Experimental modification of ion channels

(*Transgenic animal models*)

Molecular biological tools

Balázs Ördög

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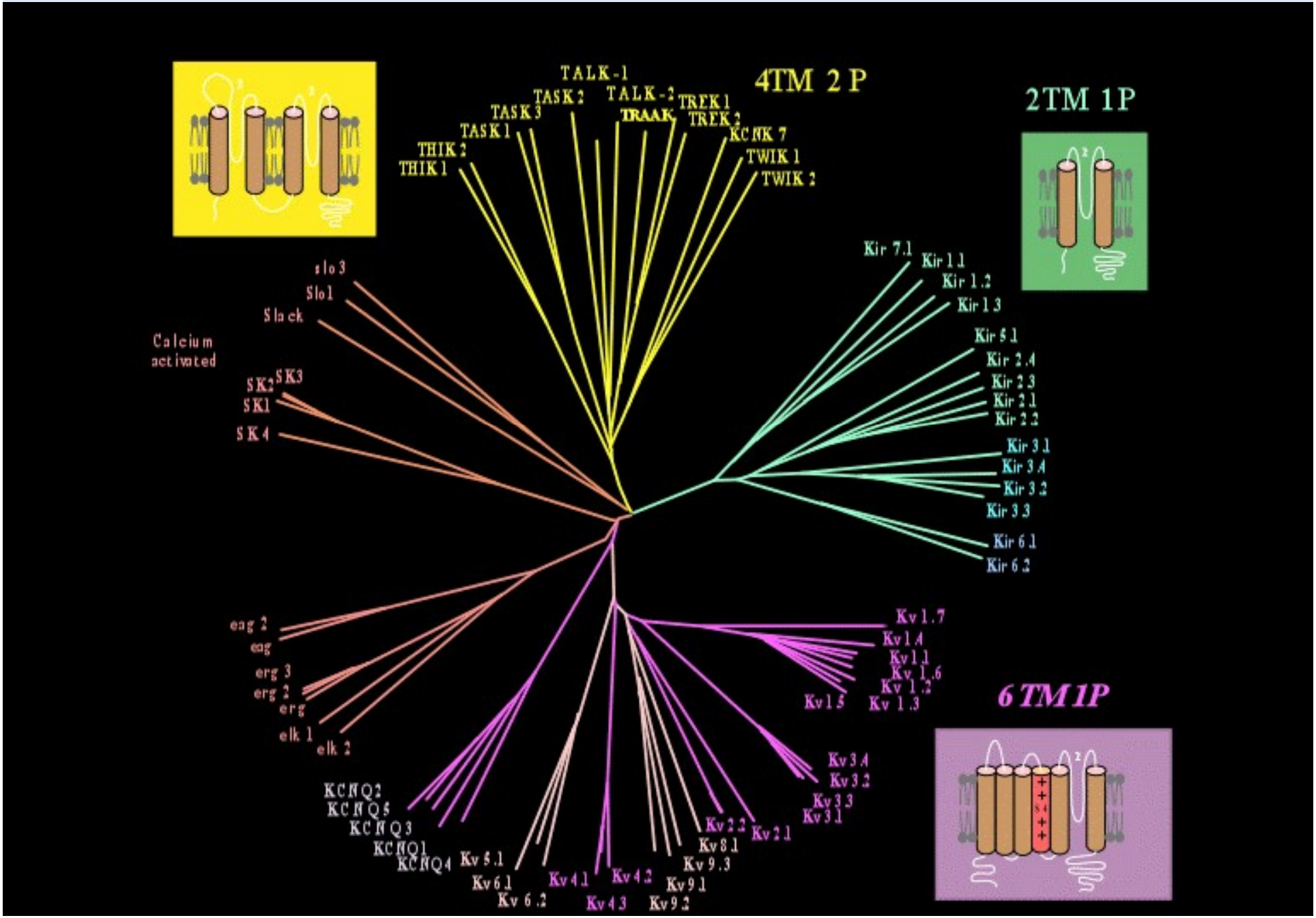
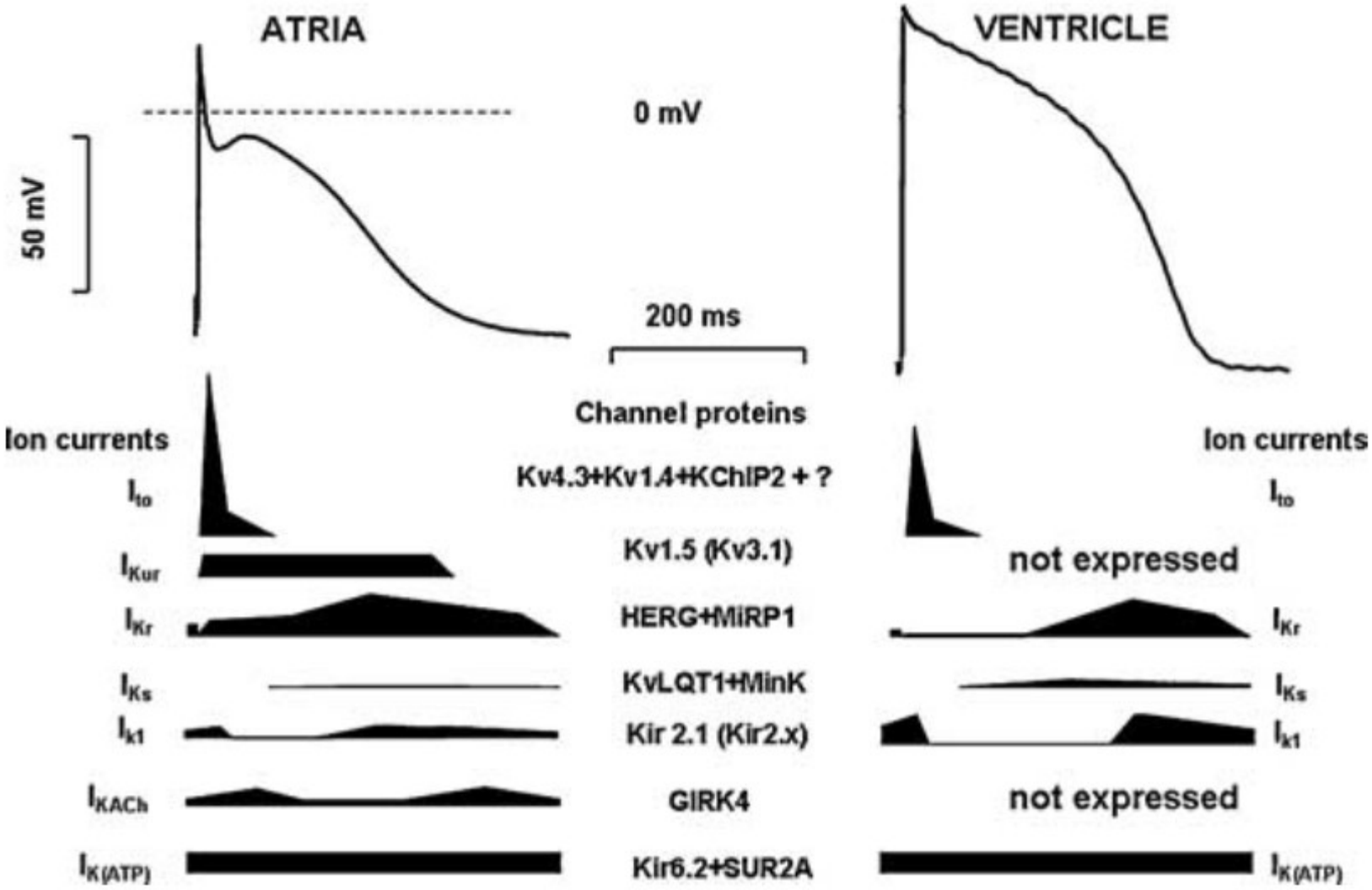
Molecular diversity of potassium channels

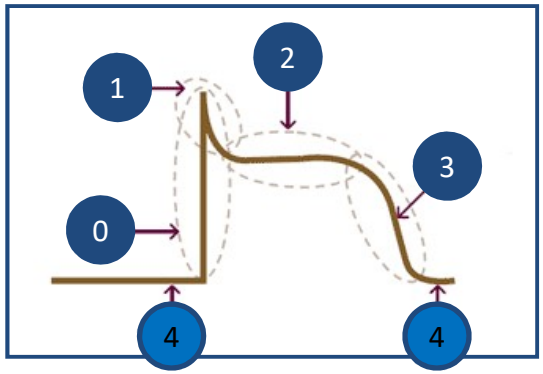


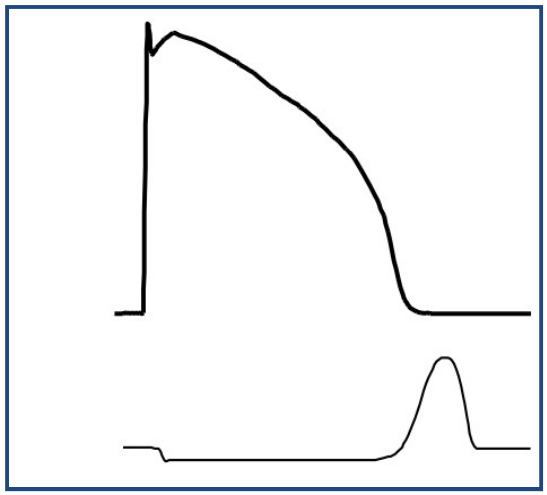
Functional diversity of potassium channels

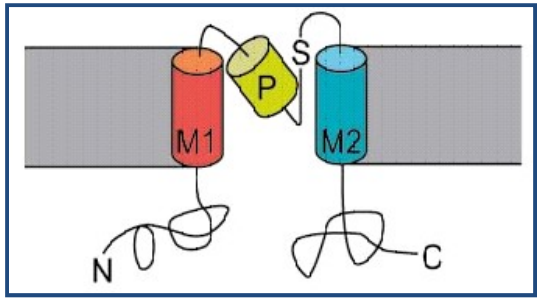


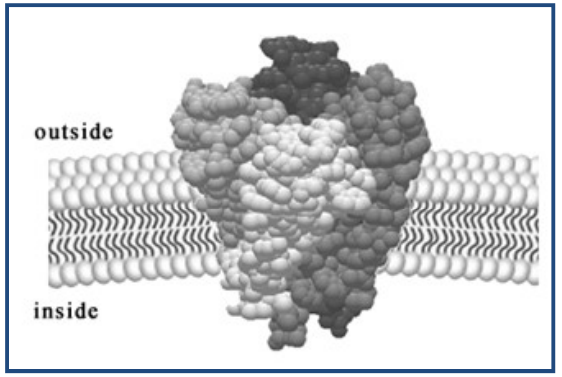
Inward rectifier potassium channels

- I_{K1}
- Subunits:
  - Kir2.1 (KCNJ2)
  - Kir2.2 (KCNJ12)
  - Kir2.3 (KCNJ4)
  - Kir2.4 (KCNJ14)
- Function:
  - AP 4. fázis
  - Nyugalmi potenciál
- Dysfunction:
  - Long QT syndrome
  - ShortQT syndrome
  - Andersen-Tawil syndrome
  - Brugada syndrome

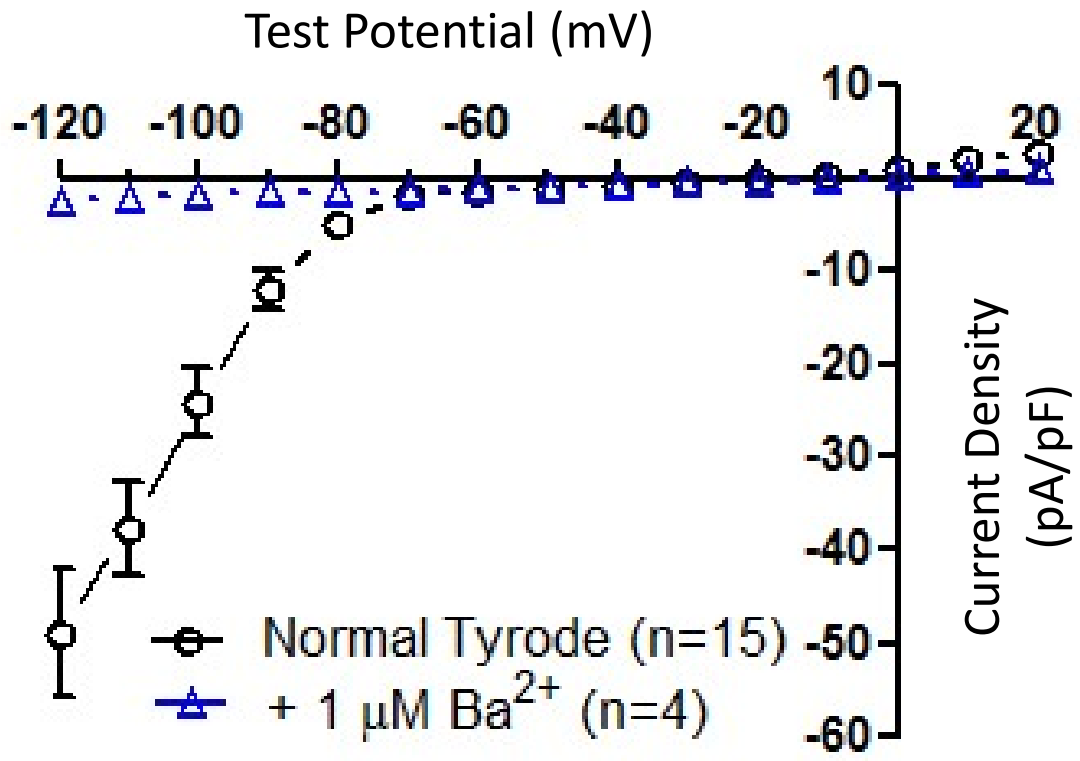






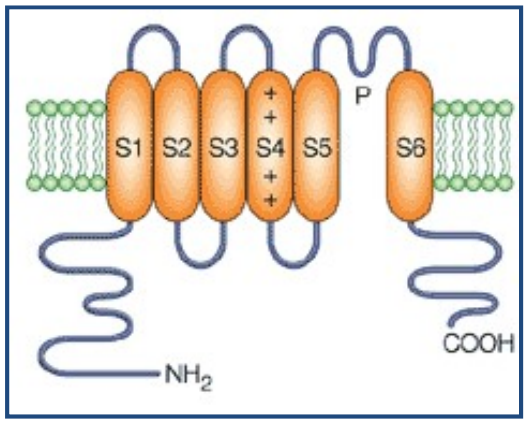


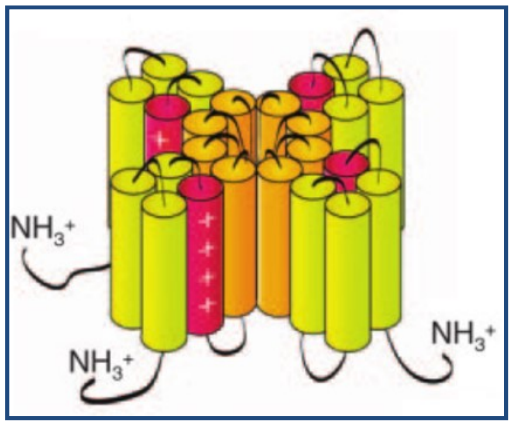
Let`s experiment with I_{K1} blocker!

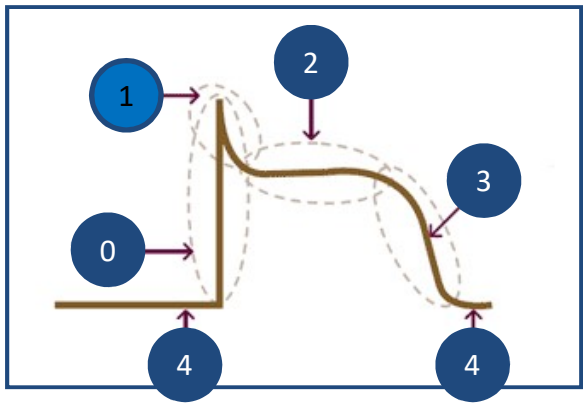


Can we extract info on the function of the Kir2.1 subunit (or Kir2.2, 2.3, 2.4)?

Transient outward K^+ channels

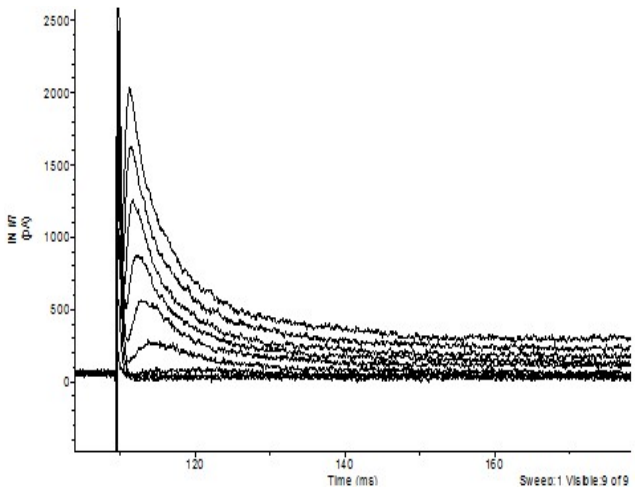




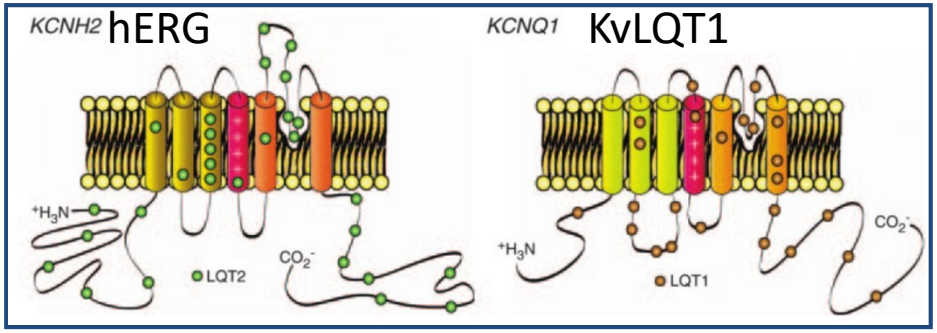


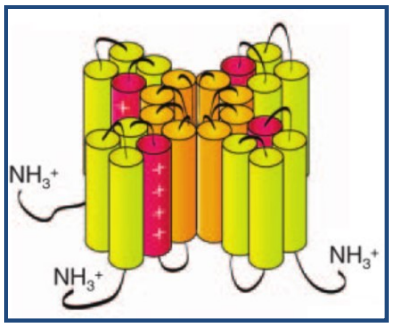
- I_{to}: „transient outward” K^+ current
- Pore-forming subunits: Kv1.4 (KCNA4), Kv4.2 (KCND2), Kv4.3 (KCND3)
- Homo- or heteromeric structure
- Sensitive to 4-aminopyridine
- Auxiliary subunits: KChIP2, DPP6, KCNE1-5 ?
- Function:
  - AP phase 1.
  - Region-specific AP configurations
- Dysfunction
  - Brugada syndrome
  - Idiopathic ventricular fibrillation

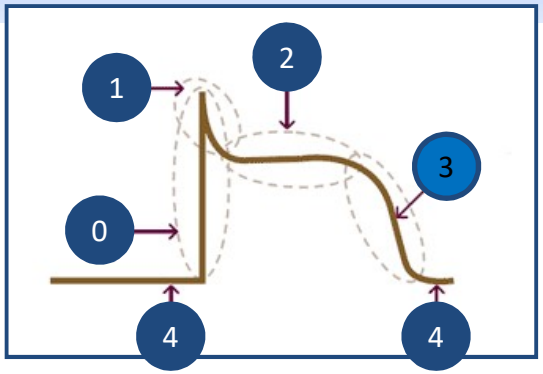
Unclosed files, case no. 1.: Subunit composition of I_{to} channels



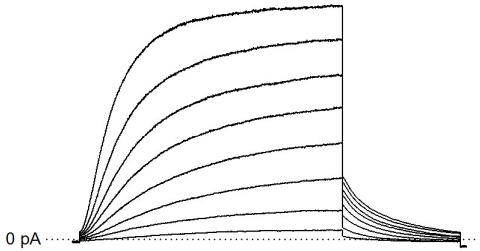
Delayed outward rectifier K^+ channels



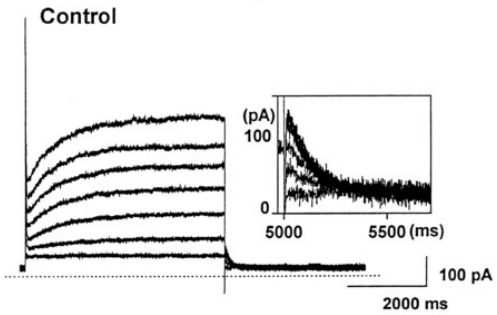


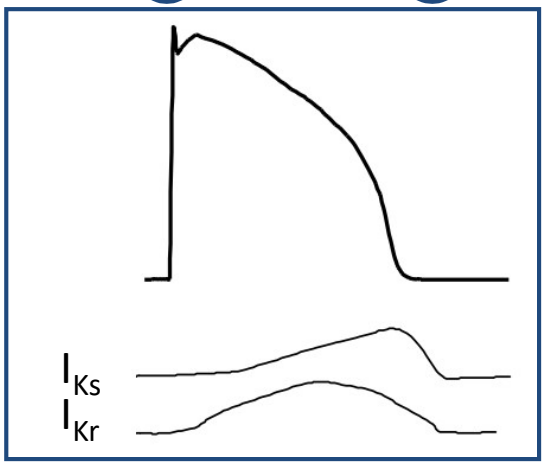


In vitro I_{KS}:
KvLQT1 + KCNE1



natív I_{KS}





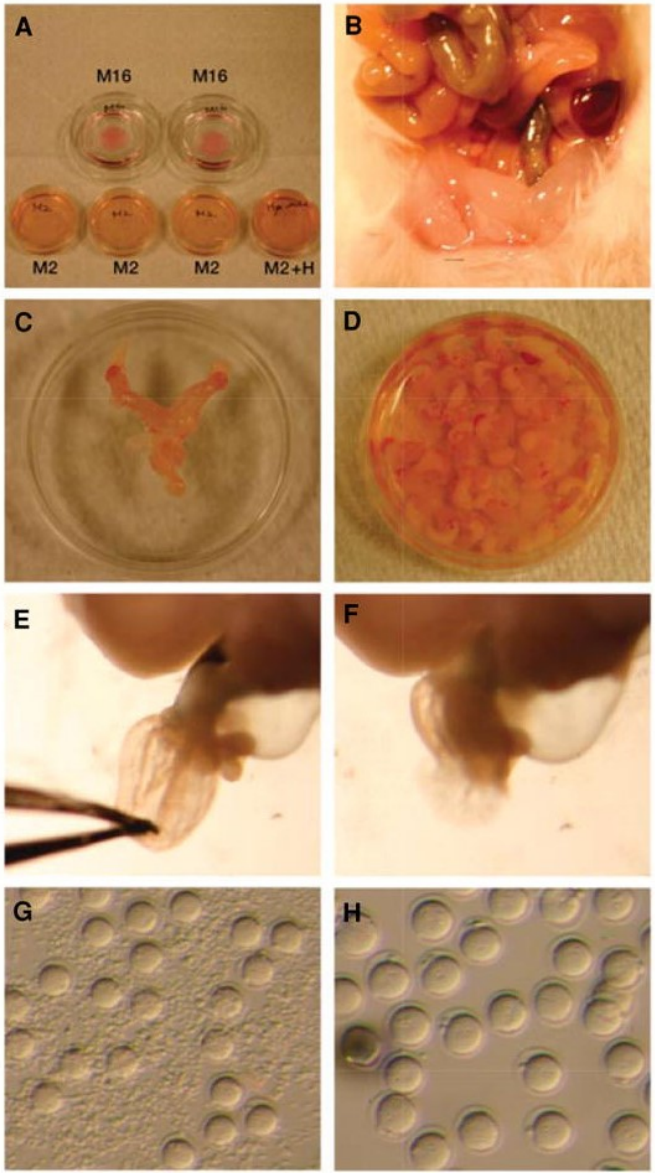
- Delayed rectifiers
  - fast component: I_{Kr}, hERG (KCNH2)
  - slow component: I_{Ks}, KvLQT1 (KCNQ1)
- Auxiliary subunits: KCNE1 – 5
- Function: AP phase 2. and 3.
- Dysfunction: long QT syndrome

Unclosed files, case no. 2.:
Subunit composition of I_K channels

Transgenic models of disease

Pronuclear injection

1.



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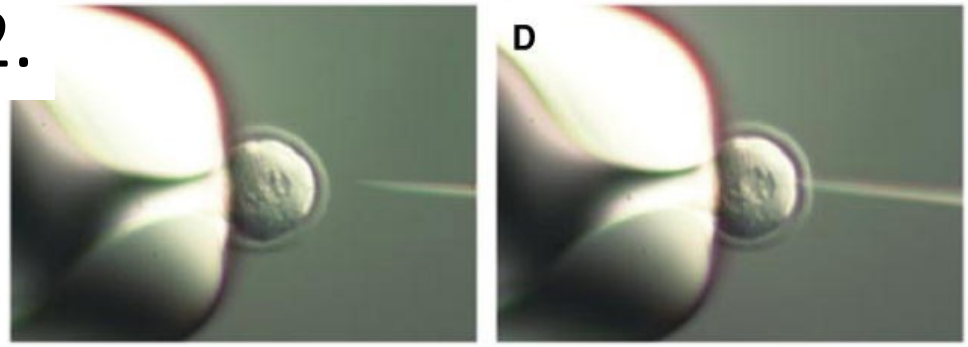

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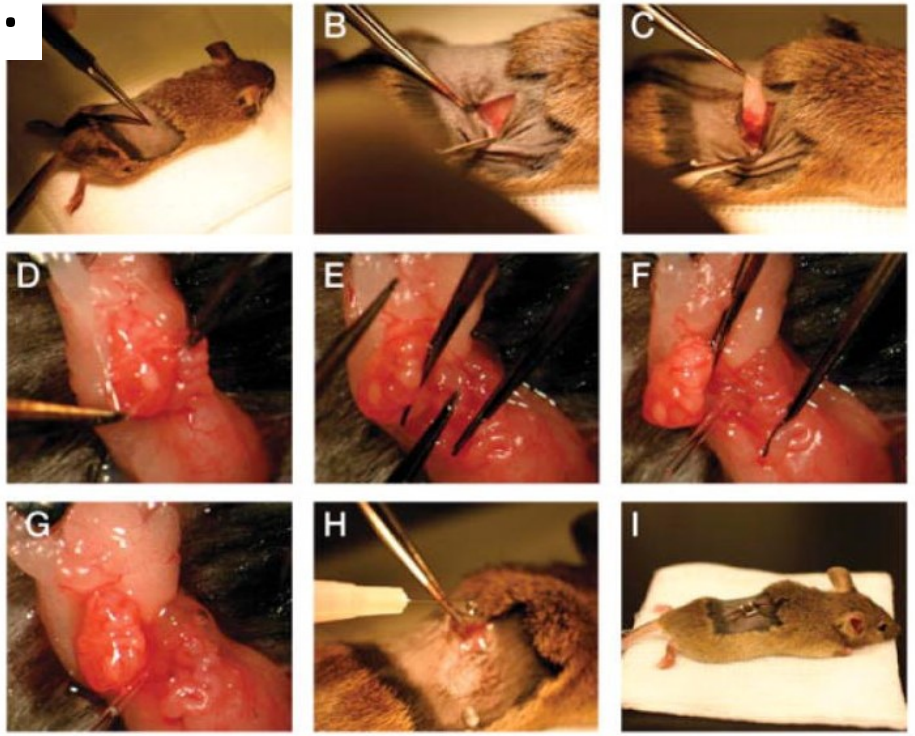
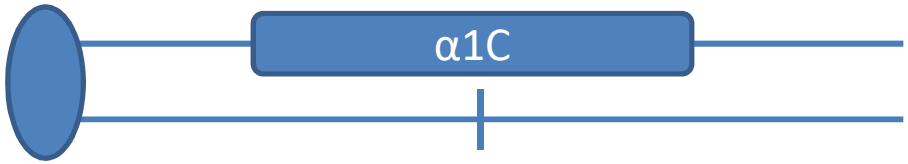


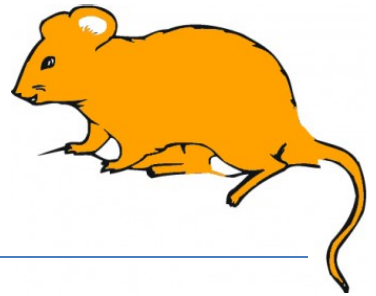
Transgenic models of disease

Knock-out variations

"Traditional" knock-out

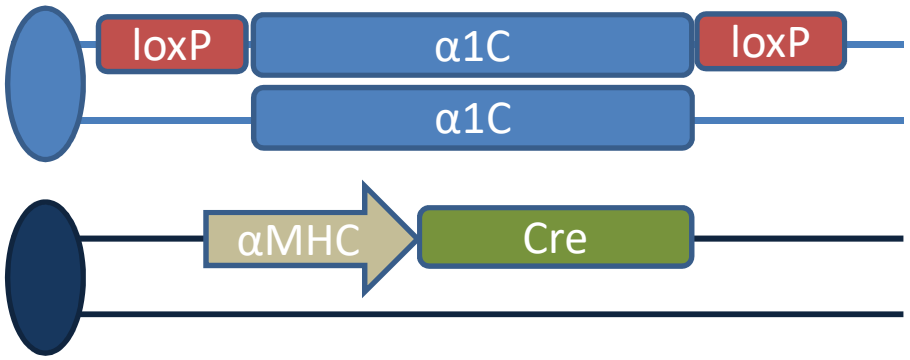
$\alpha 1C^{-/+}$





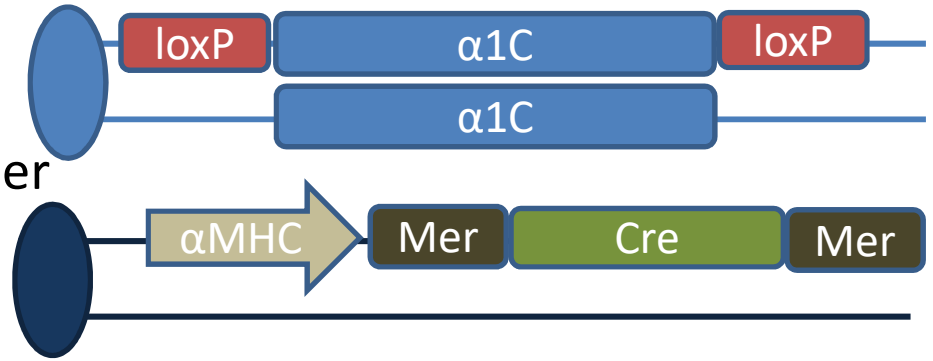
Cardiac specific KO

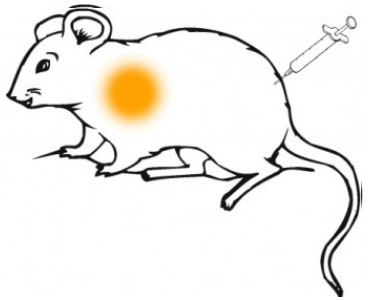
$\alpha 1C^{+/fl}$, Cre



Cardiac specific, inducible KO

$\alpha 1C^{+/fl}$, MerCreMer

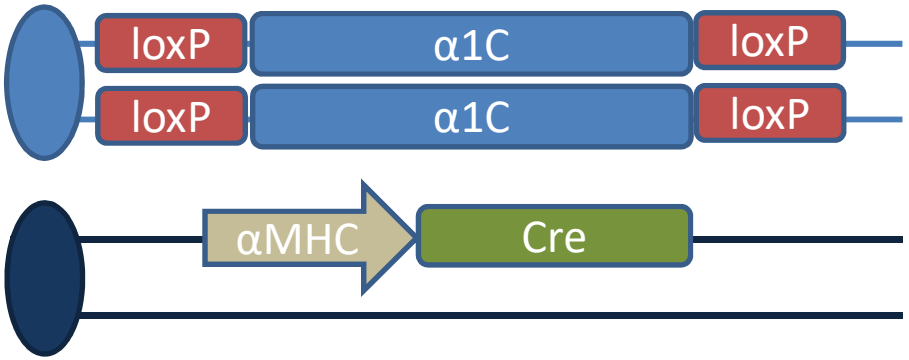


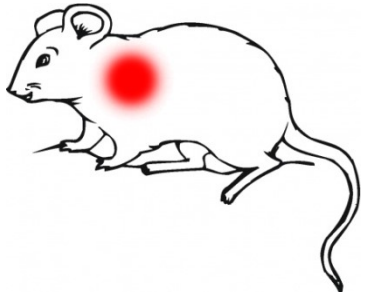


Transgenic models of disease

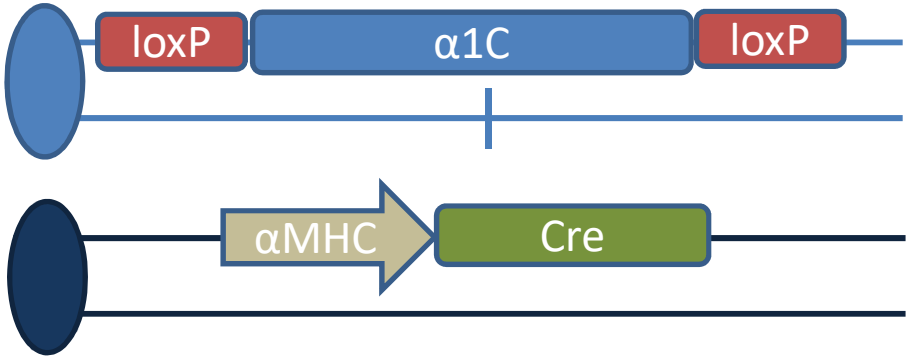
Knock-out variations

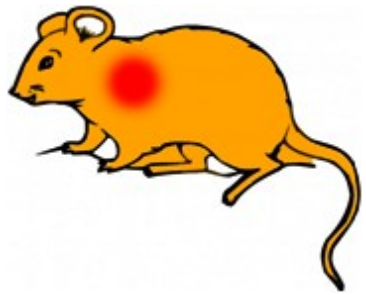
$\alpha 1C^{fl/fl,\ Cre}$



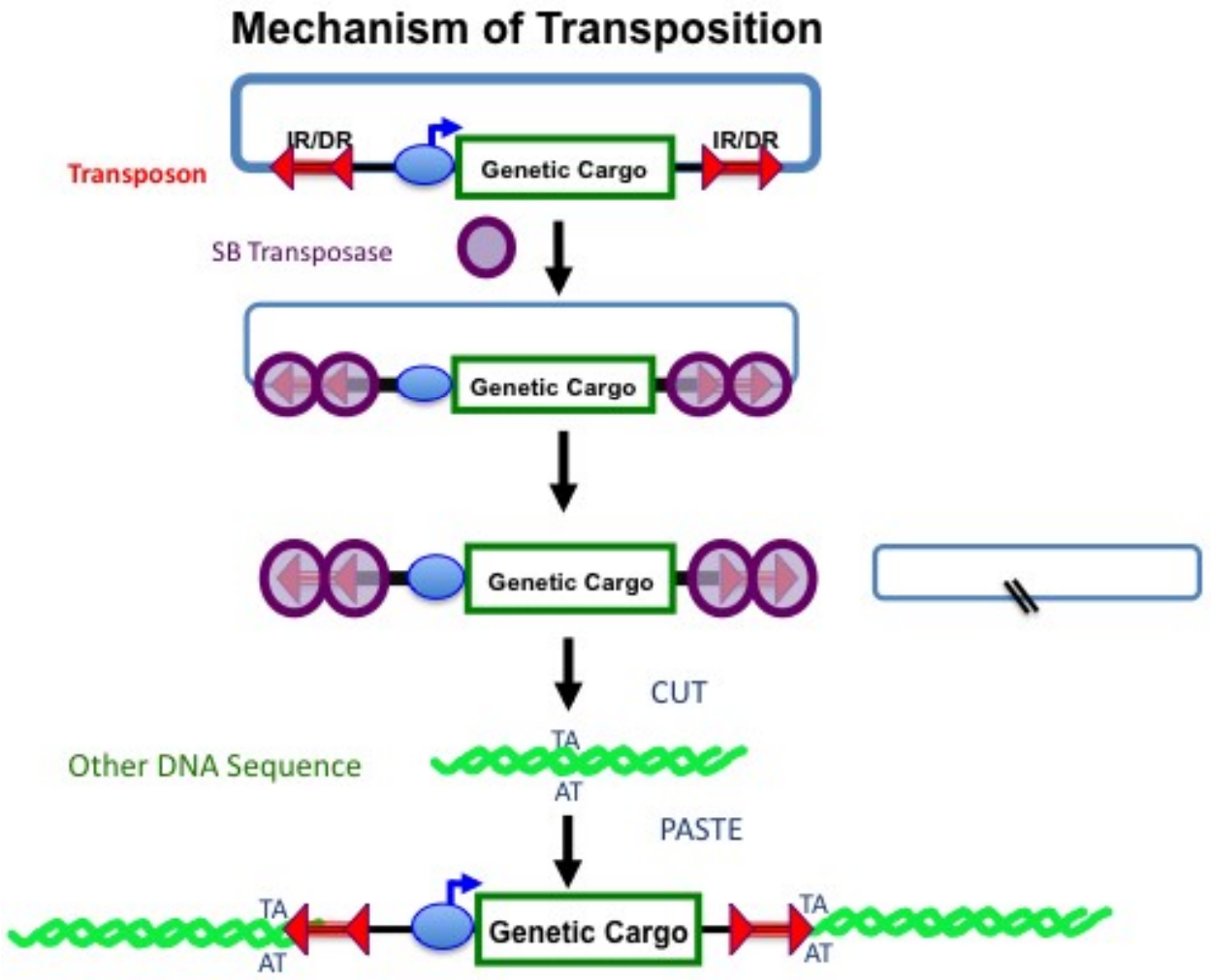


$\alpha 1C^{-/fl,\ Cre}$

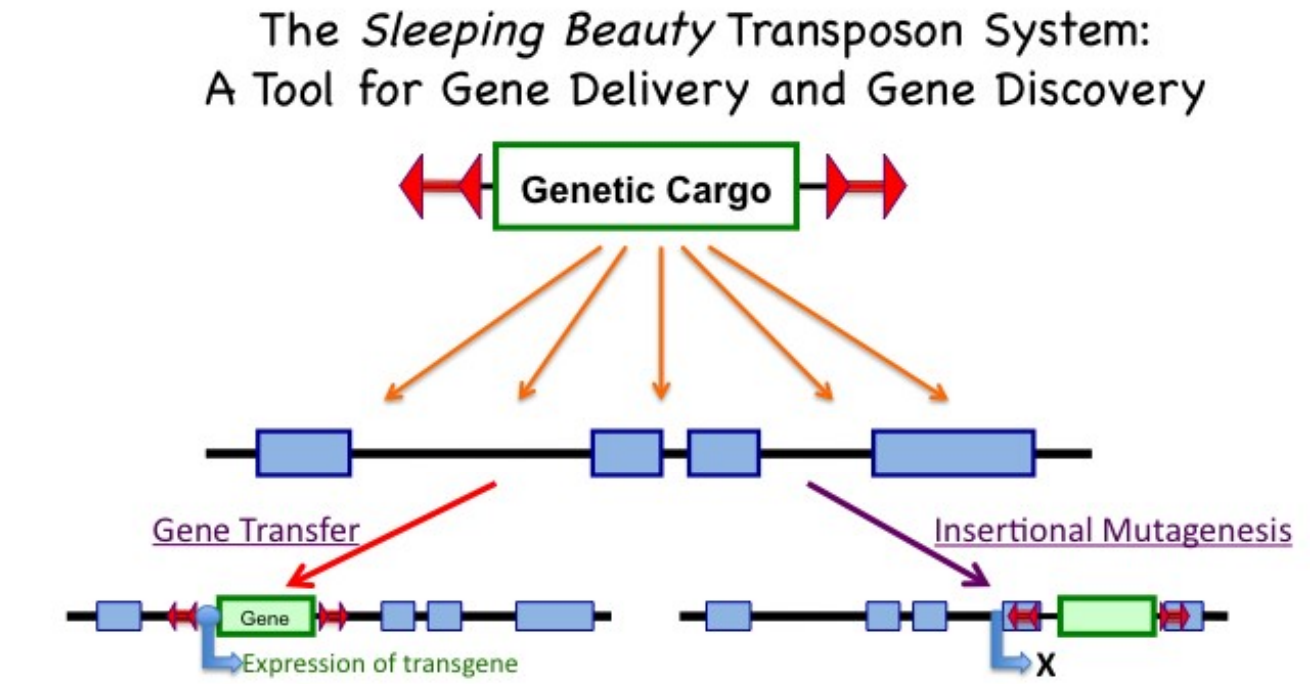




Transgenic models of disease
Sleeping Beauty transposon system



Transgenic models of disease
Sleeping Beauty transposon system

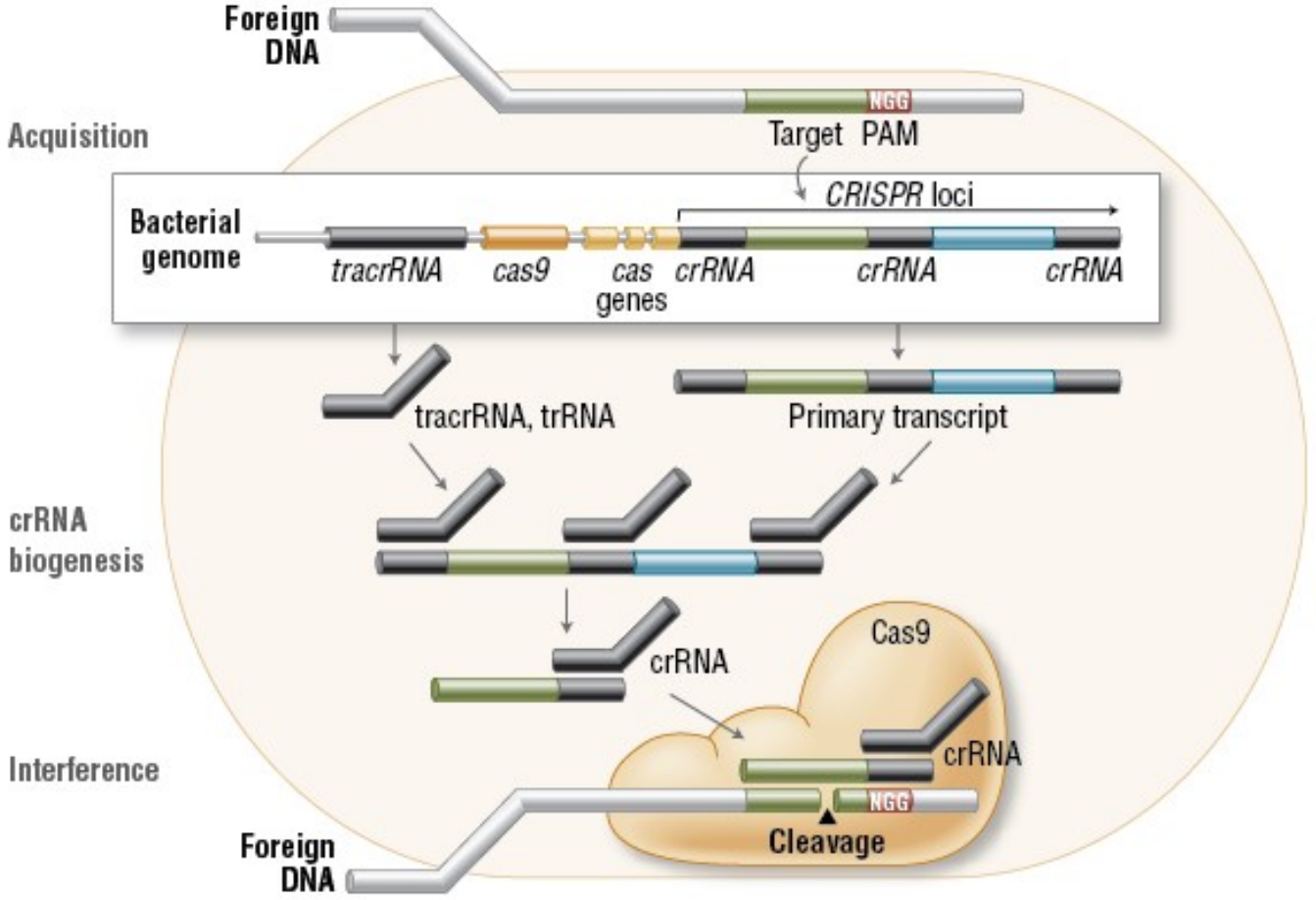


- Transgenes put into Germ-Line Cells
  (all cells have transposons)
  - Make transgenic animal lines
  - Identify roles genes play
  - Identify cancer genes

- Transgenes put into Somatic Cells
  (only some cells have transposons)
  - Gene Therapy
  - Identify roles genes play
  - Identify cancer genes

Transgenic models of disease
Genome editing with CRISPR/Cas9

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
Cas: CRISPR-associated genes



Transgenic models of disease

Pros

- All environmental parameters are present
- Subunit-specific information is well reflected

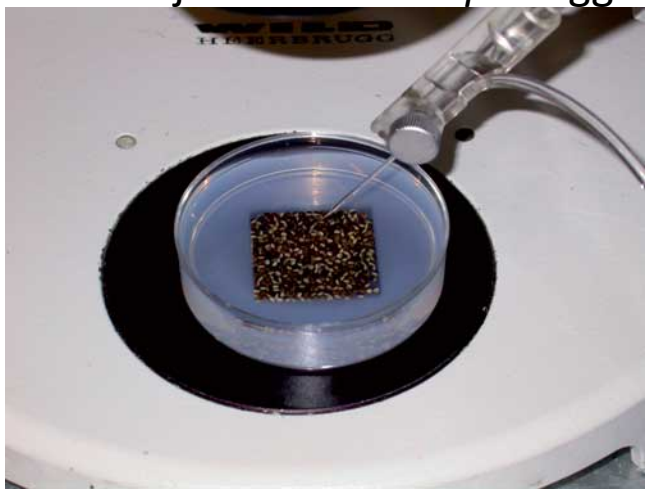
Cons

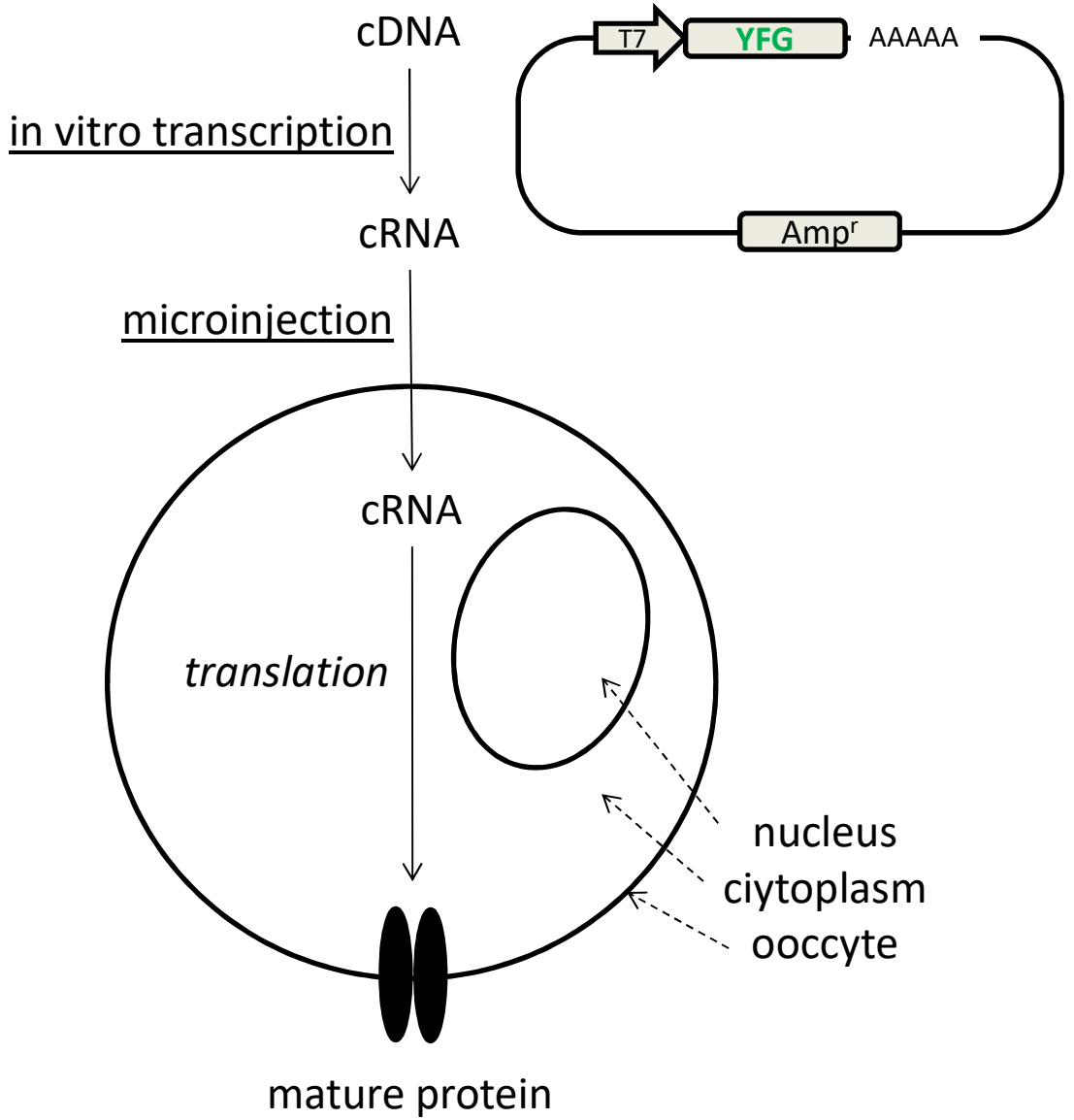
- **Not available in large animals**
- Chronic effects (compensatory mechanisms)
- Tissue- or cell-specific targeting is problematic

Heterologous expression systems
Oocyte injection

Xenopus laevis

Microinjection of *Xenopus* eggs



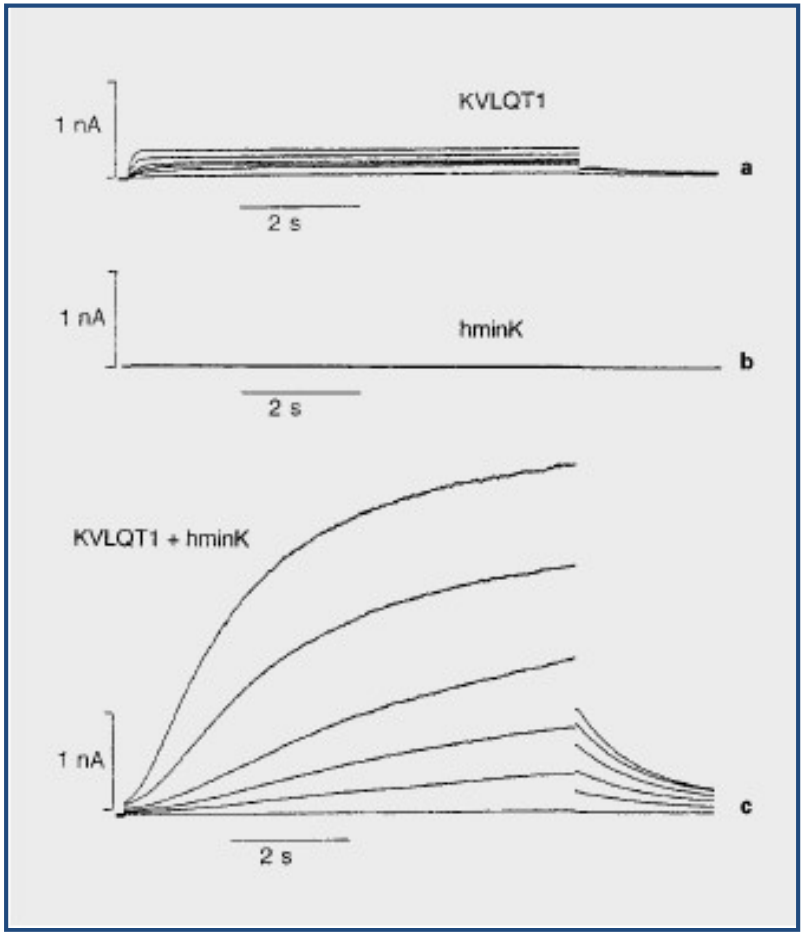


Role of auxiliary subunits: I_{Ks} channels, an illustrative example

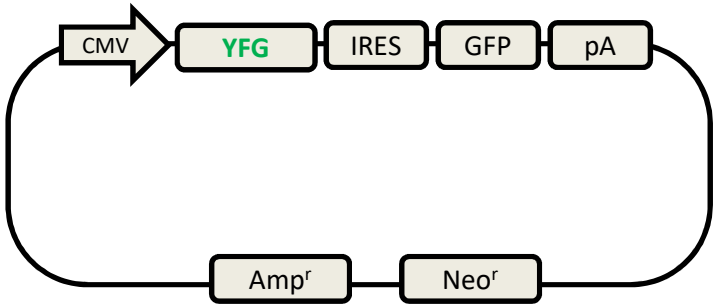
- The KvLQT1 pore-forming subunit produces no current when expressed alone.

Inject minK RNA into Xenopus eggs→ big current. How?

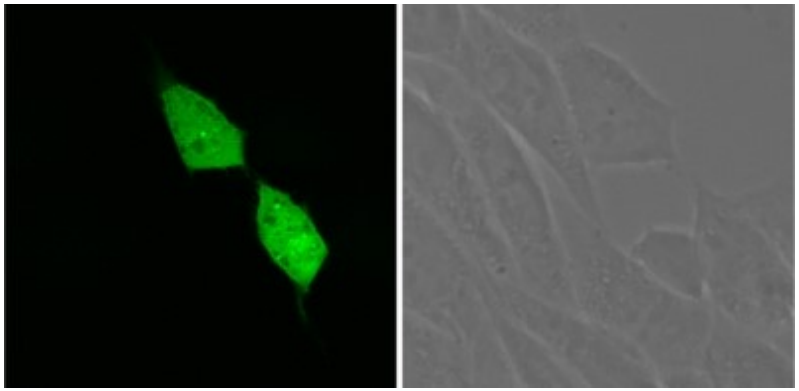
- Auxiliary subunits, fields of action:
  - Gating
  - folding, trafficking
  - Subcellular localization
  - Sensitivity to blockers
  - Receptor- and/or ligan-dependent effects

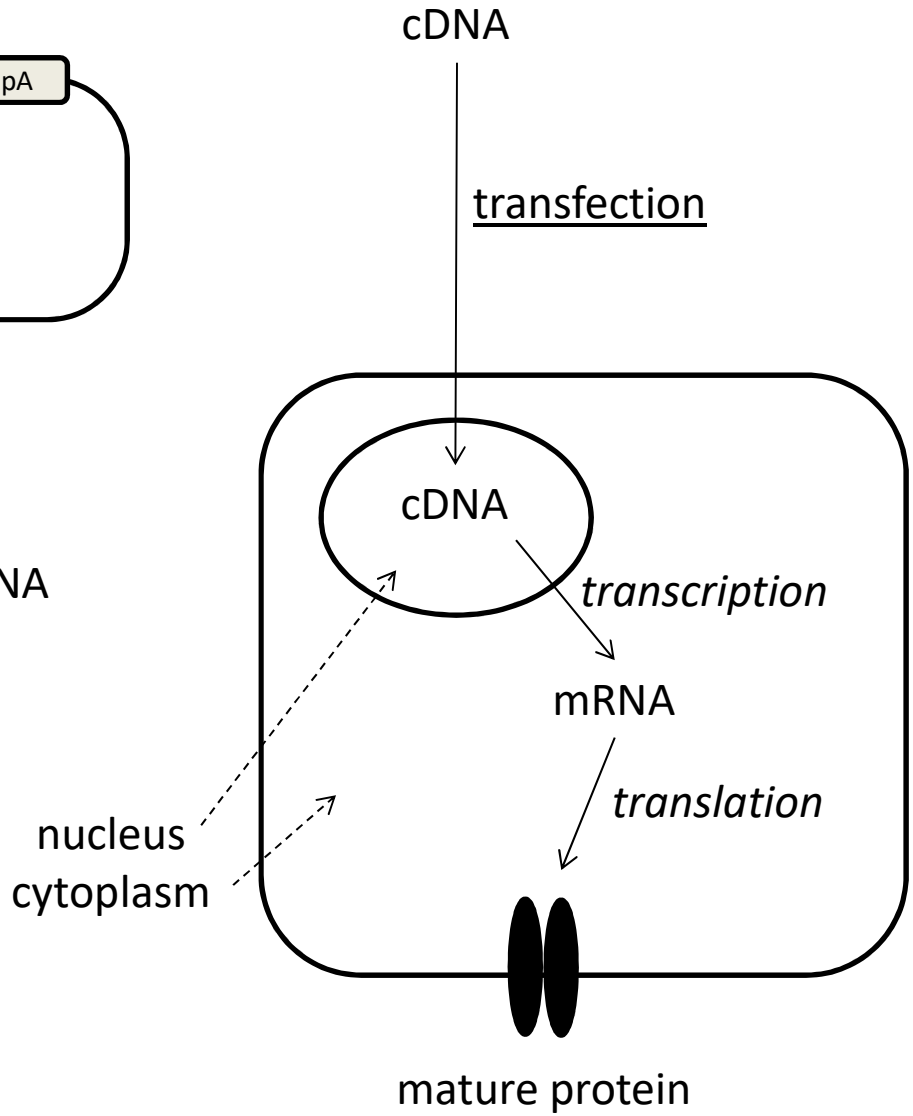


Heterologous expression systems: Transfection of mammalian cells



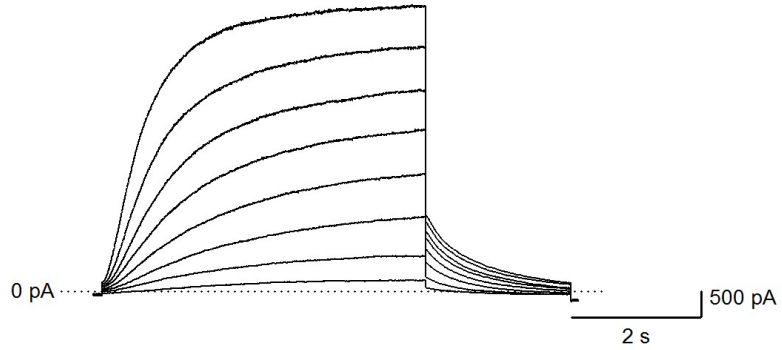
CHO cells transfected with GFP-encoding DNA

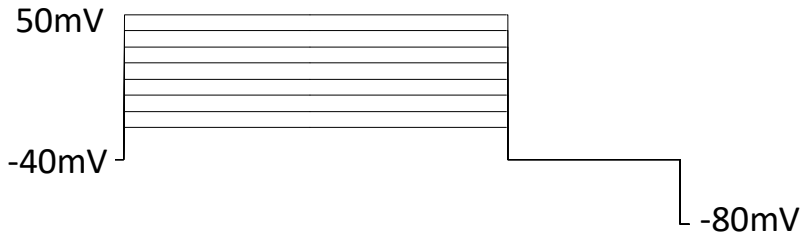




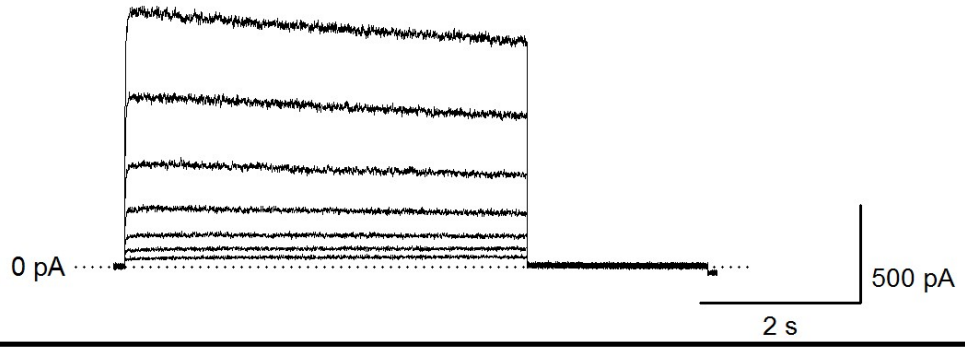
Heterologous expression systems: Investigation of subunit interactions

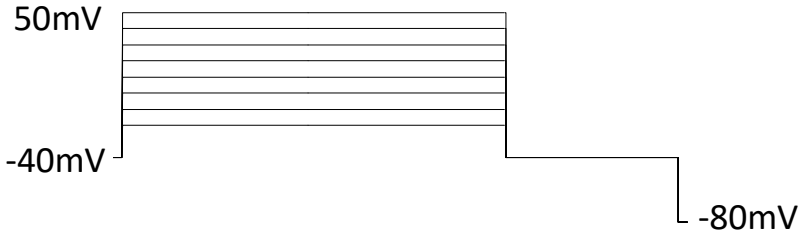
KvLQT1 + minK



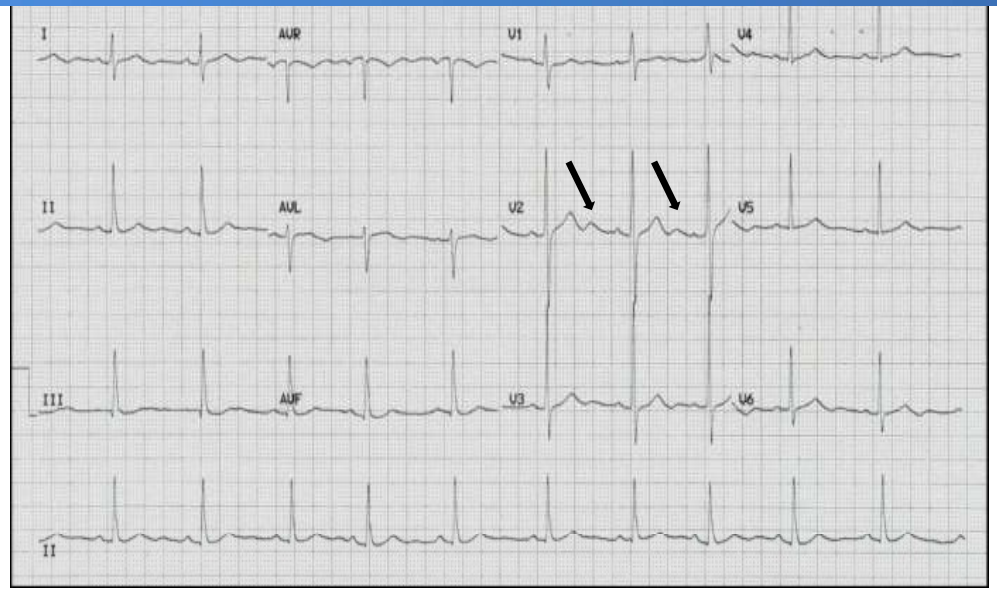


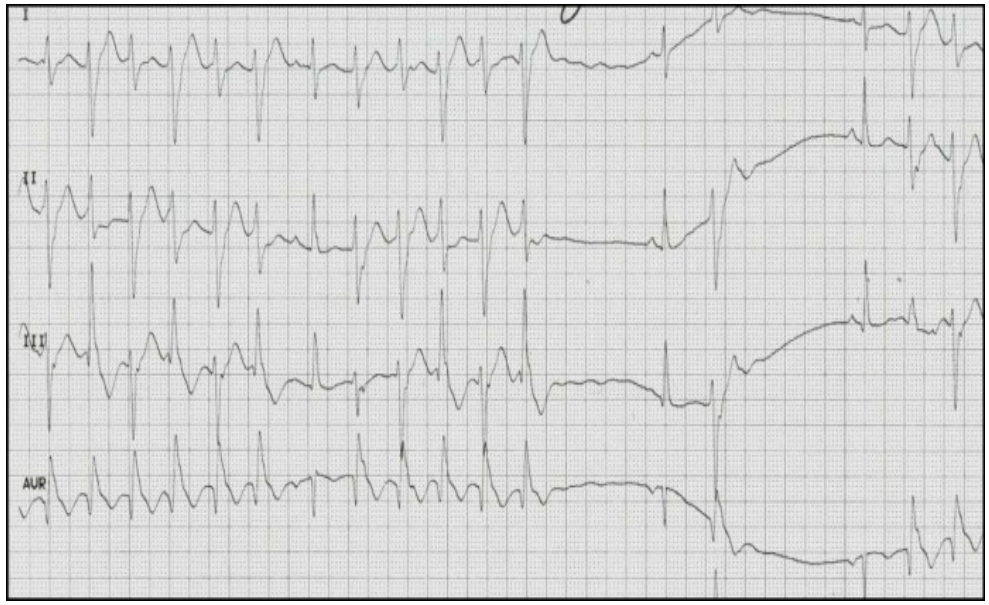
KvLQT1 + MiRP2

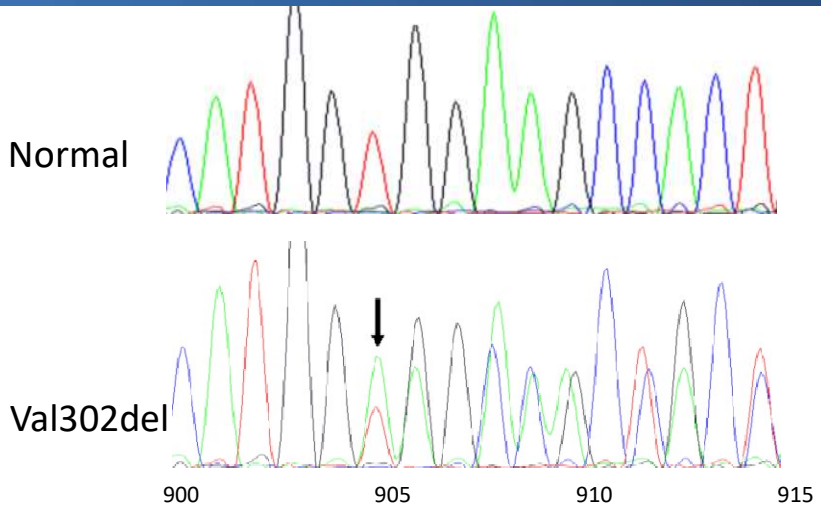




Heterologous expression systems: Characterisation of mutations







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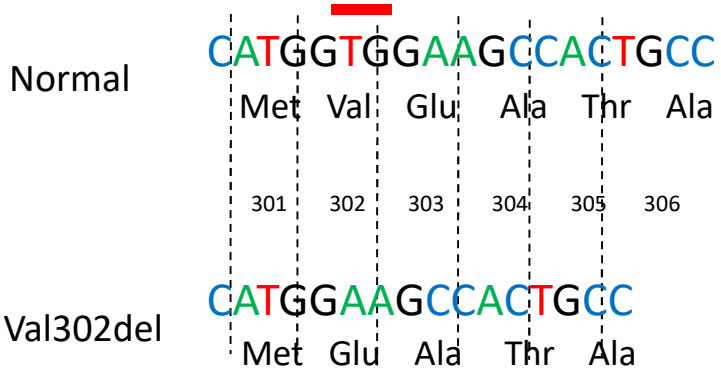
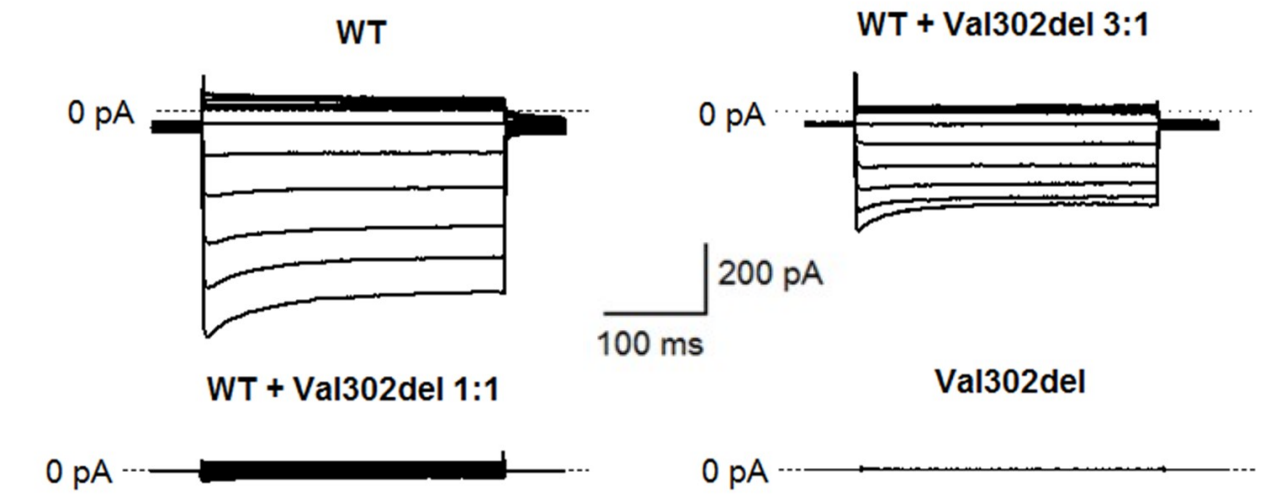
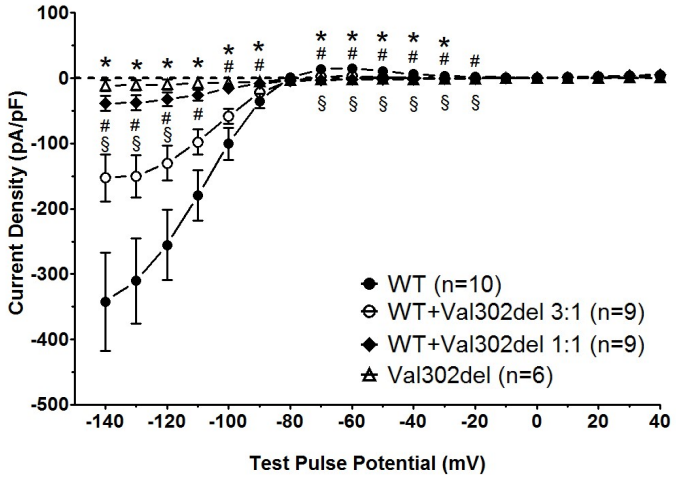
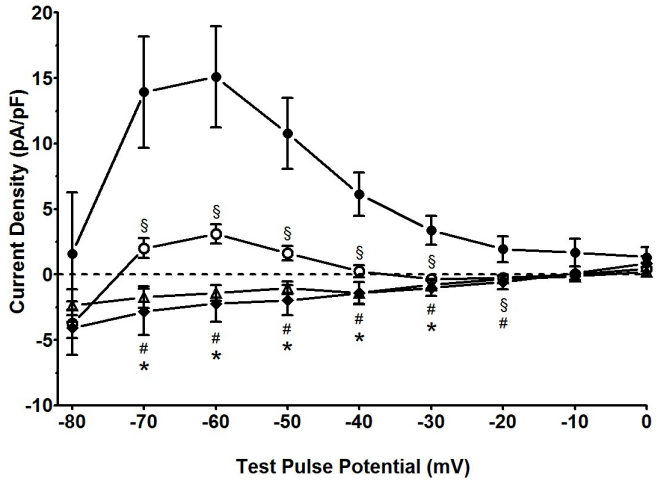


Heterologous expression systems: Characterisation of mutations







Heterologous expression systems

Pros

- Subunit-specific interactions are “absolutely” reflected
- subunit interactions can be studied
- easy to carry out

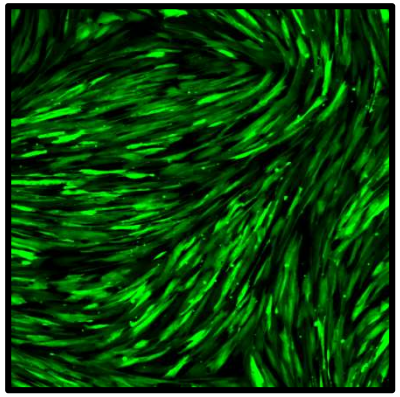
Cons

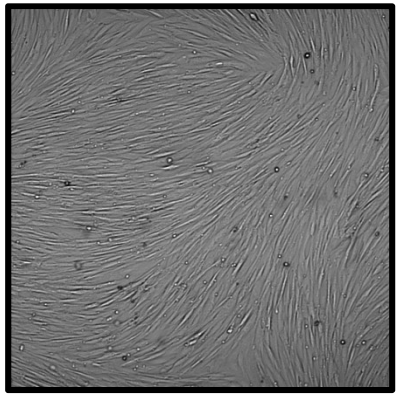
- environmental parameters are hard to model (ionic, receptorial, metabolic, protein and lipid environment)

Viral gene transfer

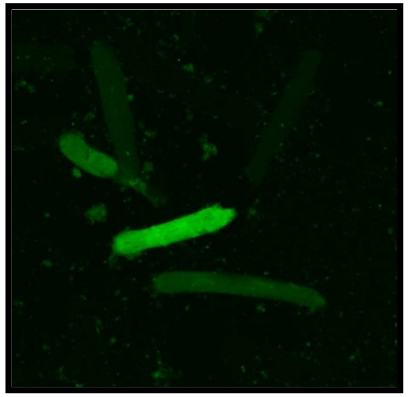
Lentiviral vectors

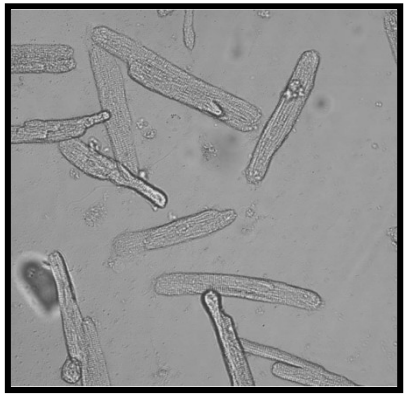
Cardiac fibroblast





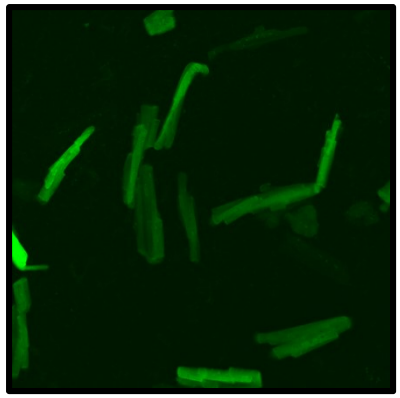
Cardiomyocyte

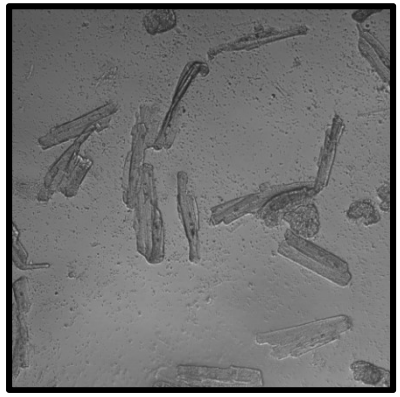




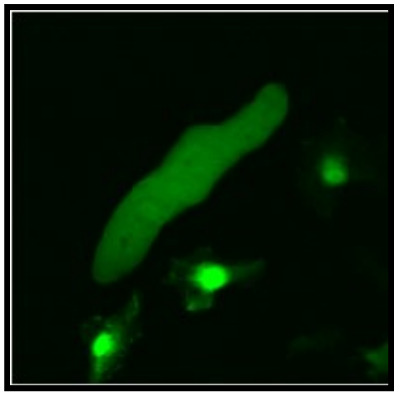
Adenoviral vectors

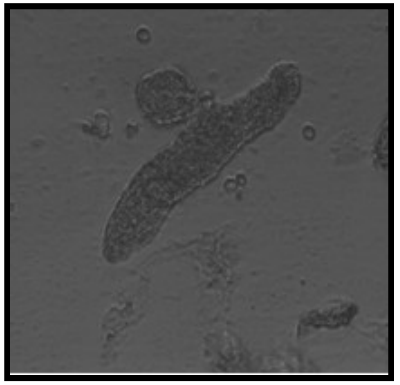
Cardiomyocyte



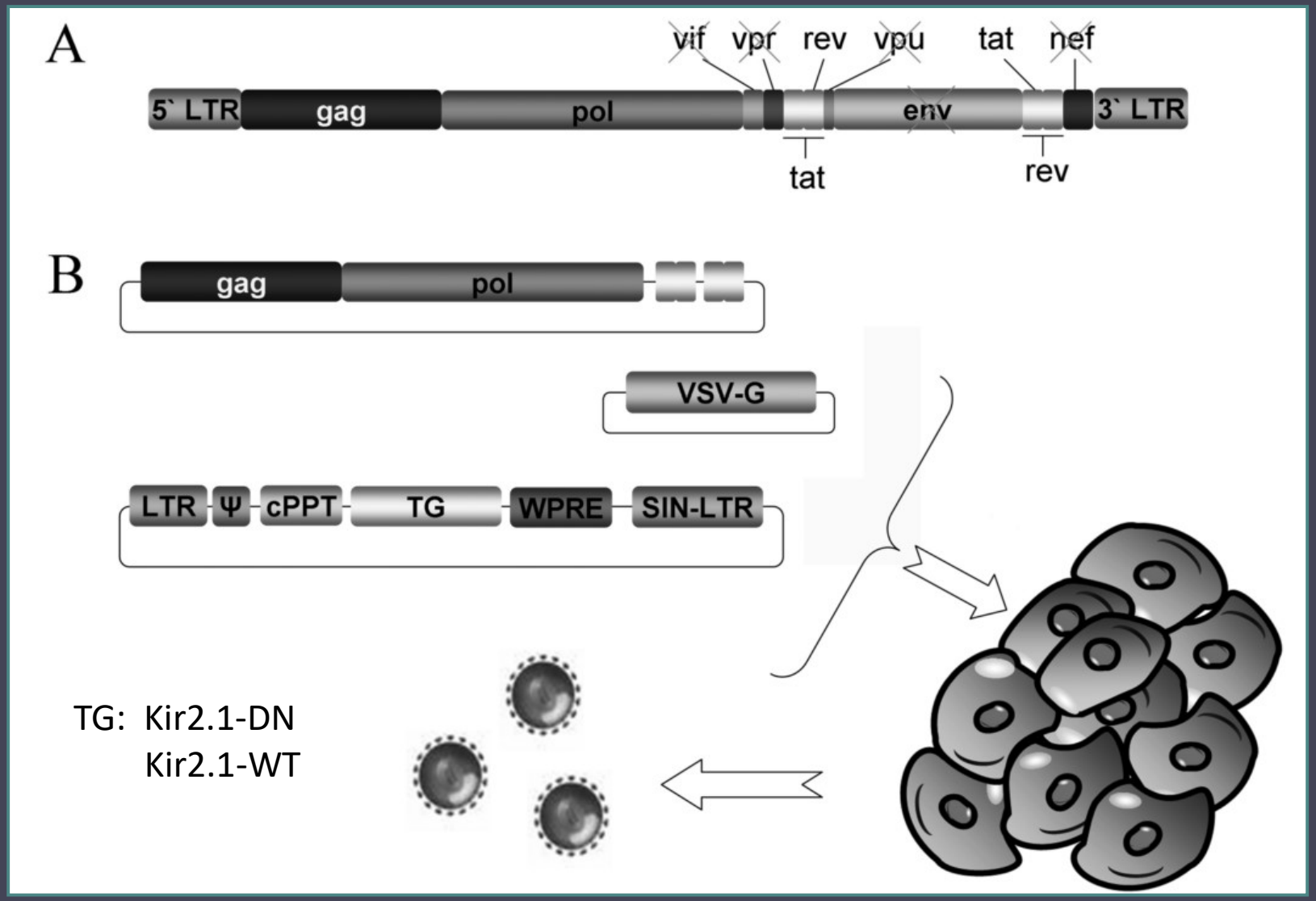


Purkinje cell

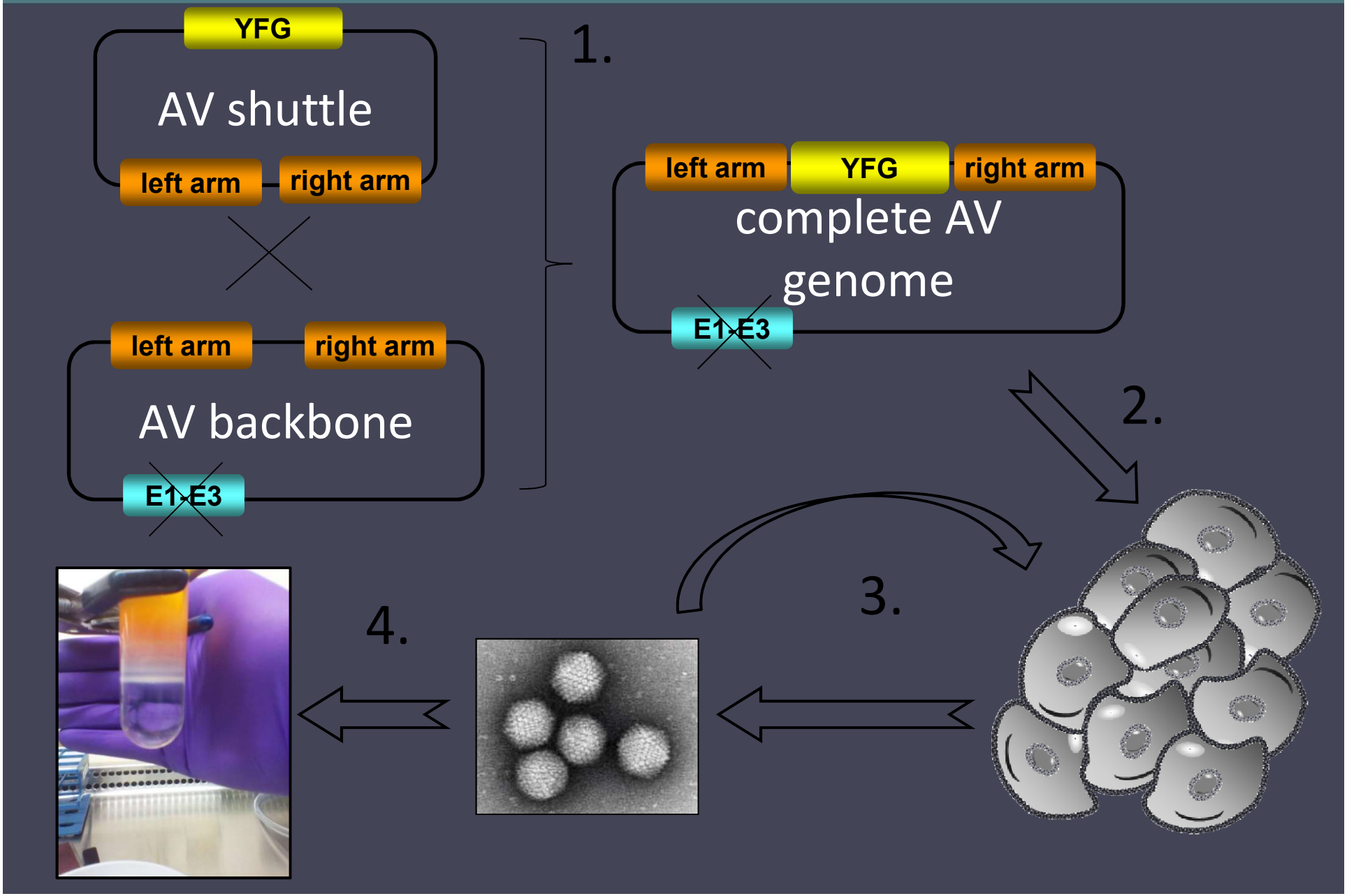




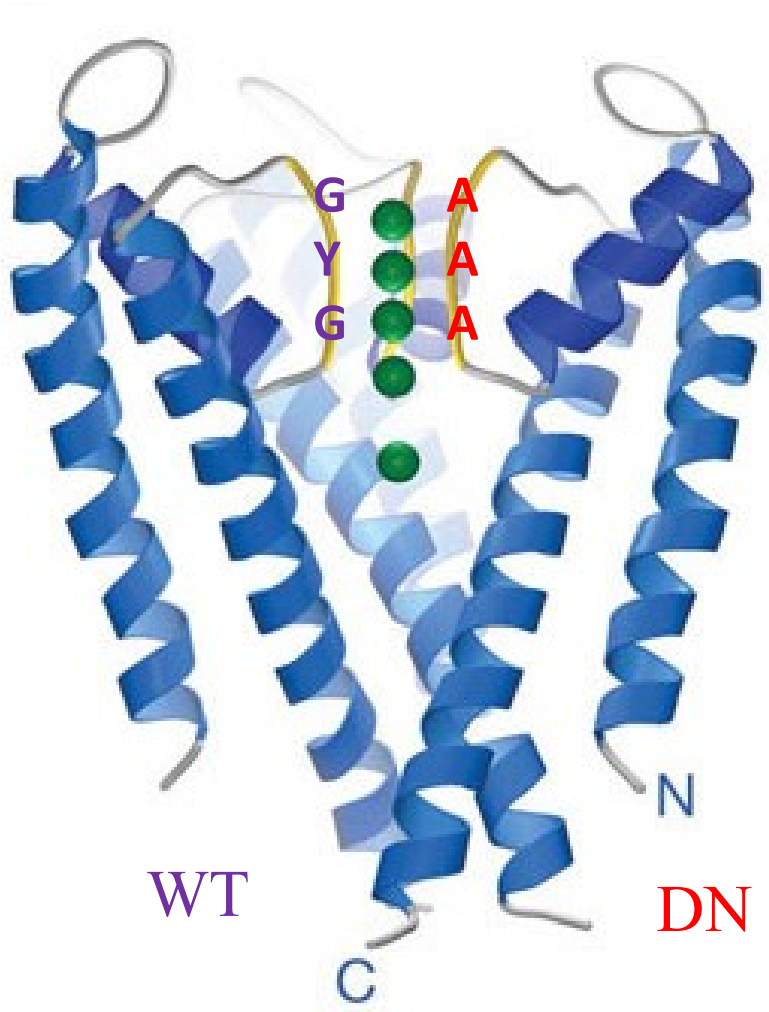
Molecular biology of lentiviral vectors

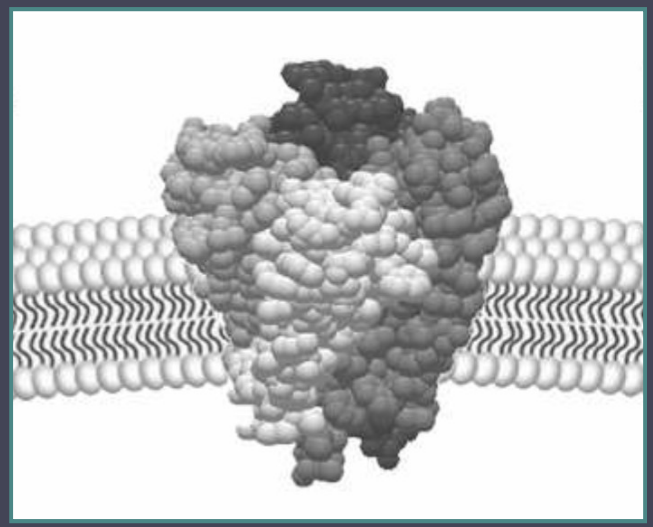


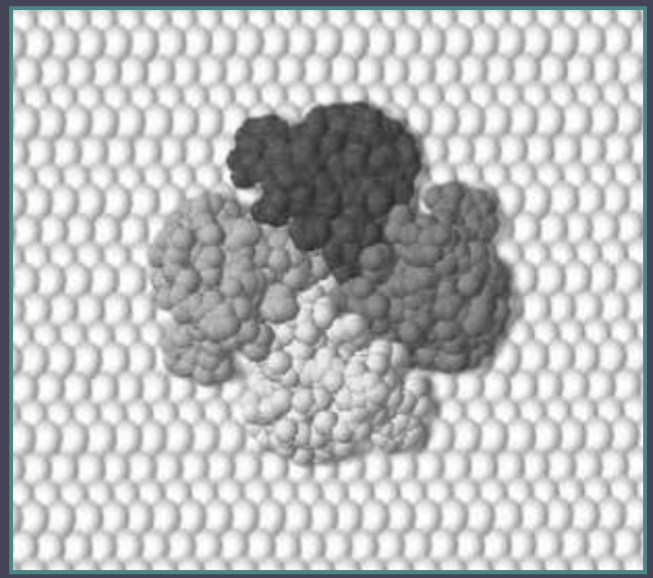
Moleceular biology of adenoviral vectors



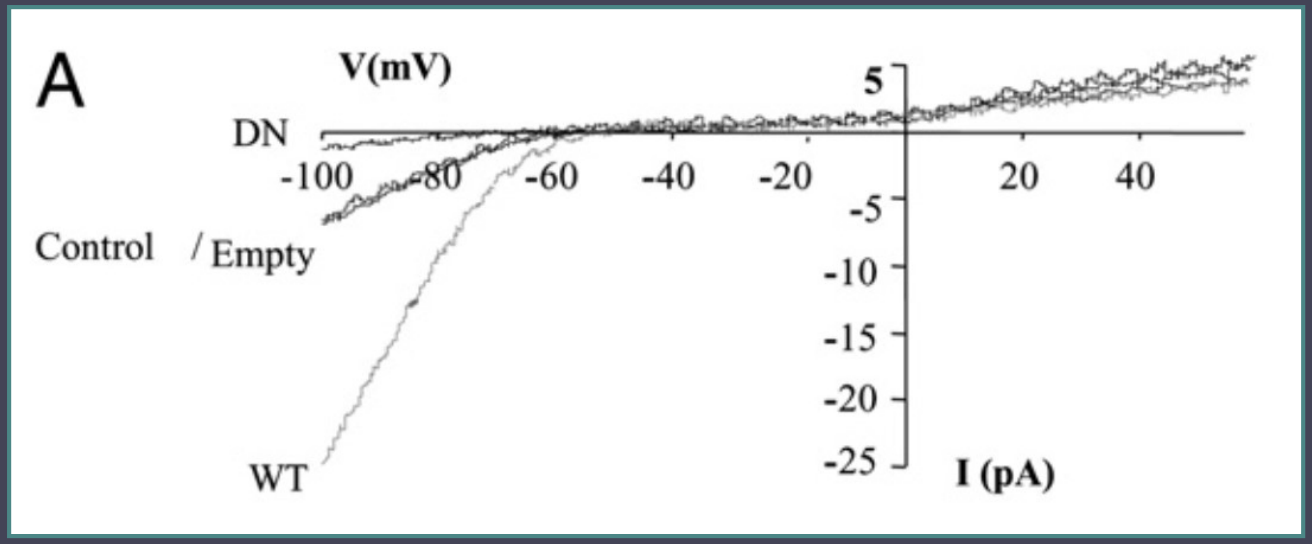
Over-expression of dominant negative mutants

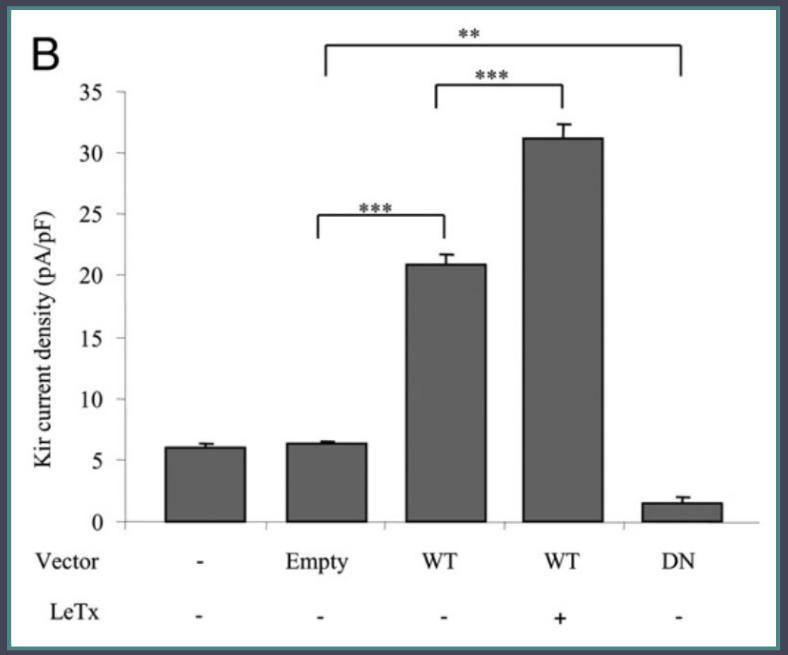


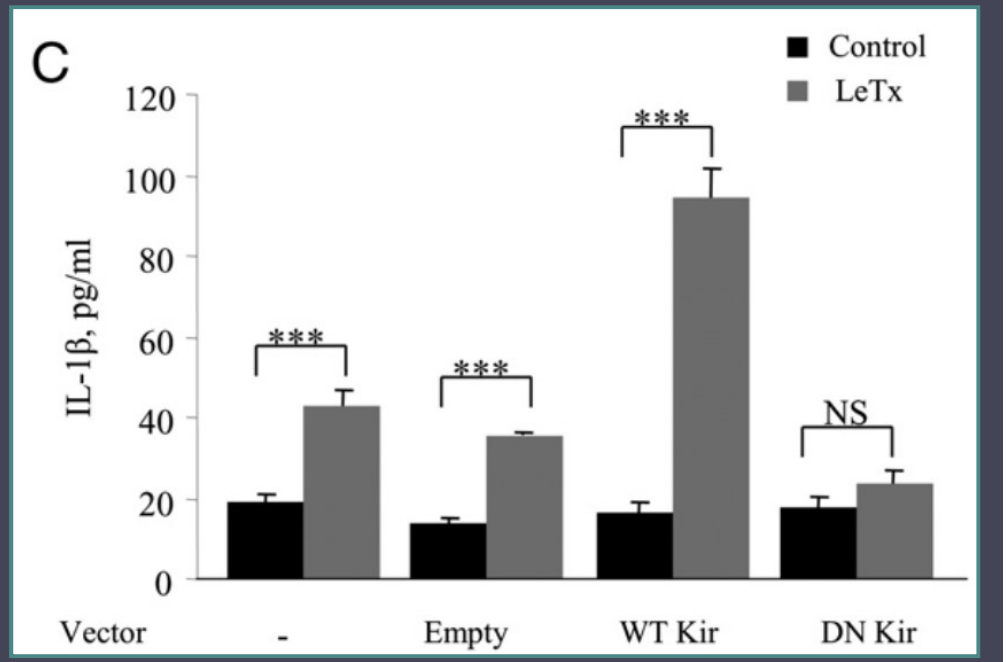




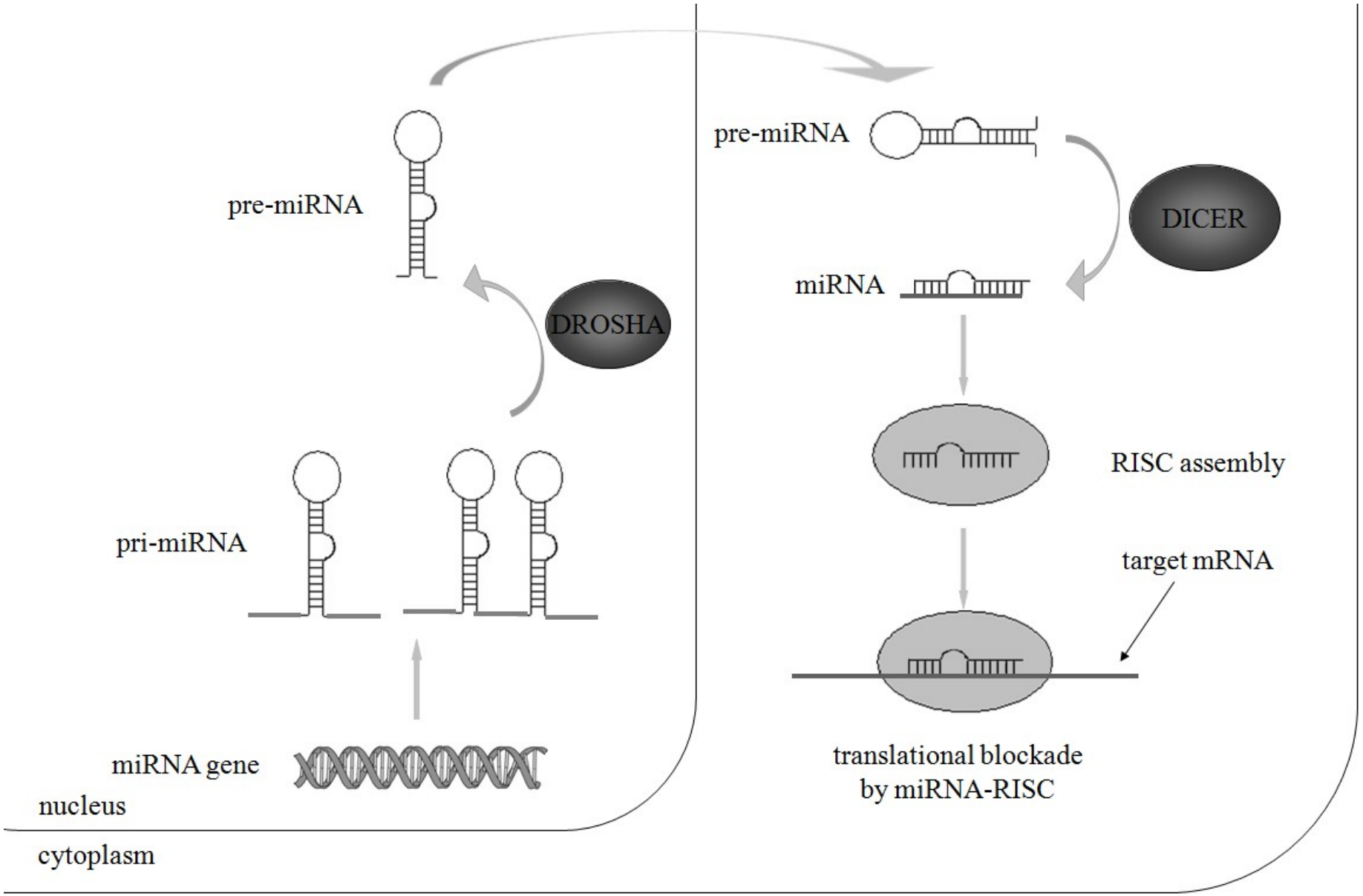
Role of I_{Kir} in IL-1β production of macrophages





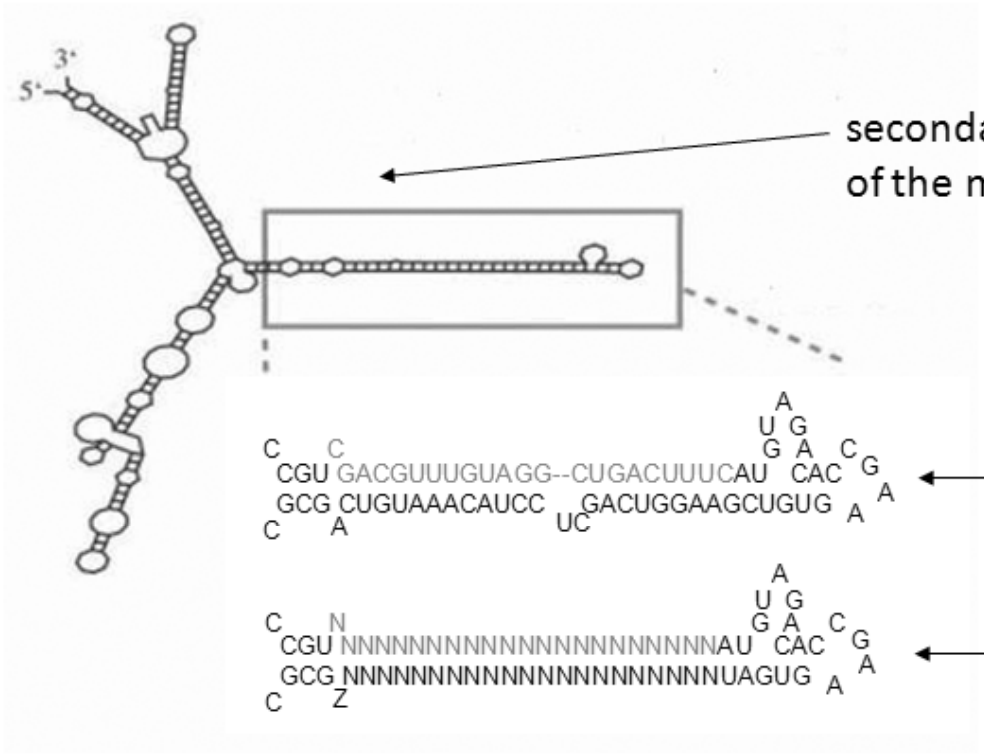


The RNAi pathway / biogenesis of microRNAs



Artificial microRNAs

A



secondary structure
of the mir30 precursor

the mir30-encoding
stem and loop

the mir30 sequence is replaced
by a targetgene-specific sequence

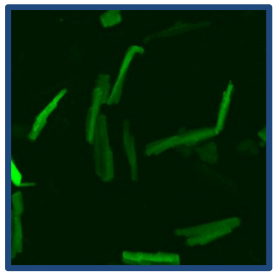
B

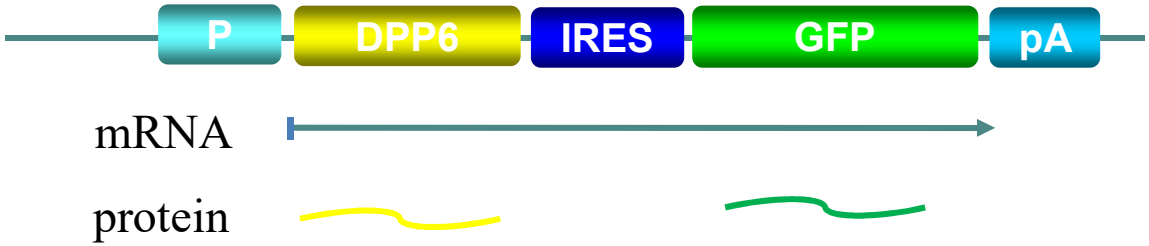
hairpin-coding part
in the shRNAmir gene

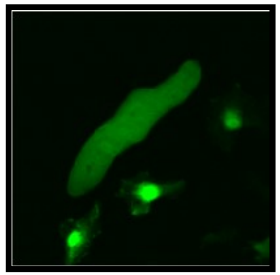
DPP6: an auxiliary subunit of I_{to} in Purkinje cells

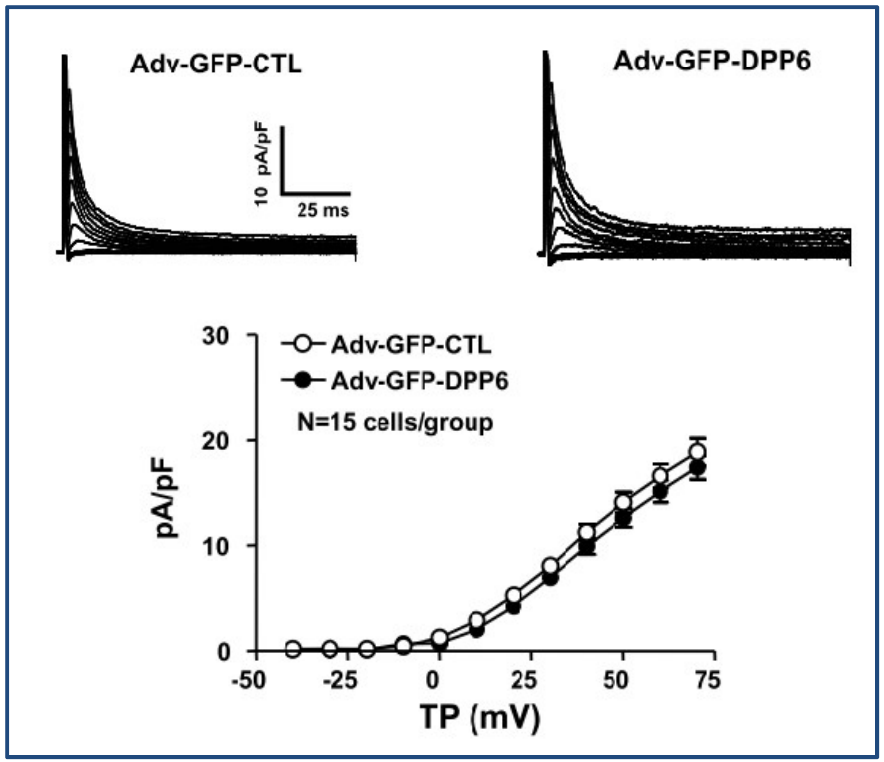
AV-GFP-DPP6

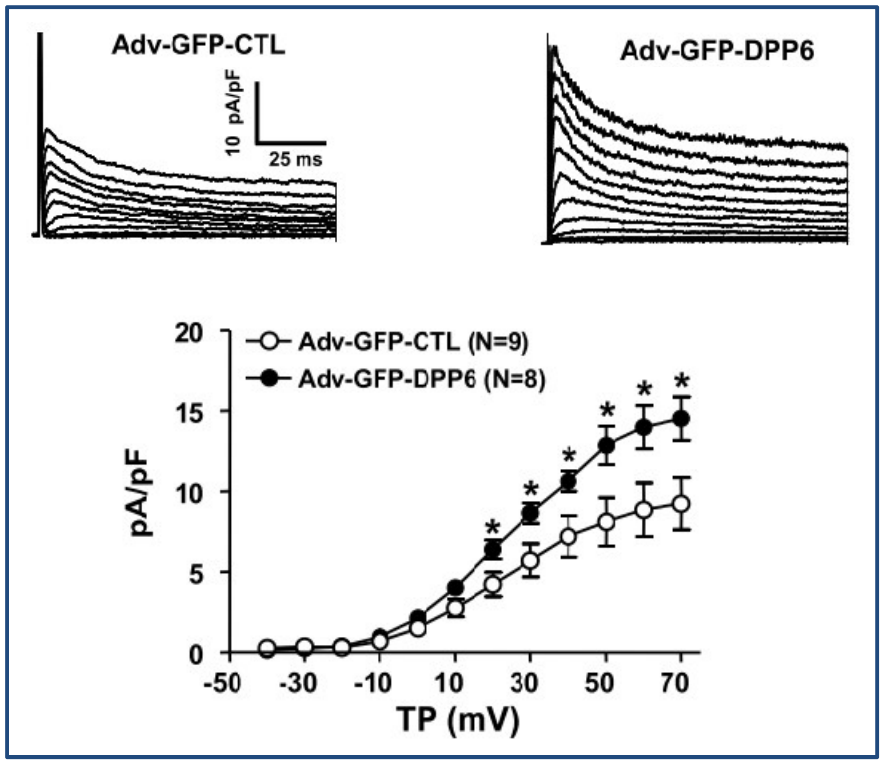
DPP6 over-expression







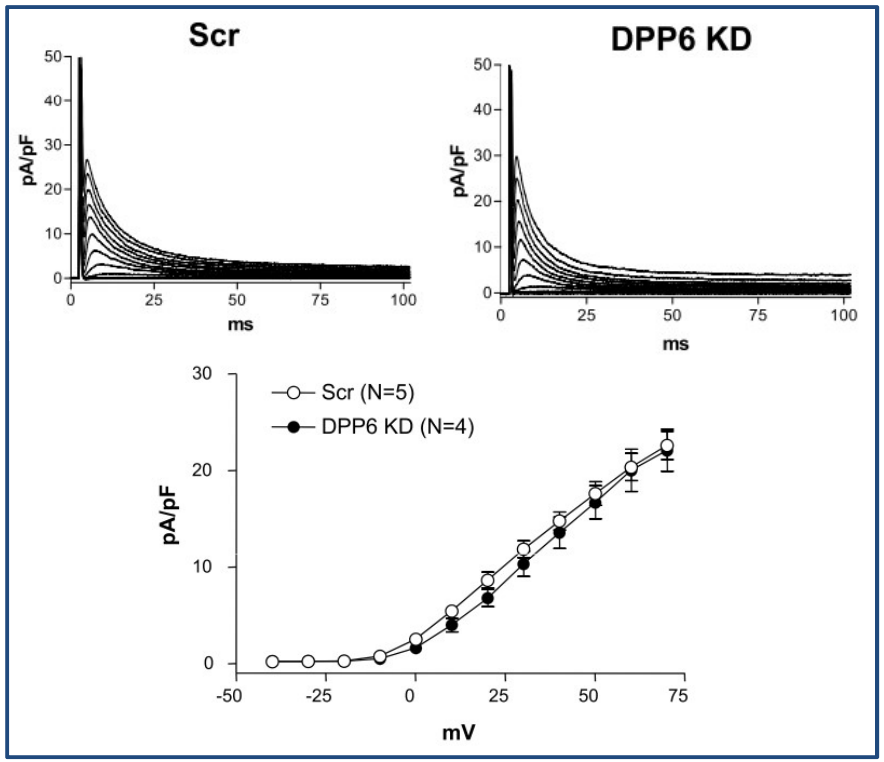


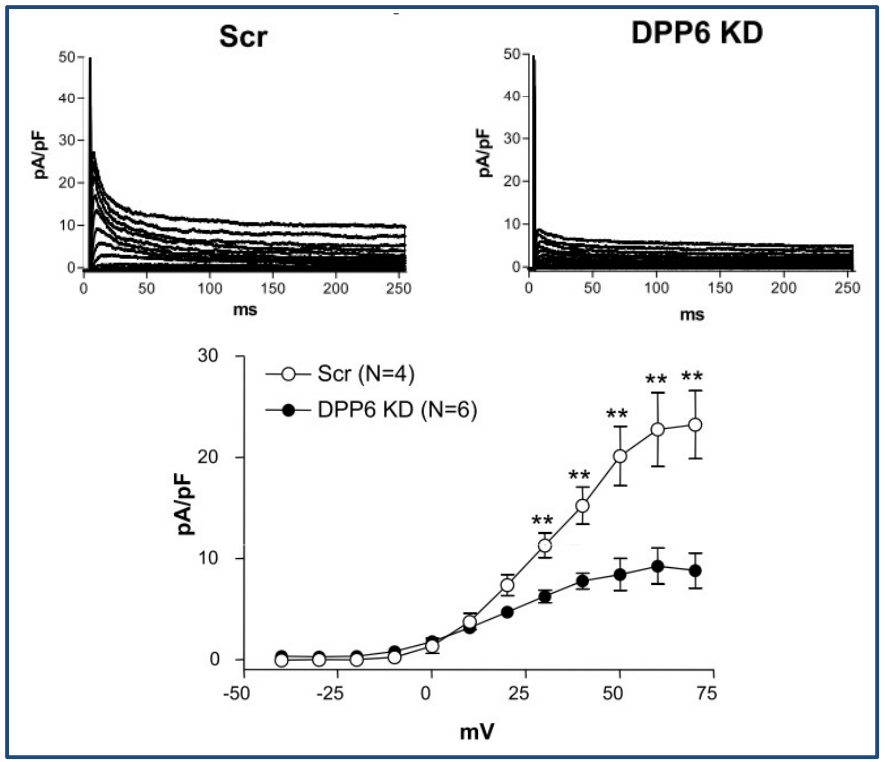


DPP6: an auxiliary subunit of I_{to} in Purkinje cells

DPP6 gene silencing

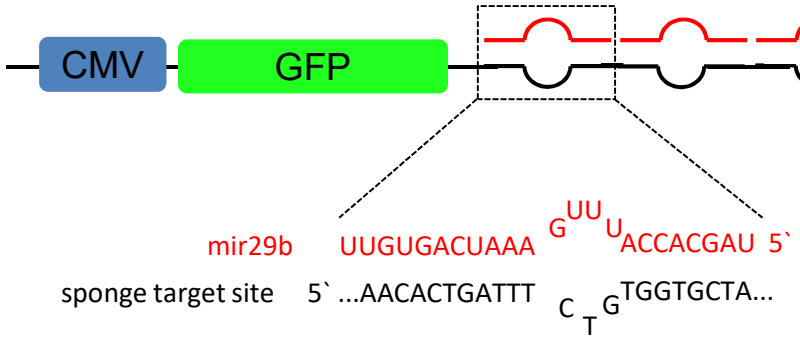
AdV-DPP6-KD

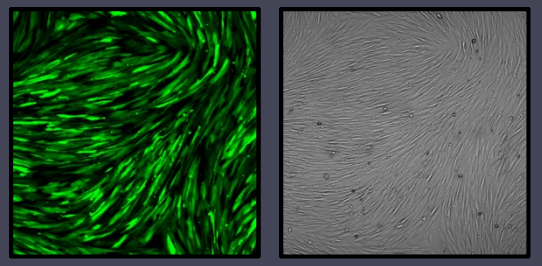




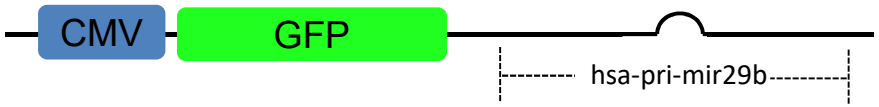
Manipulation of microRNA expression *in vitro*

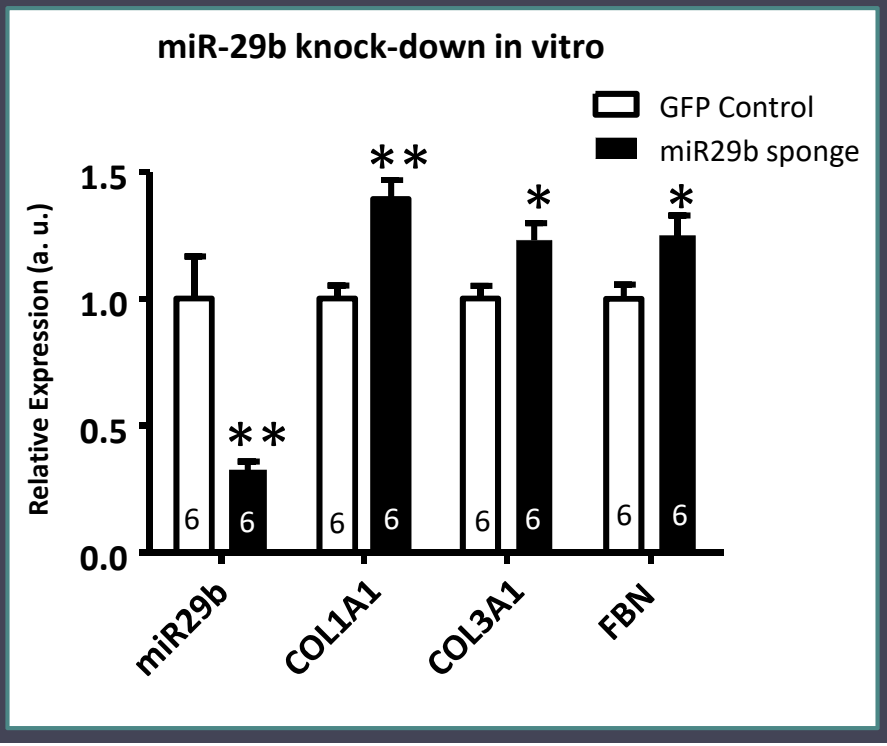
LV-mir29b szivacs

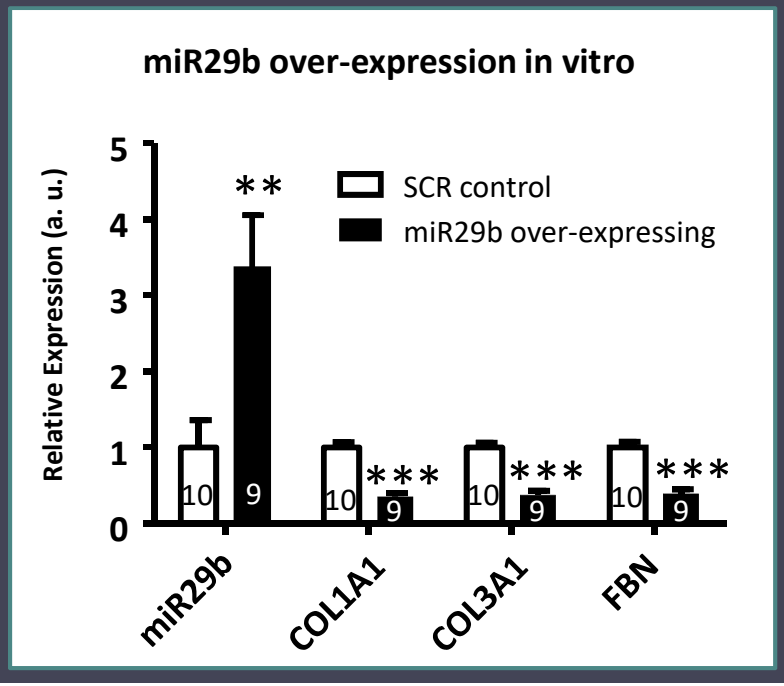




LV-mir29b-OE

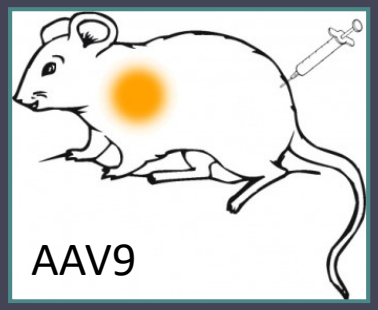




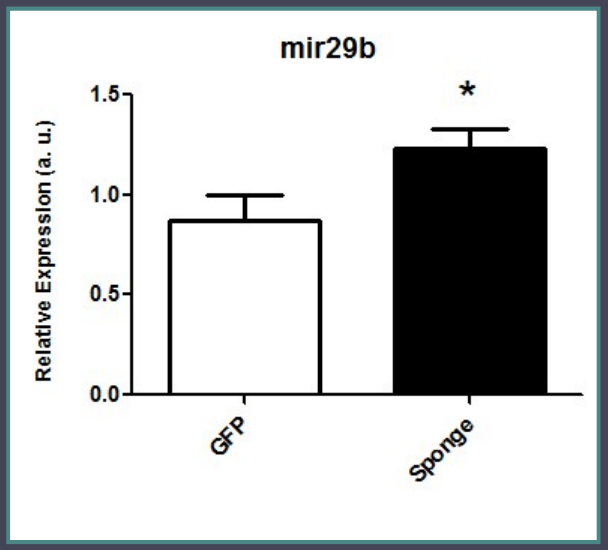


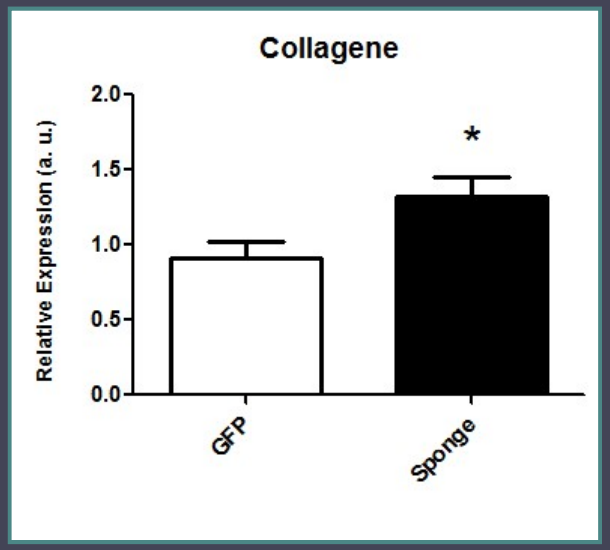
Manipulation of microRNA expression *in vivo*

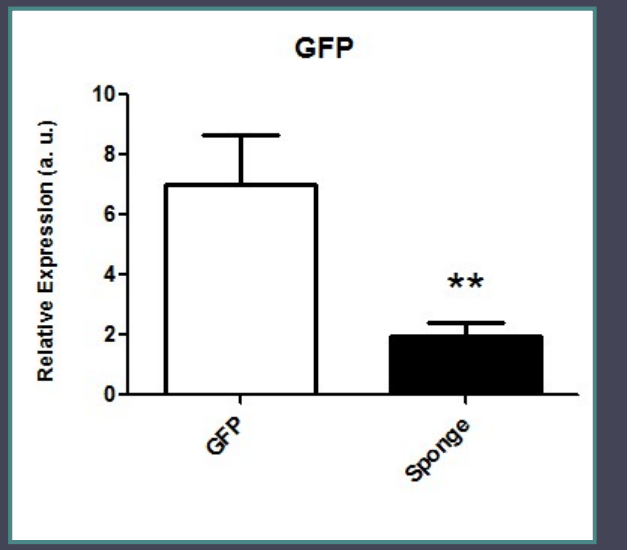
Adeno-associated virus, serotype 9



AAV-sp29b







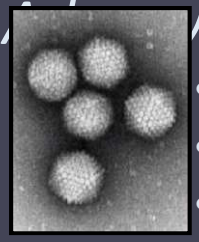
Viral gene delivery

Vector classes

Lentivirus

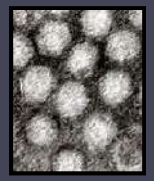
- *In vitro* applications
- for proliferating cells
- transduction is permanent -> stable cell lines
- easy production

Adenovirus



- *In vitro* and *in vivo*
- broad host range
- transduction is transient
- more difficult to produce

Adeno-associated virus (AAV9)



- cardiac gene transfer *in vivo*
- easy production
- for small payload only

Applications

Protein over-expression, e.g.:

- wild type (DPP6)
- mutant (Kir2.1-DN)

Gene silencing

RNA interference

MicroRNA manipulations

- microRNA sponge
- microRNA over-expression

Ms/Mr Have More Questions

<u>Do have questions about:</u>

Cardiac elphys
Ion channels
Gene surgery
The beauty of patch clamping
The meaning of life and everything, etc.!

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