl* genes, by an as yet unknown mechanism. TRP and TRPL opening leads to the light-induced current (LIC). Deactivation of channel activity is regulated by protein kinase C (PKC, *inaC* gene).

The *trp* phenotype. Light-induced currents in response to prolonged intense orange lights were recorded in voltage-clamped photoreceptors of wild type (WT), the *trp* mutant, and WT treated with La³⁺. A peak response and a plateau characterize the light response of WT. The rapid peak-plateau transition is a manifestation of Ca²⁺-dependent light adaptation. The response of the *trp* photoreceptor decays close to baseline during light due to exhaustion of excitation. A similar decay of the light response close to baseline is obtained by application of 10 M La³⁺ to the extracellular medium of WT photoreceptors.

Superfamily of TRP-channels

(TRP - Transient Receptor Potential)



Thermo-TRP - receptors of temperatures and pungent chemicals



Identification of a cold receptor reveals a general role for TRP channels in thermosensation

NATURE | VOL 416 | 7 MARCH 2002 | www.nature.com

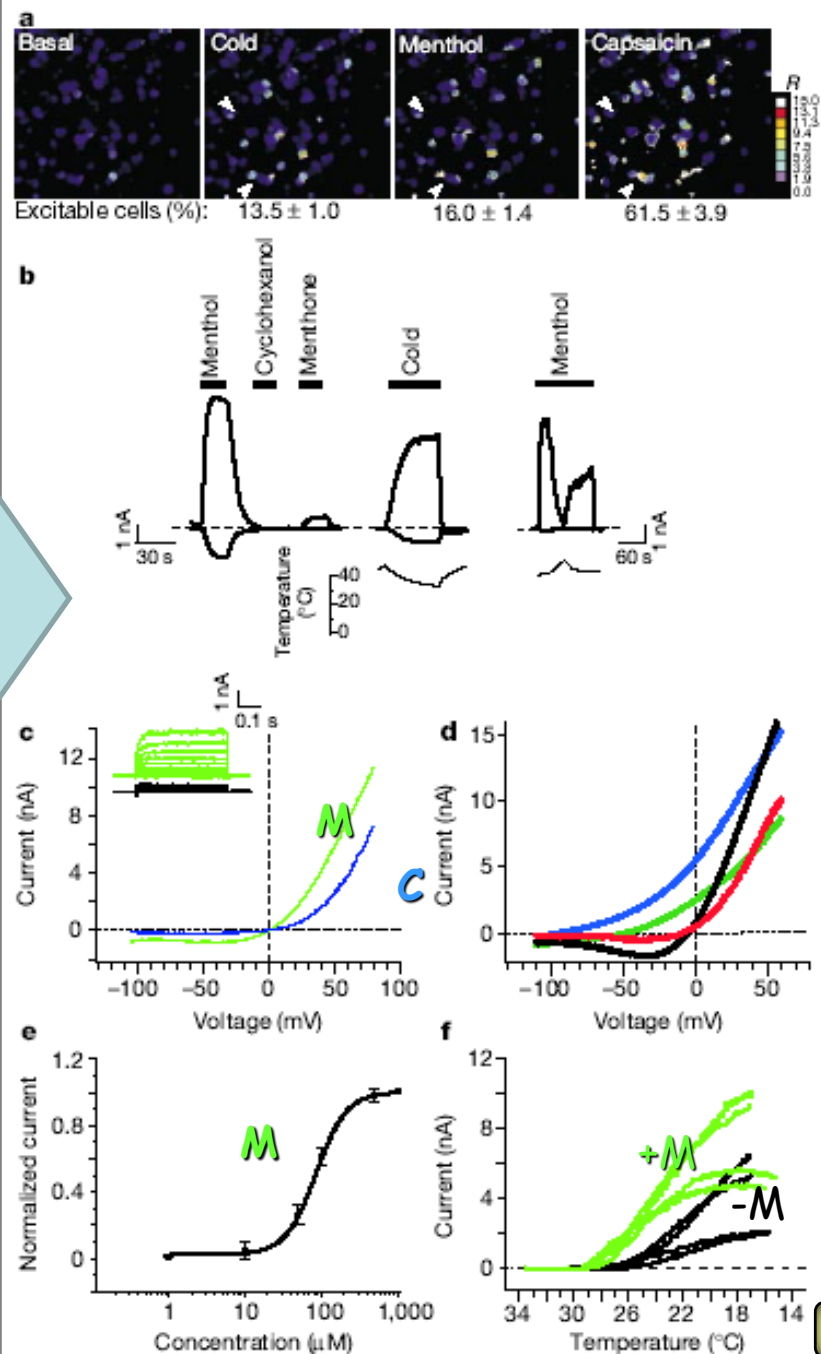
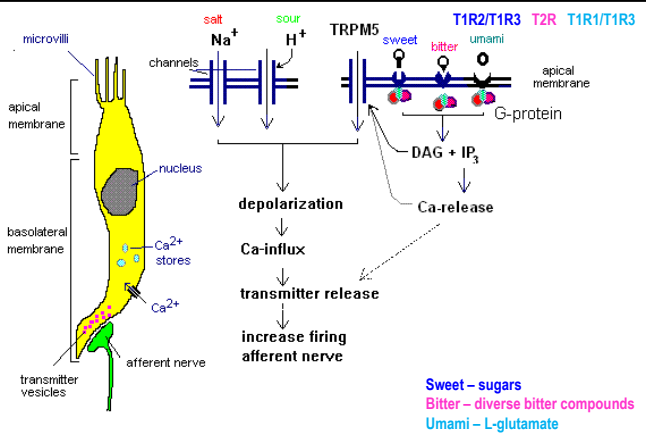
David D. McKemy[†], Werner M. Neuhauser[†] & David Julius^{*}

^{*} Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143-0450, USA
[†] These authors contributed equally to this work

How thermo-TRPs operate?

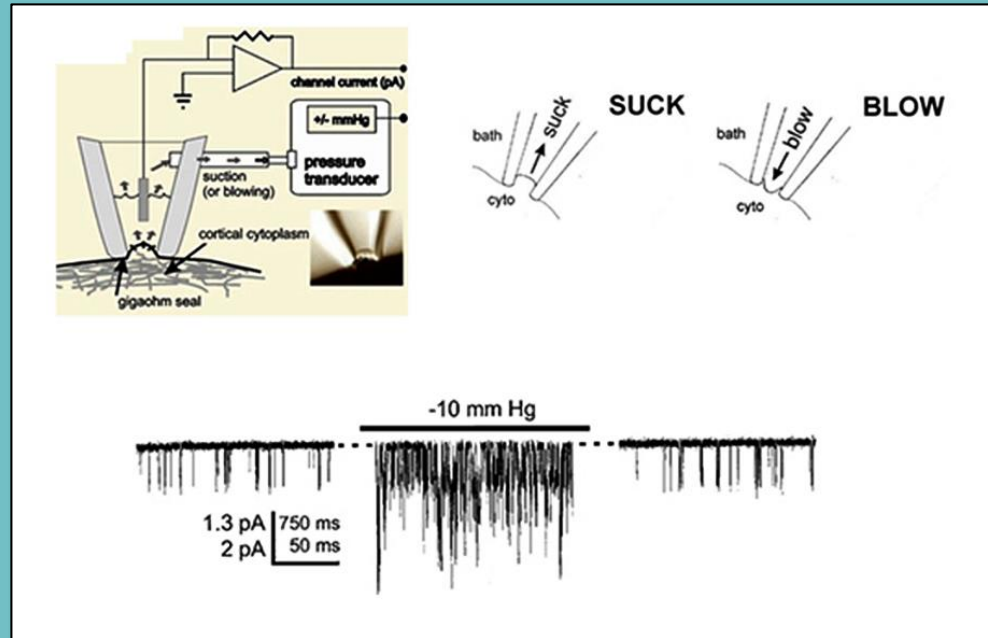
TRPM8

TRPM5 as taste transducer



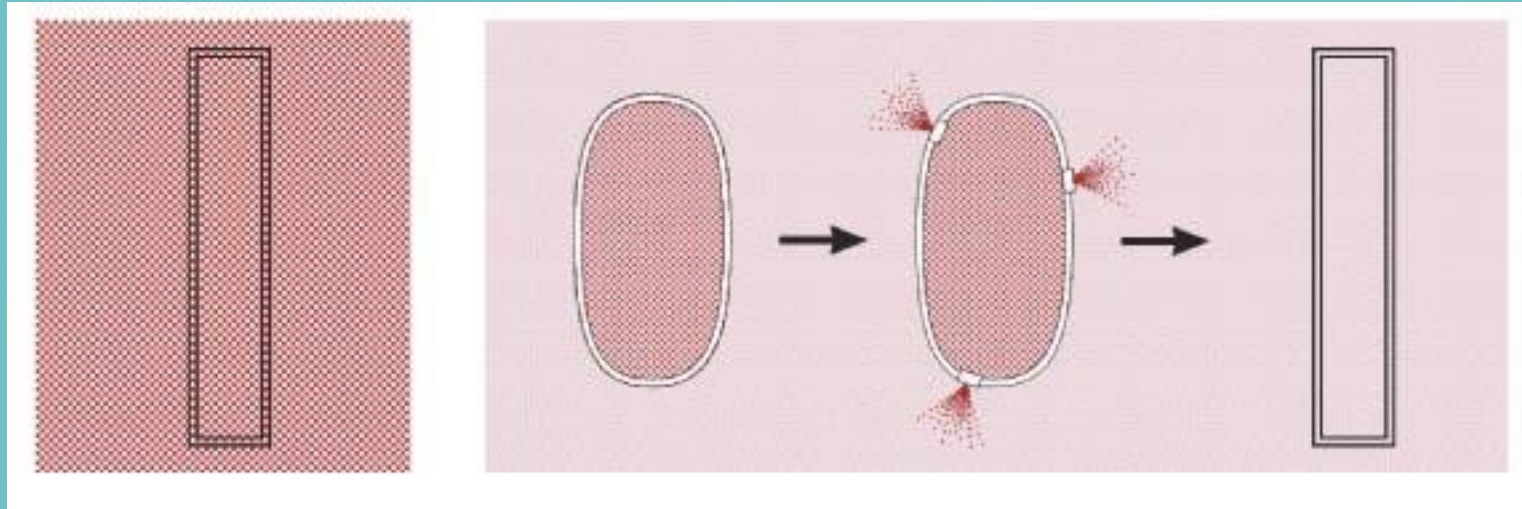
16:33:36

Mechanosensation - one of the last enigmas



Despite widespread distribution of the phenomenon of mechanosensitivity, its molecular nature for a long time remained unknown. It was long believed to rely on multiple channel types for which membrane tension was one of many modulating influences rather than the primary activating stimulus

E. coli volume regulation*



normal environment

rain or upon medium dilution



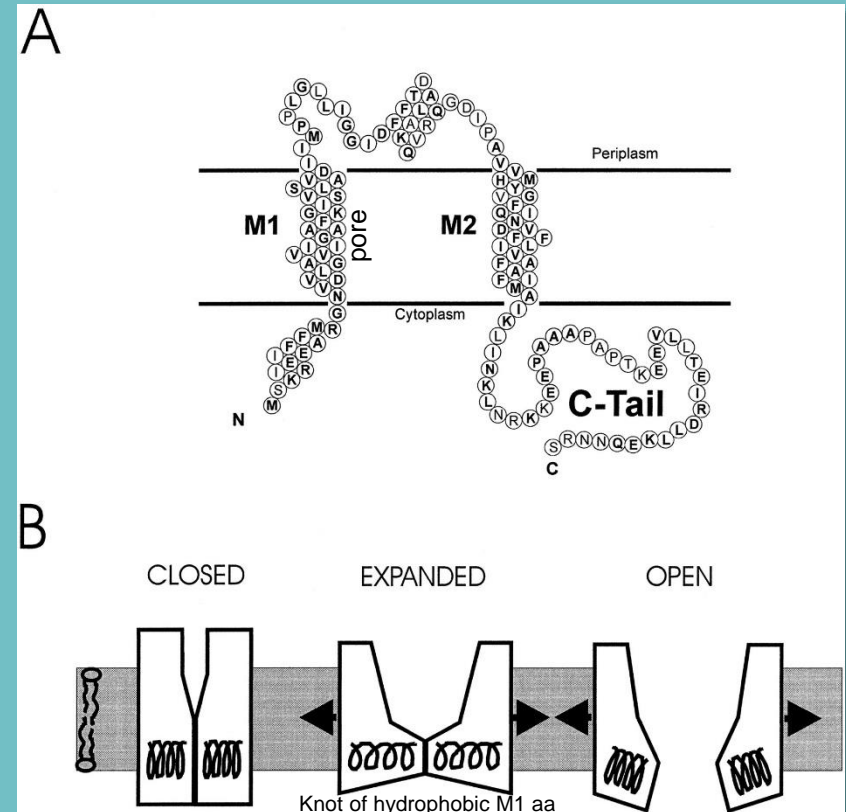
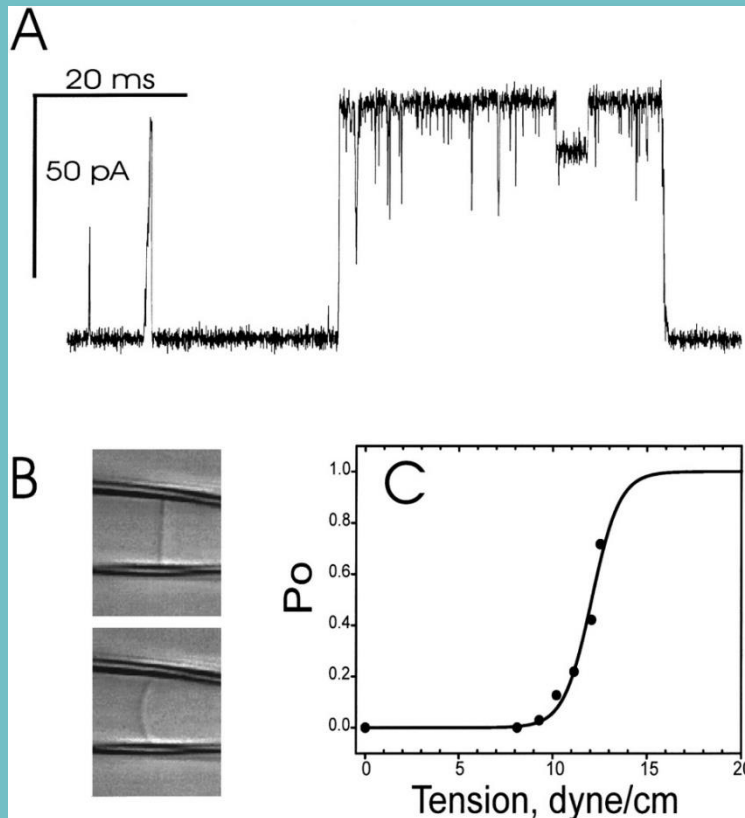
E. coli volume

Entry of water through the lipid bilayer swells the bacterium and stretches open the MA channels to release solutes, enabling it to reach a new equilibrium, escape osmolysis and return to initial shape.

*In mammals the mechanisms of reaction to membrane stretch associated with changes in cell volume (VRAC/LRRC8A/SWELL1) and to direct mechanical stimulation are different!

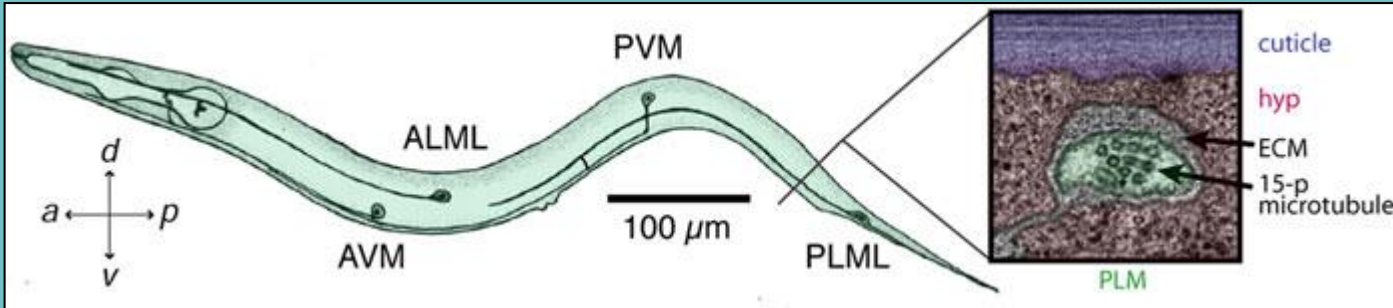
MscL* from *E. coli* - the first molecule converting mechanical tension of the membrane into the opening of aqueous pore

MscL - homo-pentamer composed of 15-kDa, two transmembrane domain subunits and 30-40 Å pore size

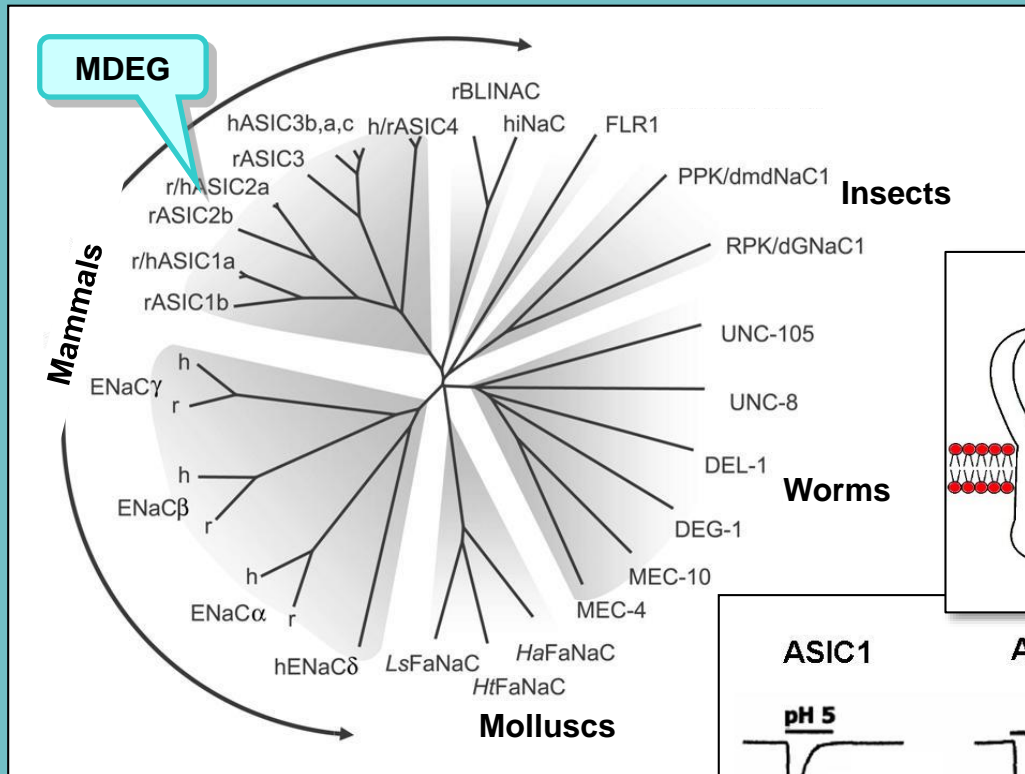


Sukharev, FASEB J, 13:S55-S61, 1999

Degenerins - molecular basis for mechanical sensitivity in nematods



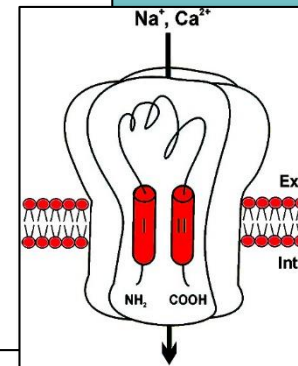
Touch sensitivity are defined by the arrangement of touch receptor neurons along the body axis. The ALMs and AVM mediate the response to touch over the anterior field whereas PLMs mediate the response to touch over the posterior field. PVM does not mediate touch response by itself



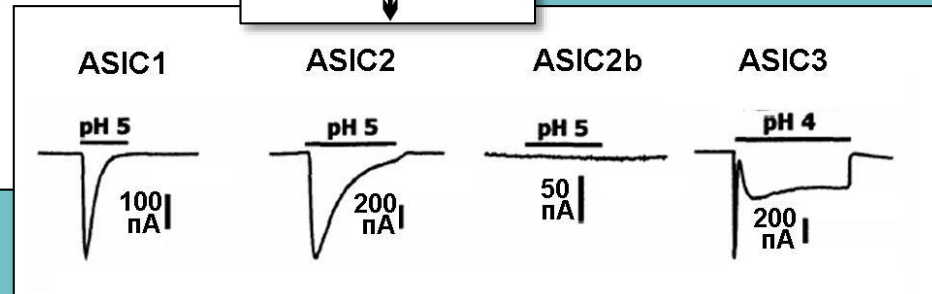
Wild-type



Degenerin mutant

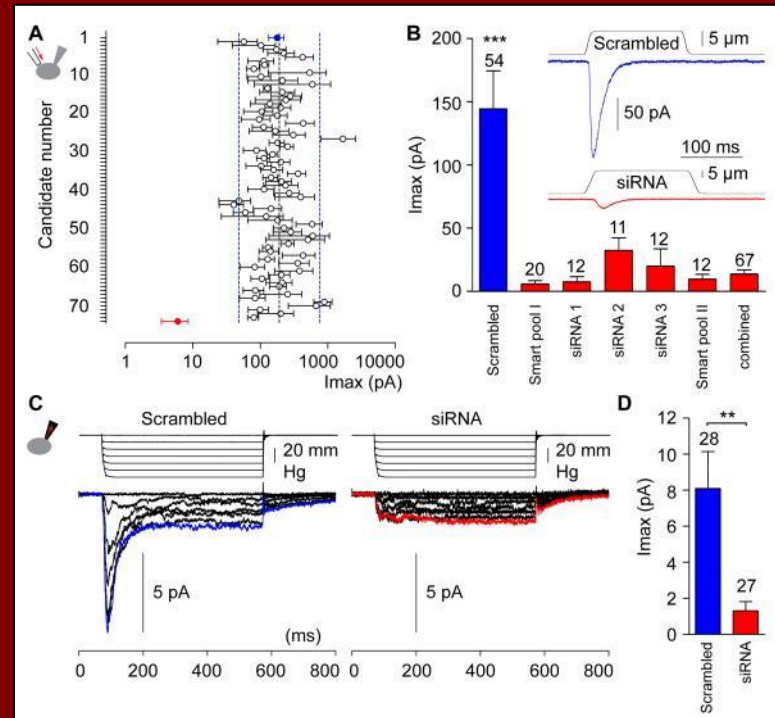
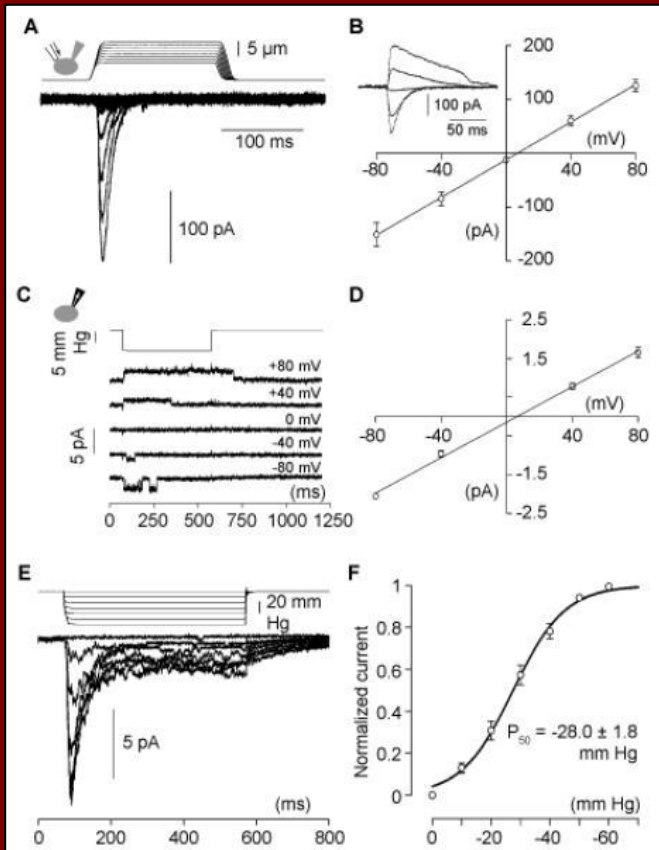
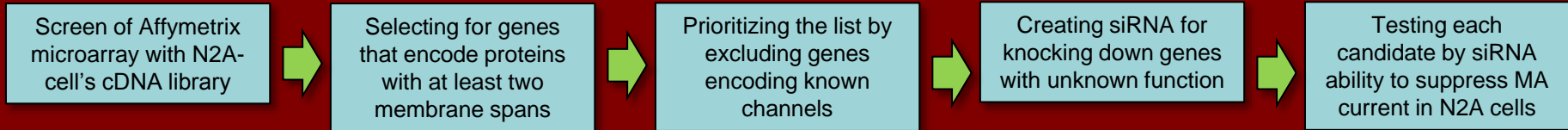


Homo-trimer of two transmembrane domain subunits



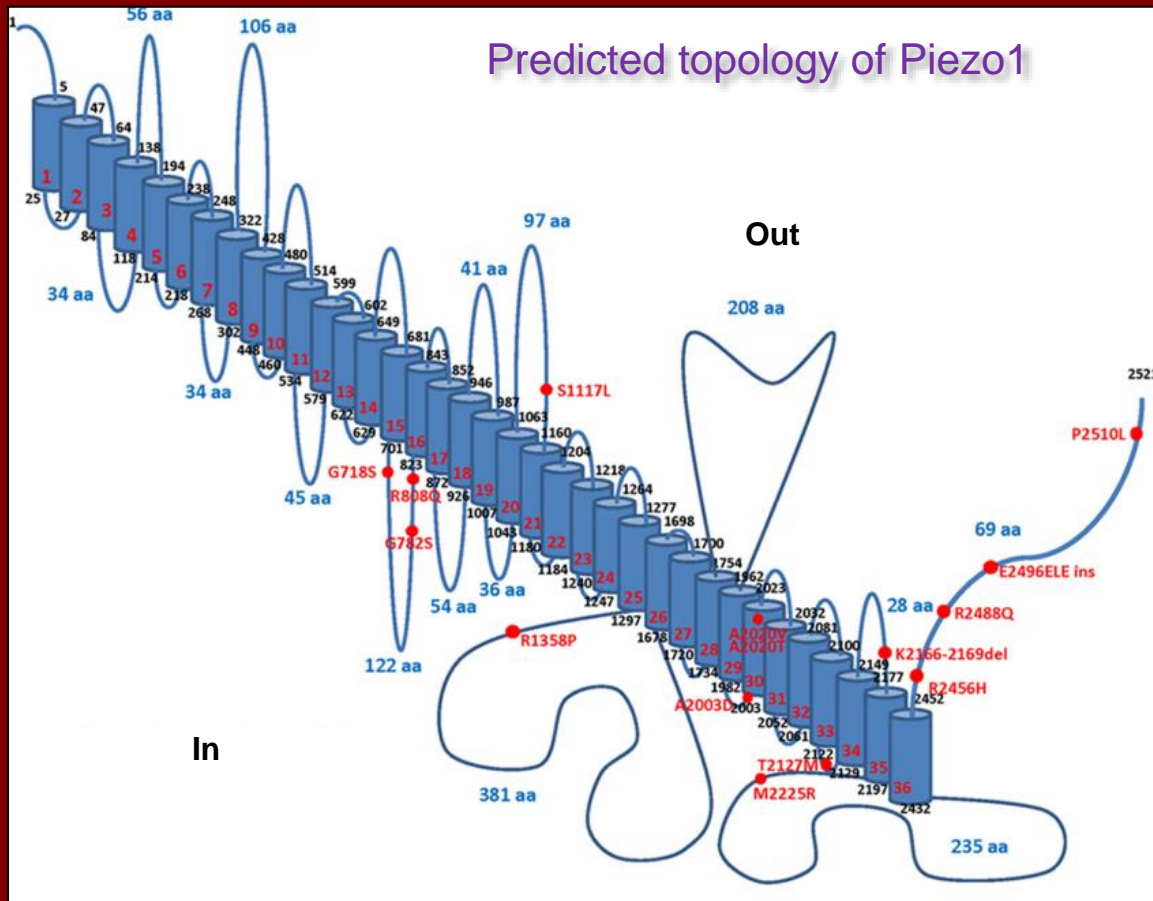
Identification of mechanoactivated (MA) channel in Neuro2A (N2A) mouse neuroblastoma cells

Strategy:



siRNA-mediated knockdown of *Fam38A* gene caused inhibition of stretch-activated current, which is why the gene was renamed to *Piezo1*. Homologous gene, *Fam38B*, was named *Piezo2*.

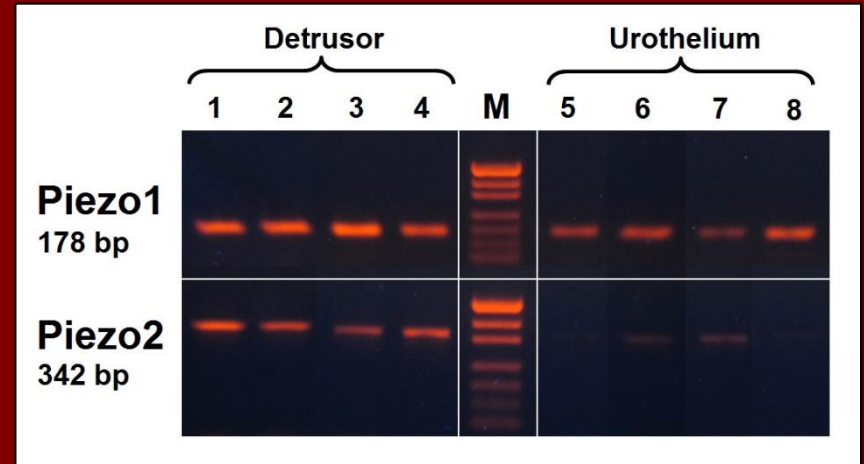
Channel forming proteins, Piezo1 and Piezo2, as molecular basis of MA channels in mammals



Piezo1 and Piezo2 consist of ~2500 and ~2800 amino acids, respectively, and share 50% of sequence identity. Functional MA channels are most probably homo-tetramers.

Coste *et al.* Piezos are pore-forming subunits of mechanically activated channels. *Nature*, 483(7388):176-181, 2012

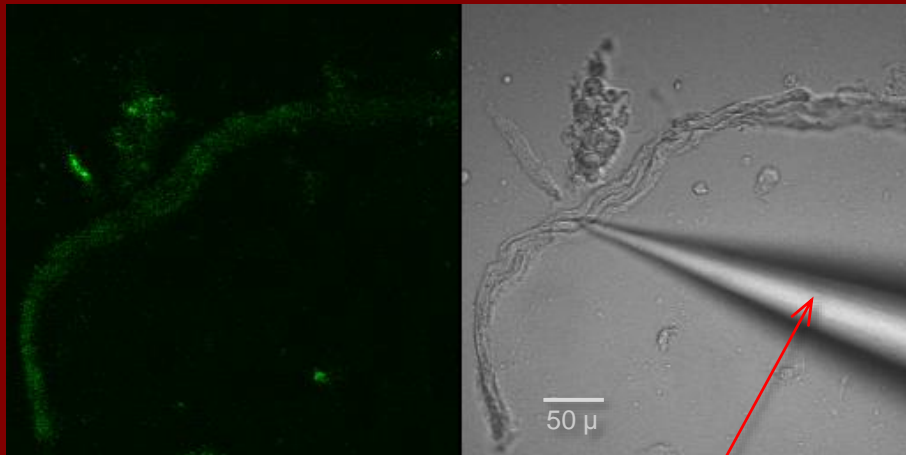
Piezo mechanoreceptors in mechanosensitivity of bladder tissues



Cluster of smooth muscle cells from rat bladder

Ca²⁺-dependent fluorescence

transmitted light

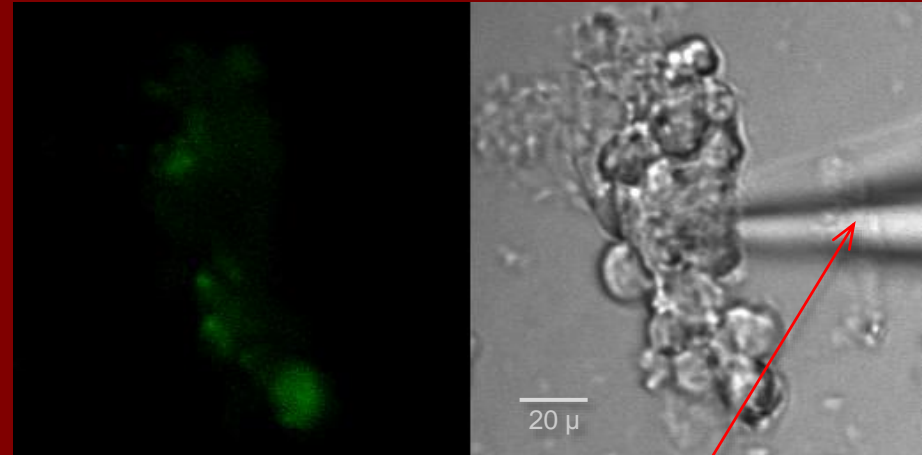


micropipette

Cluster of urothelium cells from rat bladder

Ca²⁺-dependent fluorescence

transmitted light



micropipette

Спасибо за внимание!

