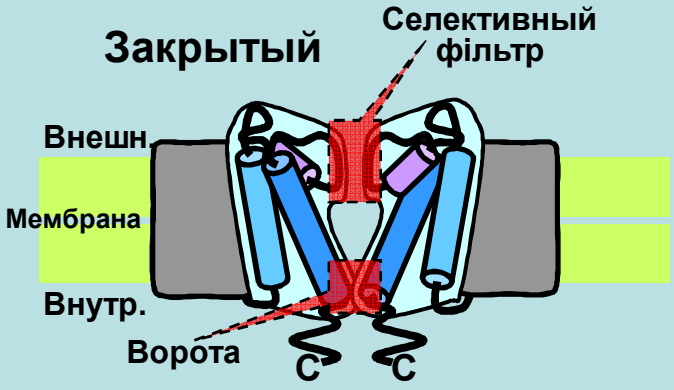
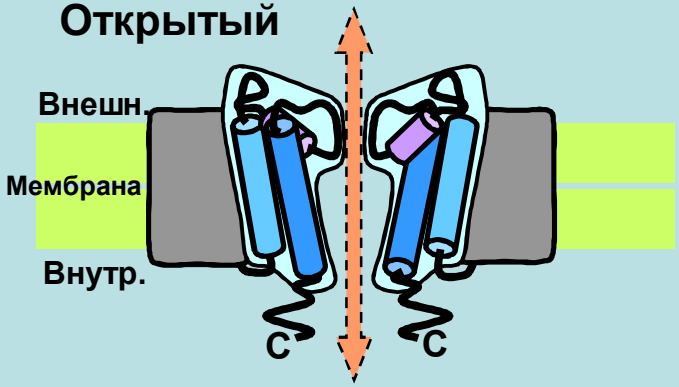
Институт физиологии им. А.А. Богомольца НАНУ
Международный центр молекулярной физиологии НАНУ

Как муха и червяк помогают изучать болезни человека?

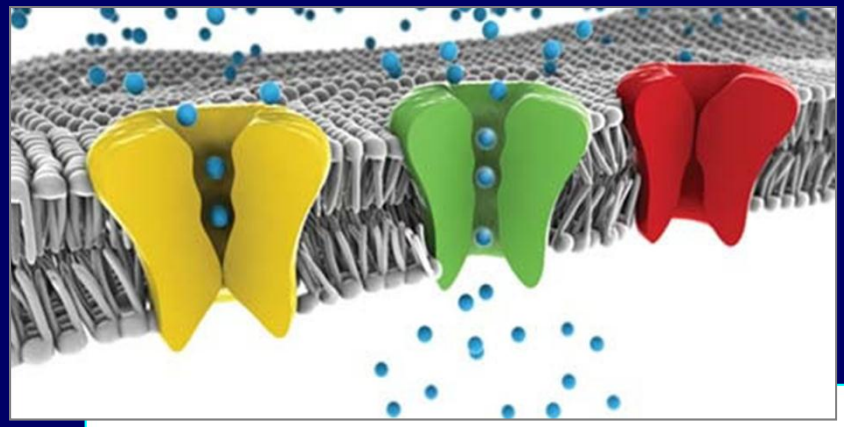
Я.М. Шуба



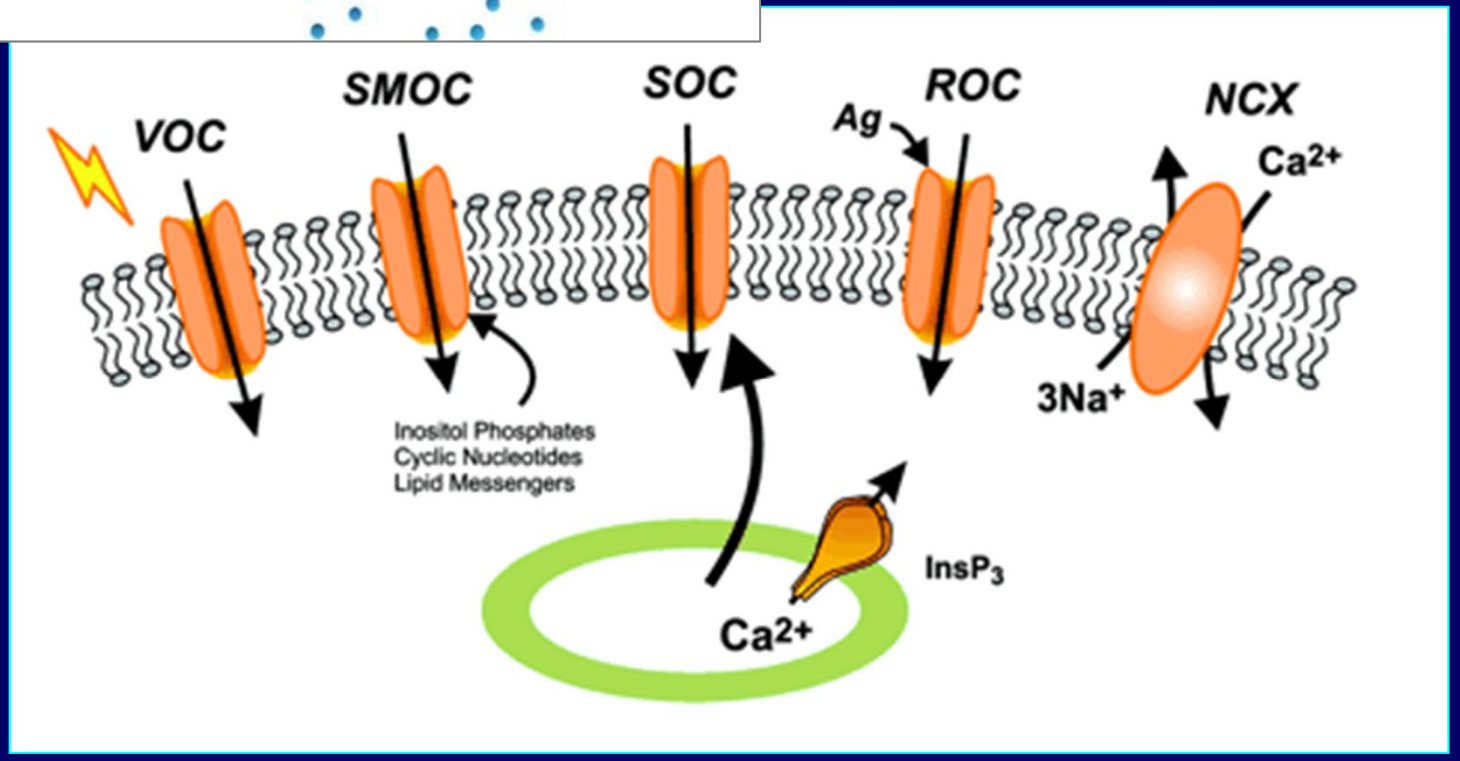


8-я студенческая школа-семинар “Биофизические методы исследования”,
Киев, 14-16 мая 2014

Классы ионных каналов



VOC - voltage-operated channels;
SMOC - second messenger-operated channels;
SOC - store-operated channels;
ROC - receptor-operated channels;
NCX – Na^+-Ca^{2+} exchanger.



Каналоспецифичаские лекарственные препараты:

Антиаритмики

- I типа: блокаторы натриевых каналов
- III типа: блокаторы калиевых каналов
- IV типа: блокаторы кальциевых каналов

Антигипертензивные

- блокаторы кальциевых каналов
- активаторы $K_{АТФ}$-каналов

Кардиопротекторы

активаторы $K_{АТФ}$-каналов

Анальгетики

- блокаторы натриевых каналов
- блокаторы кальциевых каналов
- блокаторы TRPV1 каналов
- блокаторы P2X рецепторов

Антиэпилептики

- агонисты $ГАМК_A$ рецепторов
- блокаторы натриевых каналов
- блокаторы НП кальциевых каналов
- блокаторы ионотропных глутаматных рецепторов

Протираковые

- блокаторы потенциал-независимых кальцієвых каналов (carboxyamido-triazole, CAI)
- блокаторы хлорных каналов (iodine-chlorotoxin)

Антидиабетики

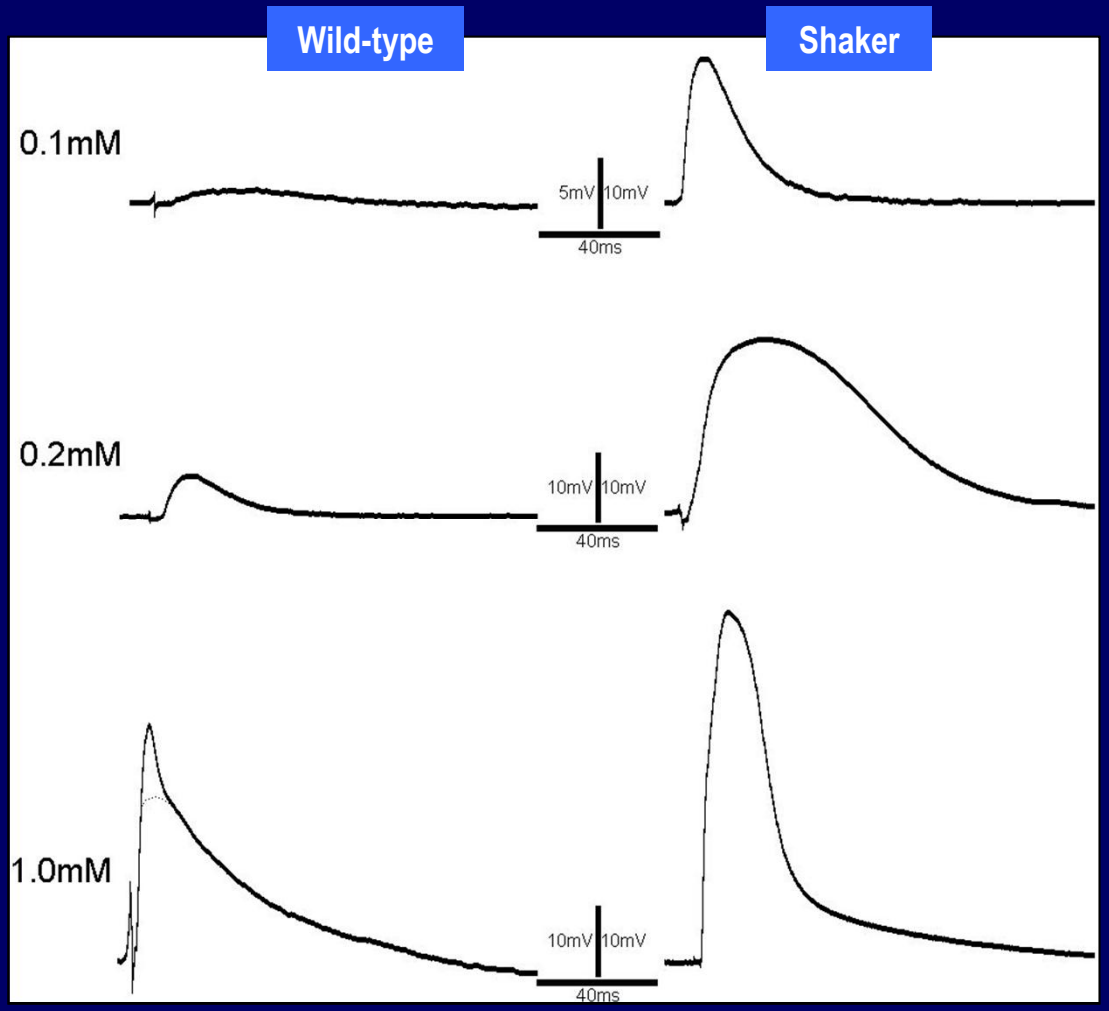
блокаторы $K_{АТФ}$-каналов (сульфанил-мочевины)

Анестетики

- Локальные: блокаторы натриевых каналов
- Общие: активаторы калиевых каналов

Shaker и *ether a go-go* фенотипы *Drosophila* и их молекулярные основы

ВПСП в нервно-мышечном синапсе мухи



[illegible] 1969 [illegible] 3 [illegible] *Drosophila*:

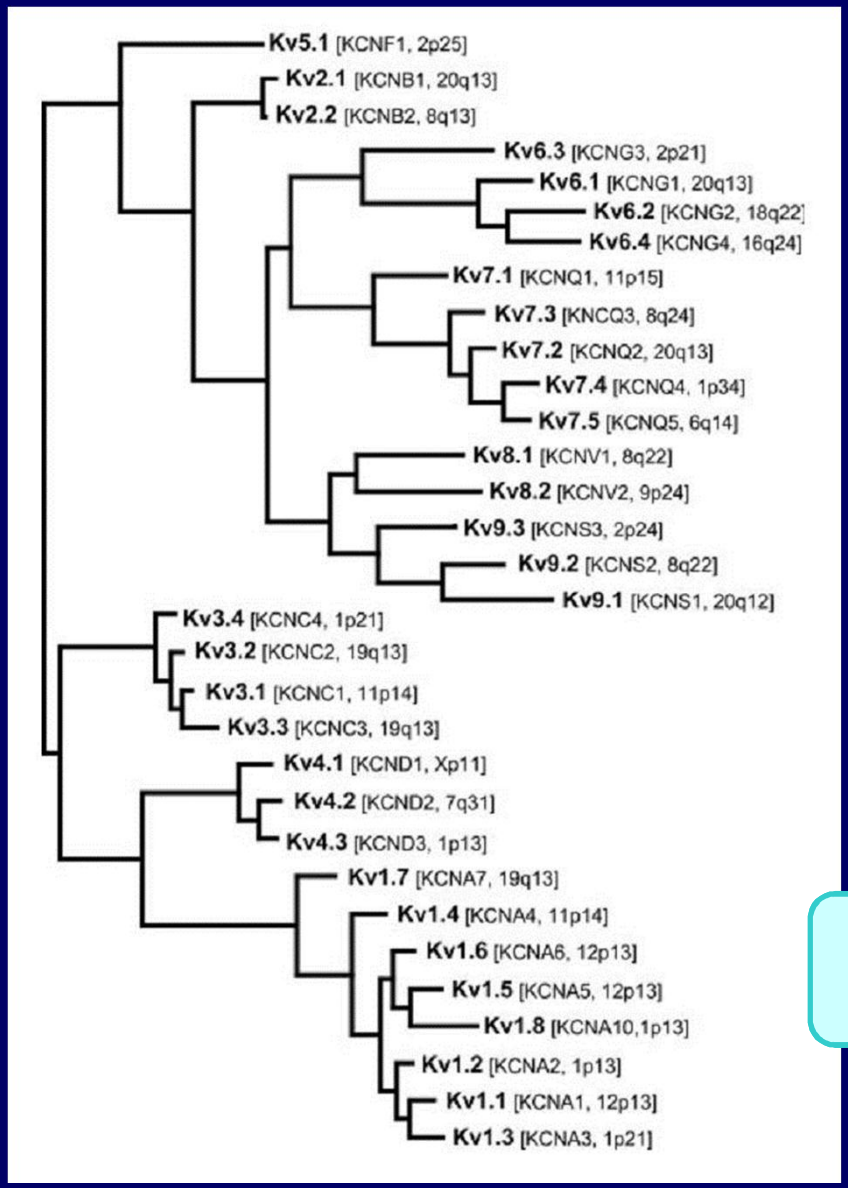
Shaker (*Sh*),
ether a go-go (*eag*),
Hyperkinetic (*Hk*)

[illegible]

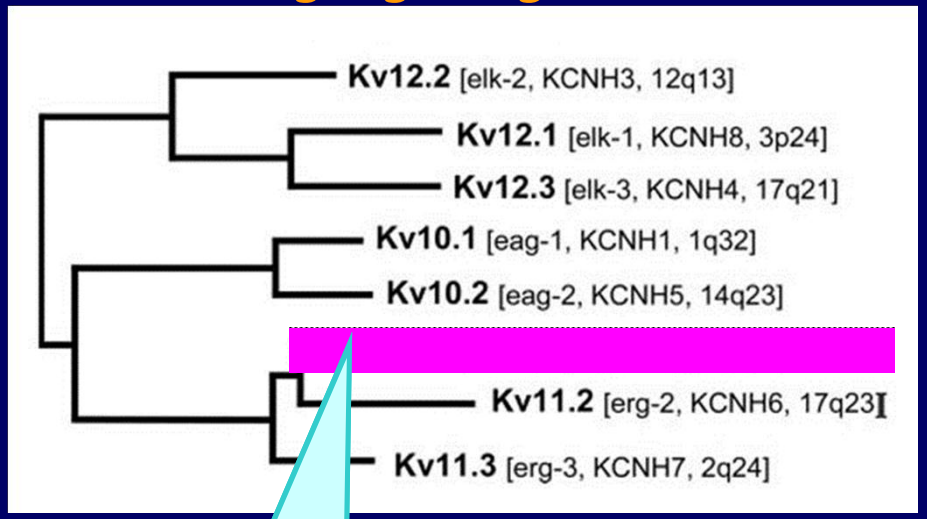
Ganetzky B. There's a whole lot of shaking going on. *Genetics.* 1989 Feb;121(2):201-4.

Калиевые каналы млекопитающих

Shaker-подобные



Ether-a-go-go(eag)-подобные

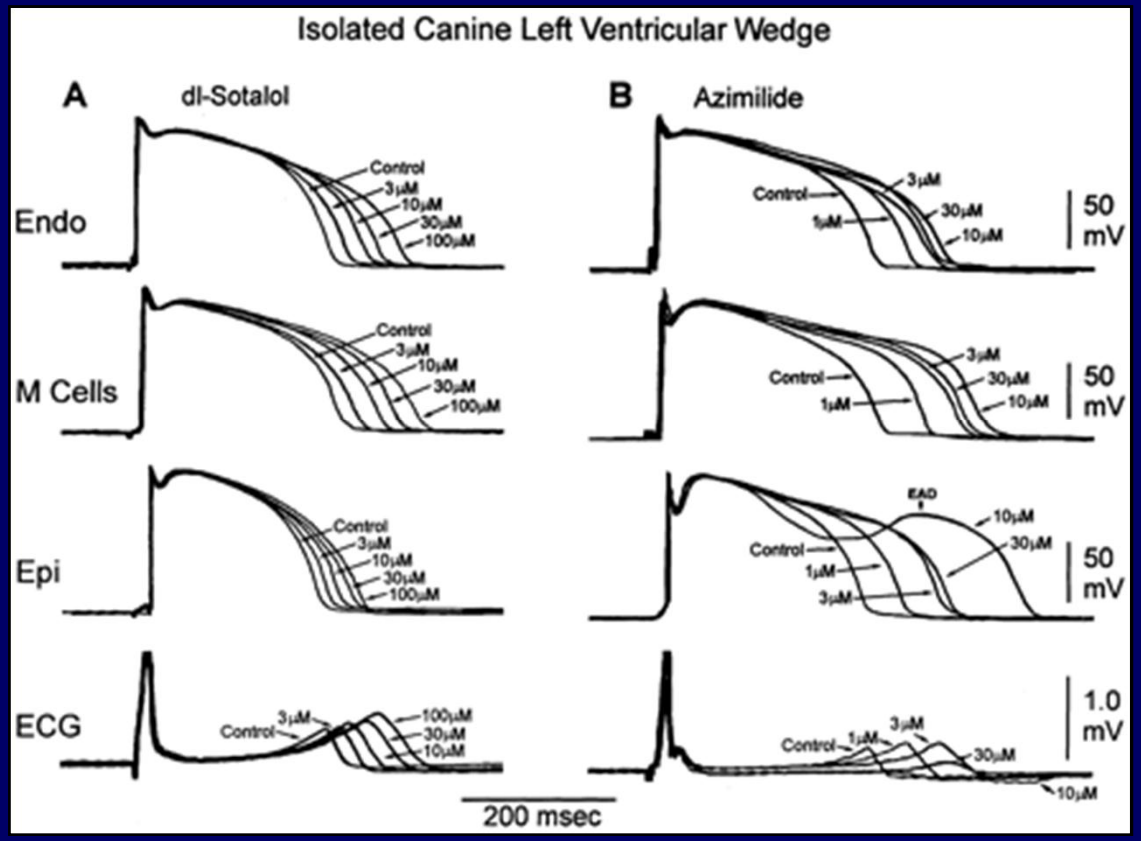


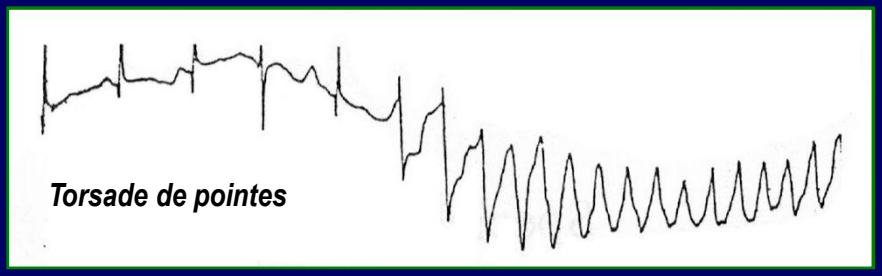
Human Ether-a-go-go-Related Gene (**HERG**)

Связь между ЭКГ, потенциалом действия и токами в сердце

HERG-опосредованный I_{Kr} - один из основных реполяризирующих калиевых токов в сердце

LQTS (**L**ong **QT** **S**yndrom): синдром удлиненного QT интервала электрокардиограммы - фактор риска и причина возникновения летальных вентрикулярных аритмий *Torsad de Pointes*



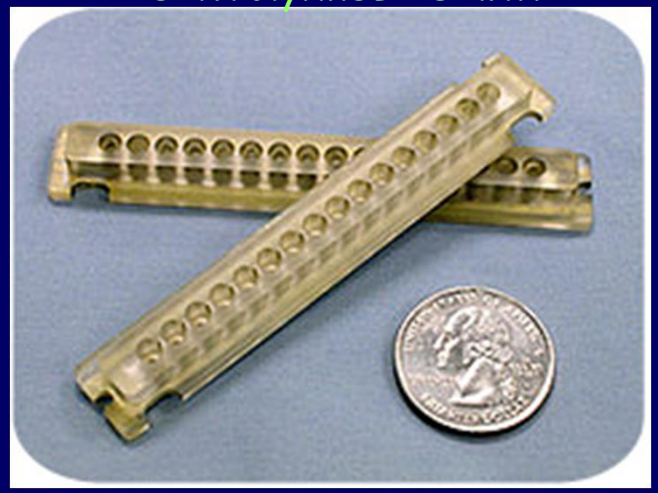


Автоматизированная электрофизиология

Patch-clamp

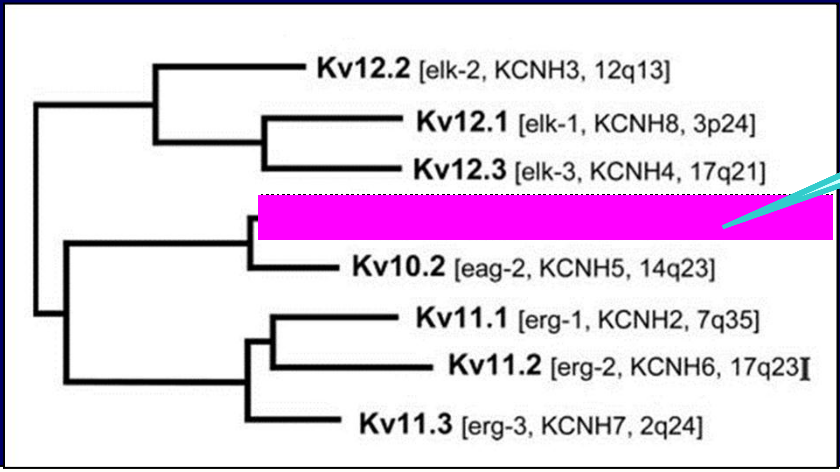
Микроэлектродная

16-ти лунковые чипы



16-ти электродный робот

Ether a go-go(eag)-подобные K⁺ каналы



Asher *et al. World Journal of Surgical Oncology* 2010, **8**:113
http://www.wjso.com/content/8/1/113

WORLD JOURNAL OF SURGICAL ONCOLOGY

REVIEW — Open Access

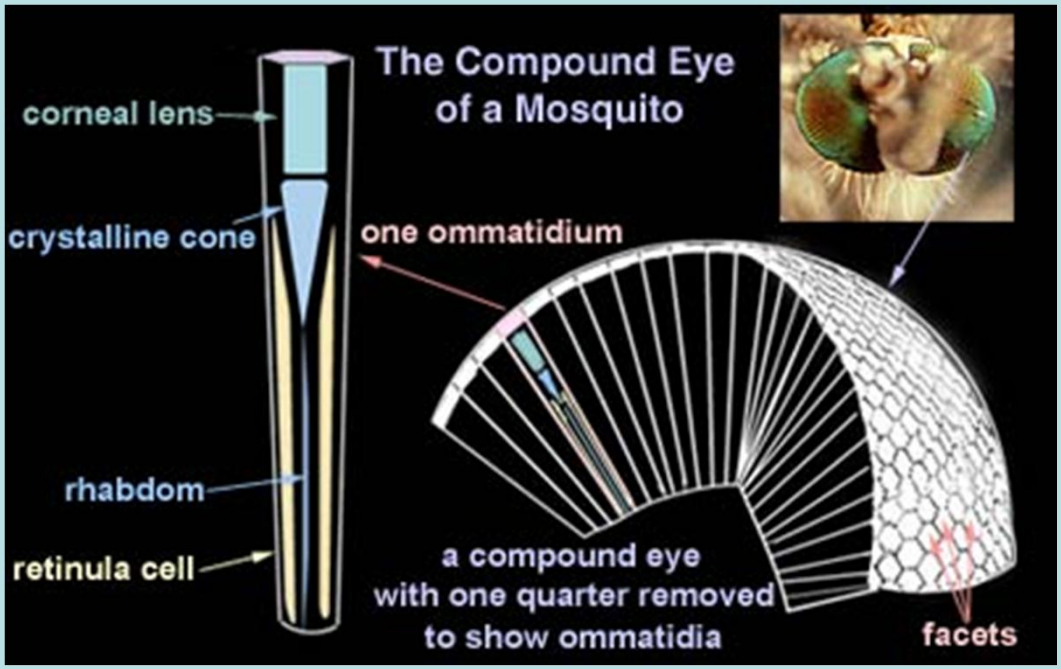
Eag and HERG potassium channels as novel therapeutic targets in cancer

Viren Asher[1*], Heidi Sowter[2], Robert Shaw[3], Anish Bali[4], Raheela Khan[5]

Abstract

Voltage gated potassium channels have been extensively studied in relation to cancer. In this review, we will focus on the role of two potassium channels, Ether à-go-go (Eag), Human ether à-go-go related gene (HERG), in cancer and their potential therapeutic utility in the treatment of cancer. Eag and HERG are expressed in cancers of various organs and have been implicated in cell cycle progression and proliferation of cancer cells. Inhibition of these channels has been shown to reduce proliferation both in vitro and vivo studies identifying potassium channel modulators as putative inhibitors of tumour progression. Eag channels in view of their restricted expression in normal tissue may emerge as novel tumour biomarkers.

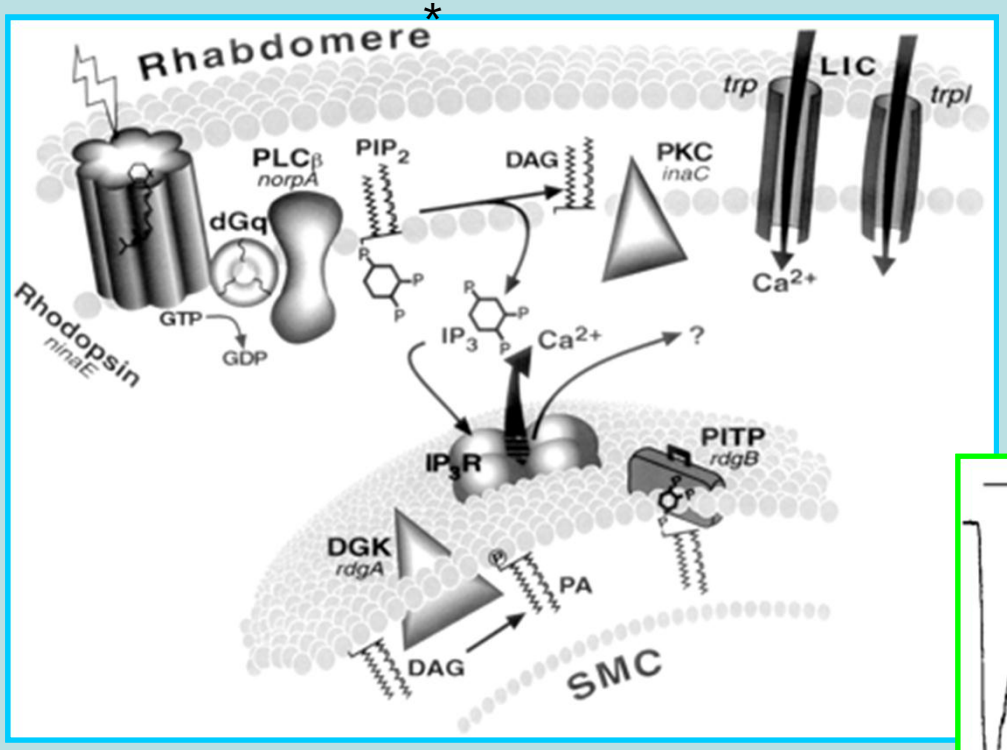
Compound eye (фасеточный глаз) of arthropods



The type of eye comonly found in arthropods. A compound eye has a meshlike appearance because it consists of hundreds or thousands of tiny lens-capped optical units called ommatidia. Each ommatidium has its own cornea, lens, and photoreceptor cells for distinguishing brightness and color. Individual ommatidia guide light through a lens and cone into a channel, known as a rhabdom, which contains light-sensitive cells. These are connected to optical nerve cells to produce the image. The ommatidia are seperated from each other by varying degrees of pigment.

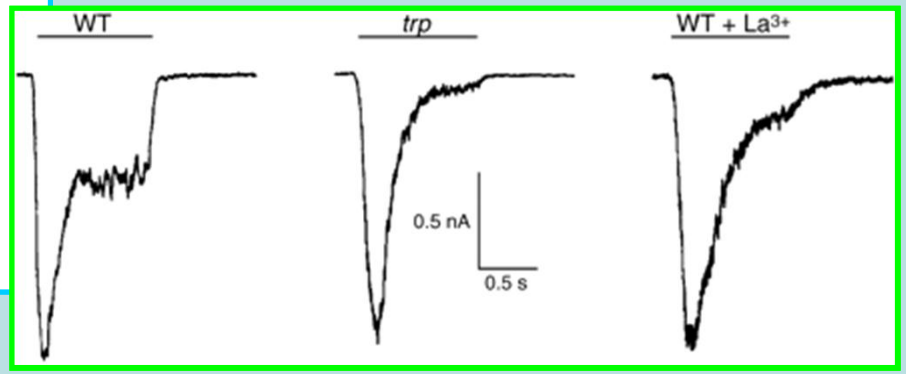
Transient Receptor Potential (TRP) channel in *Drosophila* vision

Phototransduction in the compound eye of arthropods *



The phosphoinositide cascade of insect's vision. Cloned genes (for all of which mutants are available) are shown in italics, alongside their corresponding proteins. 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 kinas C (PKC, *inaC* gene).

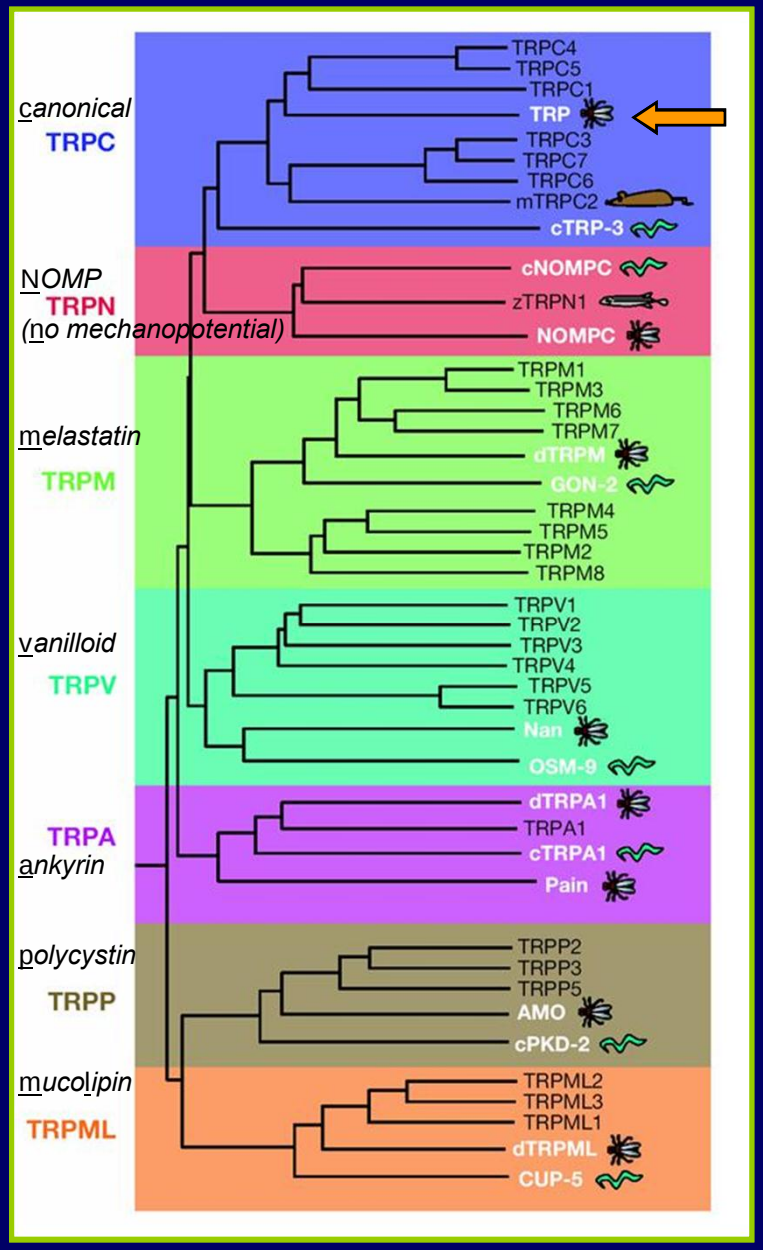
A spontaneous *Drosophila* phototransduction mutant identified in 1977 **(Minke, 1977)** displayed transient receptor potentials (trp) in response to continuous light. Identification of the gene product underlying that mutation and recognition of its function as an ion channel **(Montel and Rubin, 1989)**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

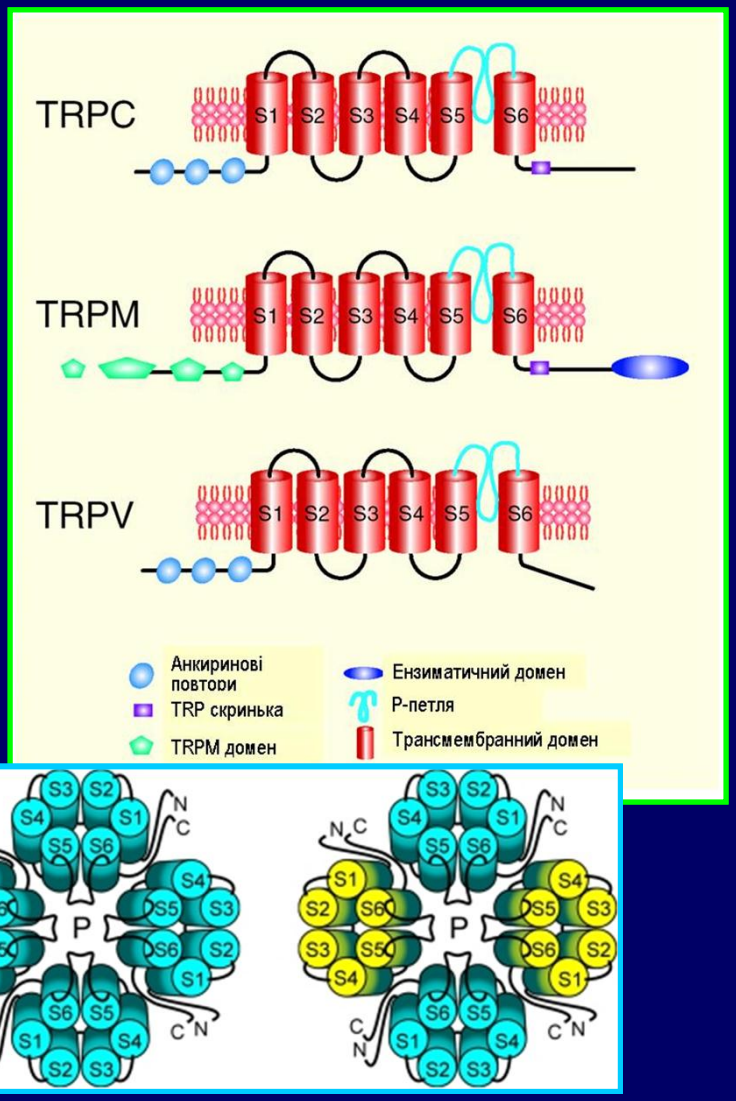


The *trp* phenotype. Light-induced currents in response to prolonged intense orange lights were recorded in voltage-clamped photoreceptors of wild type (WT), the *trp* mutant, and WT treated with La3. A peak response and a plateau characterize the light response of WT. The rapid peak-plateau transition 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.

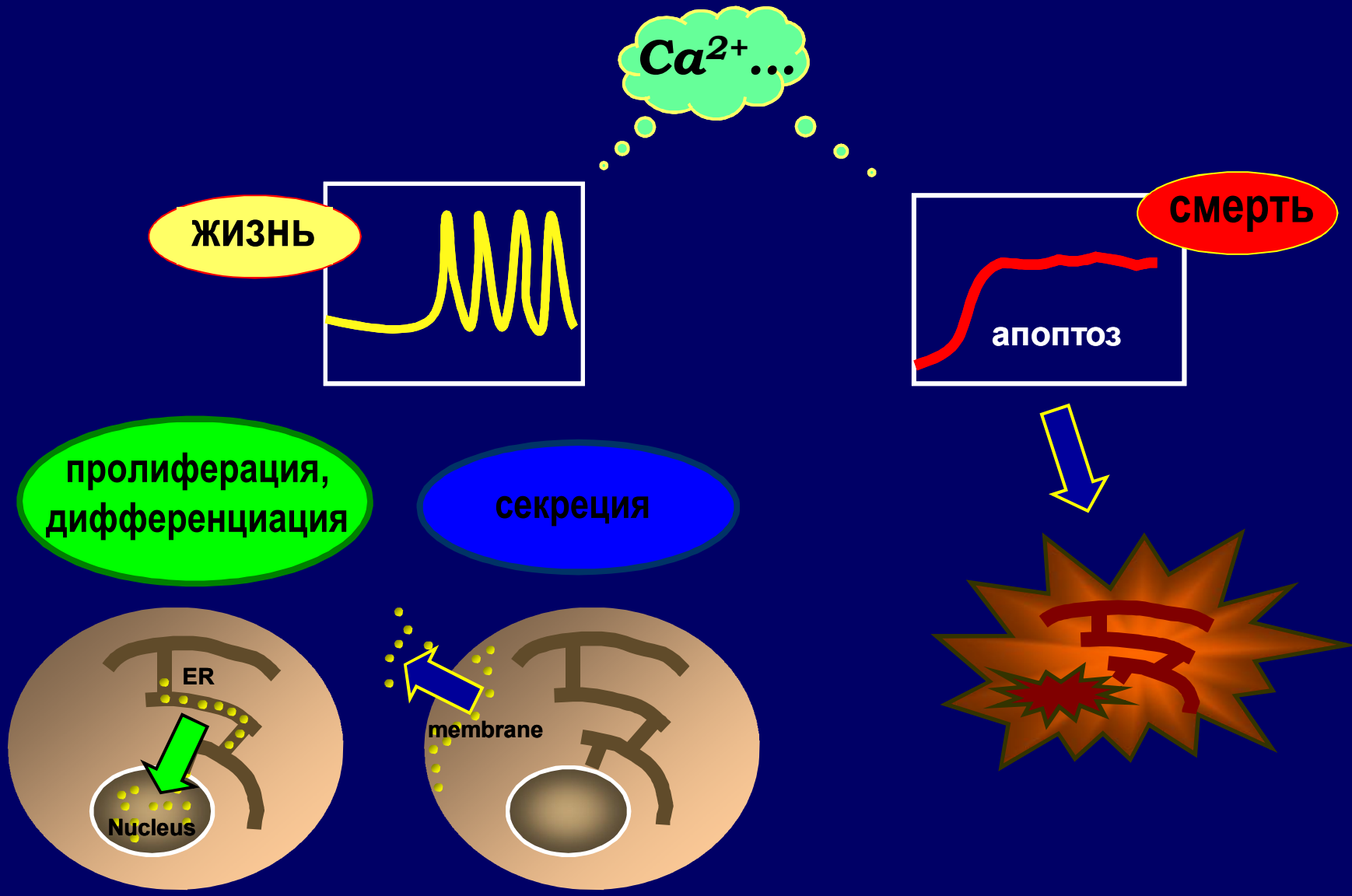
*- photocell in the compound eye of arthropods

Суперсемейство TRP каналов





TRP-каналы в кальциевой сигнализации...



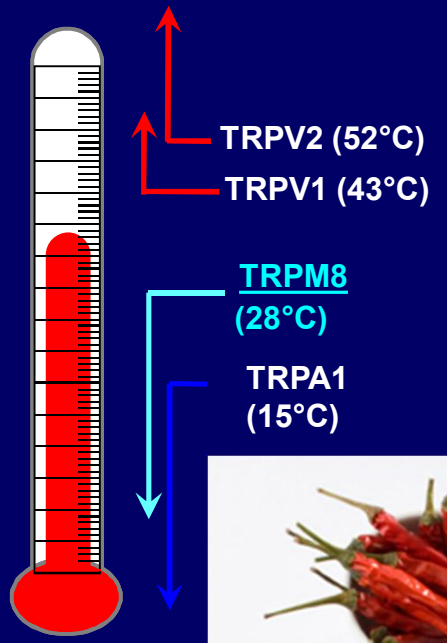
TRP-каналы ка терморецепторы и сенсоры химических раздражителей...

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. Neuhausser*† & David Julius*

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



TRPV2 (52°C)

TRPV1 (43°C)

TRPM8 (28°C)

TRPA1 (15°C)

TRP-каналы как детерминанты заболеваний человека...

TRP channels in **cancer**.
Prevarskaya N, Zhang L, Barritt G.
Biochim Biophys Acta. 2007 Aug;1772(8):937-46.

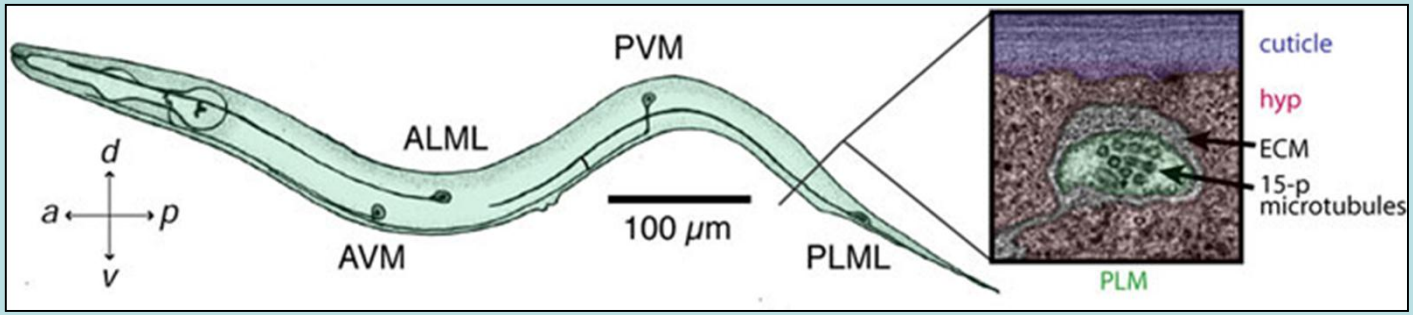
TRP channels: functions and involvement in **neurologic disease**.
Benarroch EE.
Neurology. 2008 Feb 19;70(8):648-52.

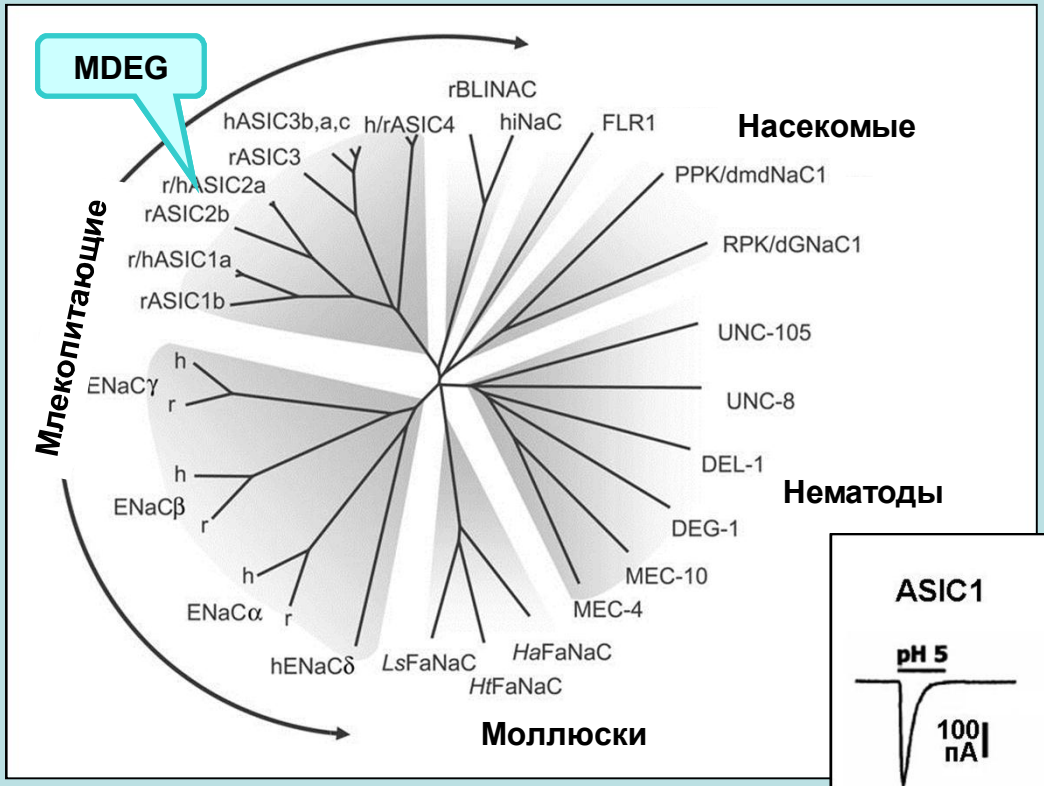
TRP channel and **cardiovascular disease**.
Watanabe H, Murakami M, Ohba T, Takahashi Y, Ito H.
Pharmacol Ther. 2008 Jun;118(3):337-51.

TRP channels as emerging targets for **pain therapeutics**.
Broad LM, Mogg AJ, Beattie RE, Ogden AM, Blanco MJ, Bleakman D.
Expert Opin Ther Targets. 2009 Jan;13(1):69-81.

TRP channels: emerging targets for **respiratory disease**.
Banner KH, Igney F, Poll C.
Pharmacol Ther. 2011 Jun;130(3):371-84.

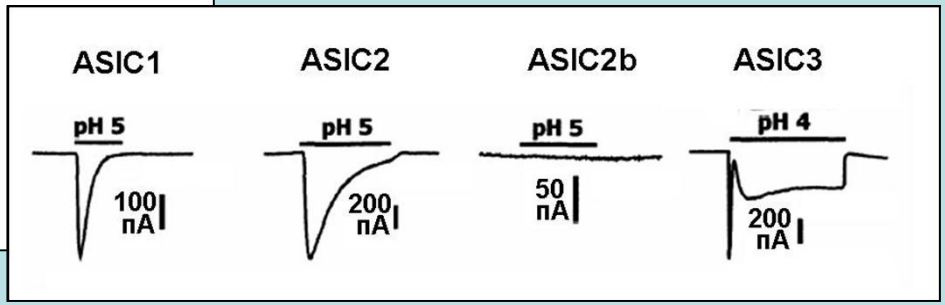
От дегенеринового фенотипа механочувствительности червяка C. elegans до ...





Wild-type

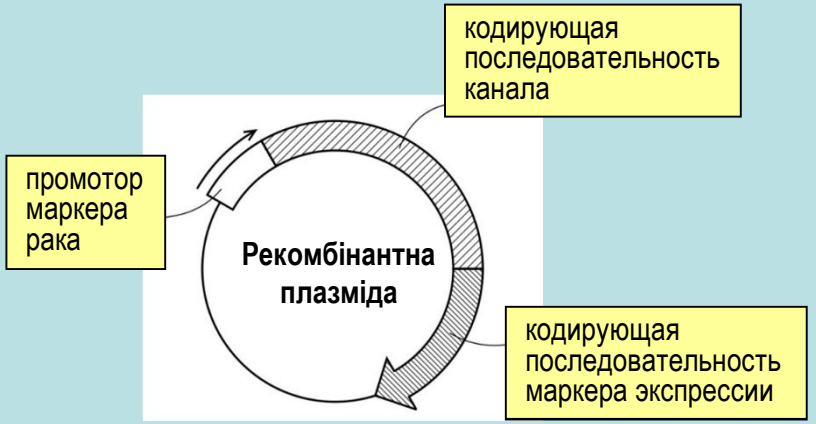
Mutant



... до возможности генной терапии рака

Маркери типів раку

1) (CEA, *CEACAM* gene family), which is produced by tumors of gastrointestinal system as well as by some other cancers such as lung, breast, ovarian;
2) **prostate specific antigen** (PSA, also known as kallikrein III, gene *KLK3*);
3) **bladder tumor antigen** (BTA);
4) **cacrcinoma antigen 15-3** (CA 15-3, gene *MUC1*) characteristic of advanced breast cancer;
5) **cancer antigen 125** (CA 125, gene *MUC16*), which is typical of ovarian cancer;
6) **carbohydrate antigen 19-9** (CA 19-9, synthesis involves three genes *FUT2*, *FUT3*, *ST3GAL1*), which is the most useful marker of pancreatic cancer, and is also elevated in colon cancer.



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www.nature.com/cgt

A novel strategy for cancer therapy by mutated mammalian degenerin gene transfer

Masayoshi Horimoto,[1] Yutaka Sasaki,[1,2] Shinya Ugawa,[3,4] Shigeo Wada,[1] Takashi Toyama,[1] Kenya Iyoda,[2] Takayuki Yakushijin,[2] Yuki Minami,[3] Toshifumi Ito,[1] Taizo Hijioka,[5] Akiko Eguchi,[6] Mahito Nakanishi,[6] Shoichi Shimada,[4] Masaya Tohyama,[3] Norio Hayashi,[2] and Masatsugu Hori[1]

Departments of [1]Internal Medicine and Therapeutics, [2]Molecular Therapeutics, and [3]Anatomy and Neuroscience, Osaka University Graduate School of Medicine, Osaka, Japan; [4]Department of Anatomy II, Nagoya City University Medical School, Nagoya, Japan; [5]Department of Gastroenterology, Osaka-minami National Hospital, Osaka, Japan; and [6]Department of Neurovirology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Mammalian degenerin (MDEG) is a member of the amiloride-sensitive sodium ion channel family, and its site-directed active mutant (MDEG-G430F) induces massive Na^+ influx into cells, leading to cell ballooning and cell bursting. We attempted a novel therapeutic approach for gastric cancers by transferring MDEG-G430F into cancer cells using tumor-specific promoters. In carcinoembryonic antigen (CEA)-producing gastric cancer cells, the level of cell death observed when MDEG-G430F was used with a CEA promoter was similar to that observed when using a potent nonspecific promoter such as the cytomegalovirus promoter. In an *in vivo* study, fusogenic liposome complexes containing MDEG-G430F driven by the CEA promoter were injected intraperitoneally into CEA-producing gastric cancer cells in a mouse peritoneal dissemination model. Although all 15 of the control mice were dead by 50 days postinoculation, 13 of the 15 mice treated with MDEG-G430F survived. These results indicate that transferring MDEG-G430F into cancer tissues using tumor-specific promoters can achieve striking and selective cancer cell death irrespective of the transcriptional efficiency of the promoters used *in vivo*, and suggest that this approach is a promising new strategy for cancer gene therapy. **Cancer Gene Therapy (2000) 7, 1341–1347**

Key words: Mutated ion channel; degenerin; carcinoembryonic antigen promoter; fusogenic liposome; peritoneal dissemination.

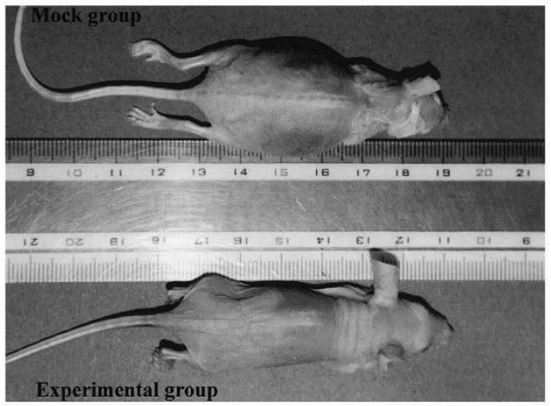

Figure 6. Mice with peritoneal dissemination of MKN45-P cells. **Top:** Representation of a mouse in the mock group at 30 days postinoculation. Mice inoculated with 1×10^6 MKN45-P cells were injected i.p. with fusogenic liposomes containing CEApCDNA3 plasmid each week. All mice in the mock and control groups exhibited substantial peritoneal dissemination with massive ascites. **Bottom:** Representative demonstration of a mouse in the experimental group at 30 days postinoculation. Mice inoculated with 1×10^6 MKN45-P cells were injected i.p. with fusogenic liposomes containing CEApCDNA3 MDEG-G430F plasmid each week. Mice in the experimental group exhibited substantially less peritoneal dissemination.

Київ, “Наукова думка”, 2010

Спасибо за внимание!