Tübingen Hearing Research Centre

University of Tübingen

Mol Aspects of Hearing

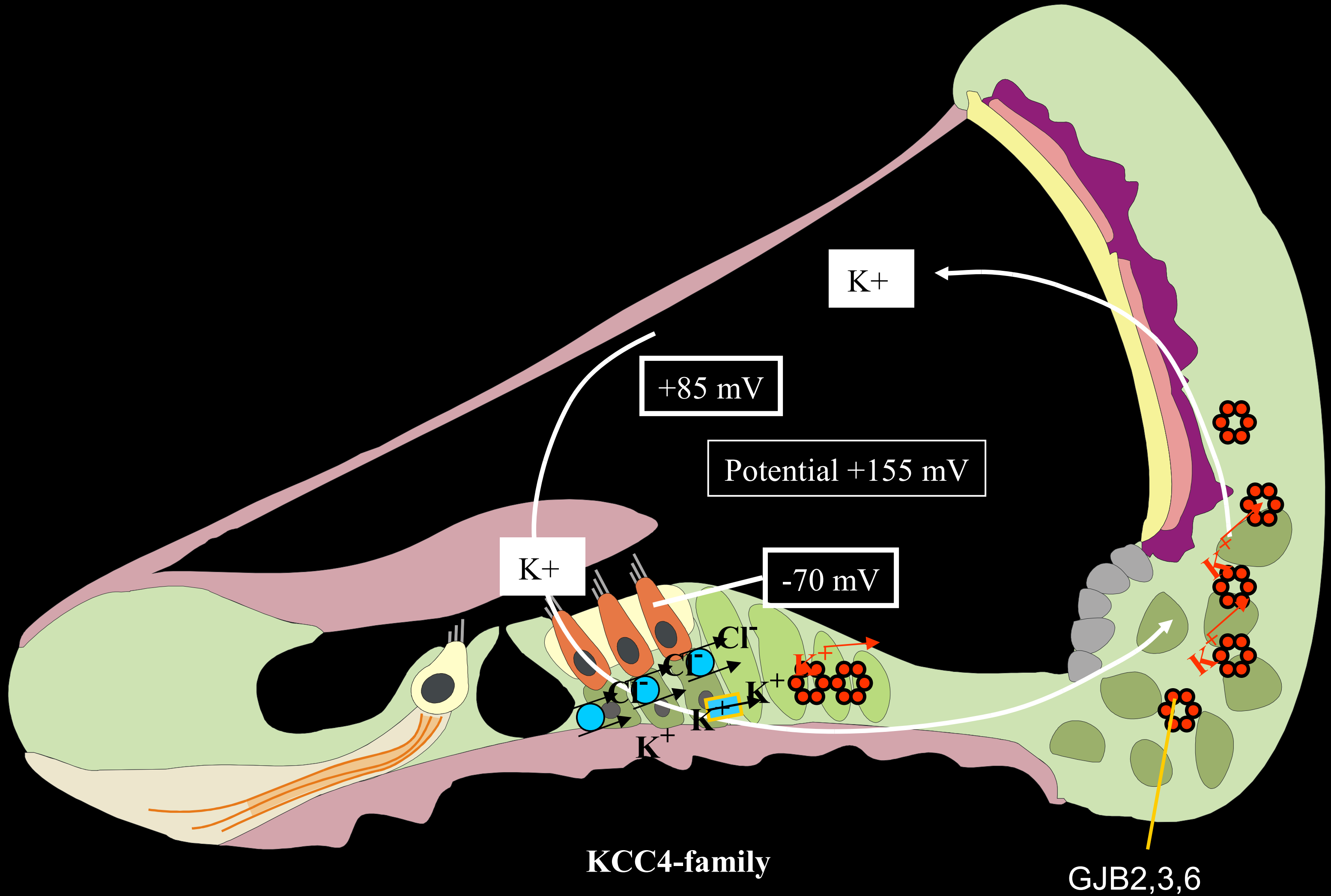
NeuroSensory Curriculum
2009/ 2010

Attempto

Singularity of our human being is not due to the individuality or higher number of genes

Singularity is the result from the tremendous possibilities to combine different genes, their subtypes and their splice variants

	Genomsize	Coding Genes	Genes per MB
Human	**2724 MB**	**31.780**	**12**
C elegans		**19.099**	**197**
Drosophila	**116 MB**	**13.601**	**117**
Plant ***Arabidopsis thaliana***		**25.498**	**221**



KCC4-family

K+- Cl- Cotransport →

→ Deafness in mice mutants (Jentsch, ZMNH Hamburg, 2002)

Non-syndromal deafness in heterozygous and homozygous patients
Connexin 31 (GJB3) →Chromosome 1p34 → DFNA2 locus ?? *Cochlea; auditory nerve*
Connexin 26 (GJB2) →Chromosome 13q12 →DFNB1 locus *spiral ligament;supporting cell*
Connexin 30 (GJB6) →Chromosome 13q12 →DFNA3 locus *spiral ligament ;supporting cell*

GJB = Connexin – Cx = 49% aller nicht-syndromalen Hördefekte→ Connexin

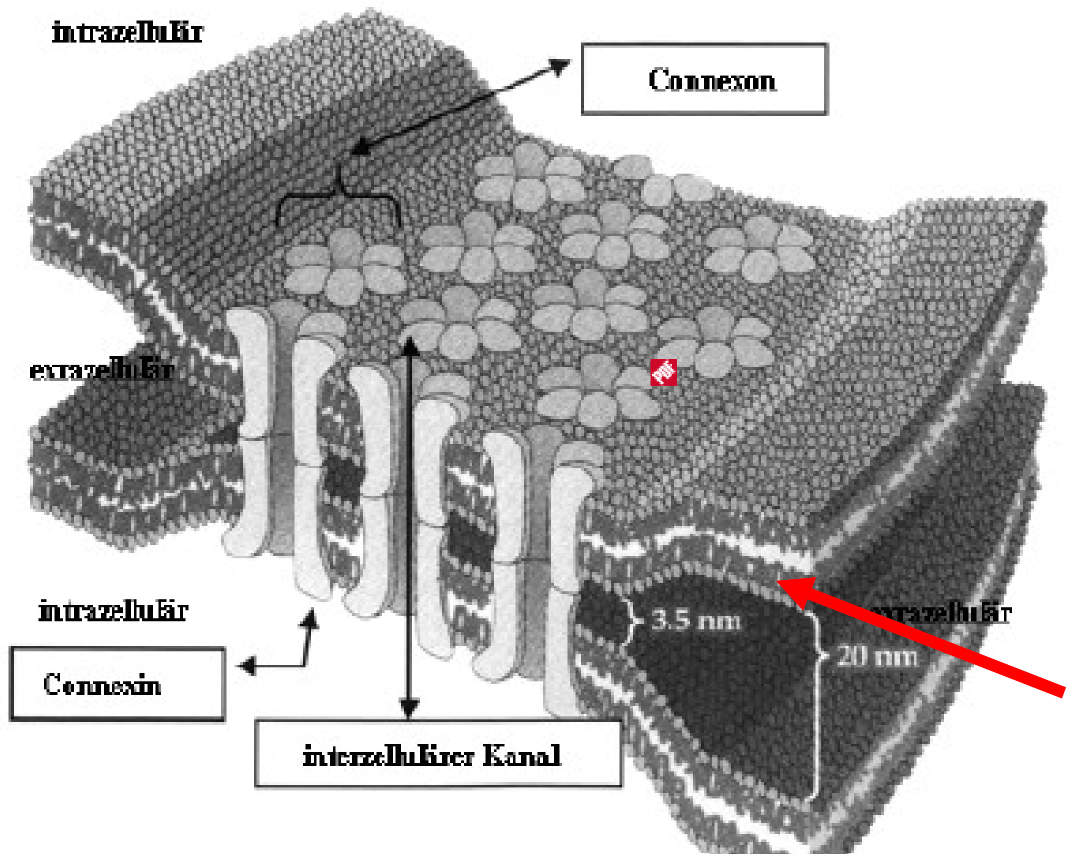
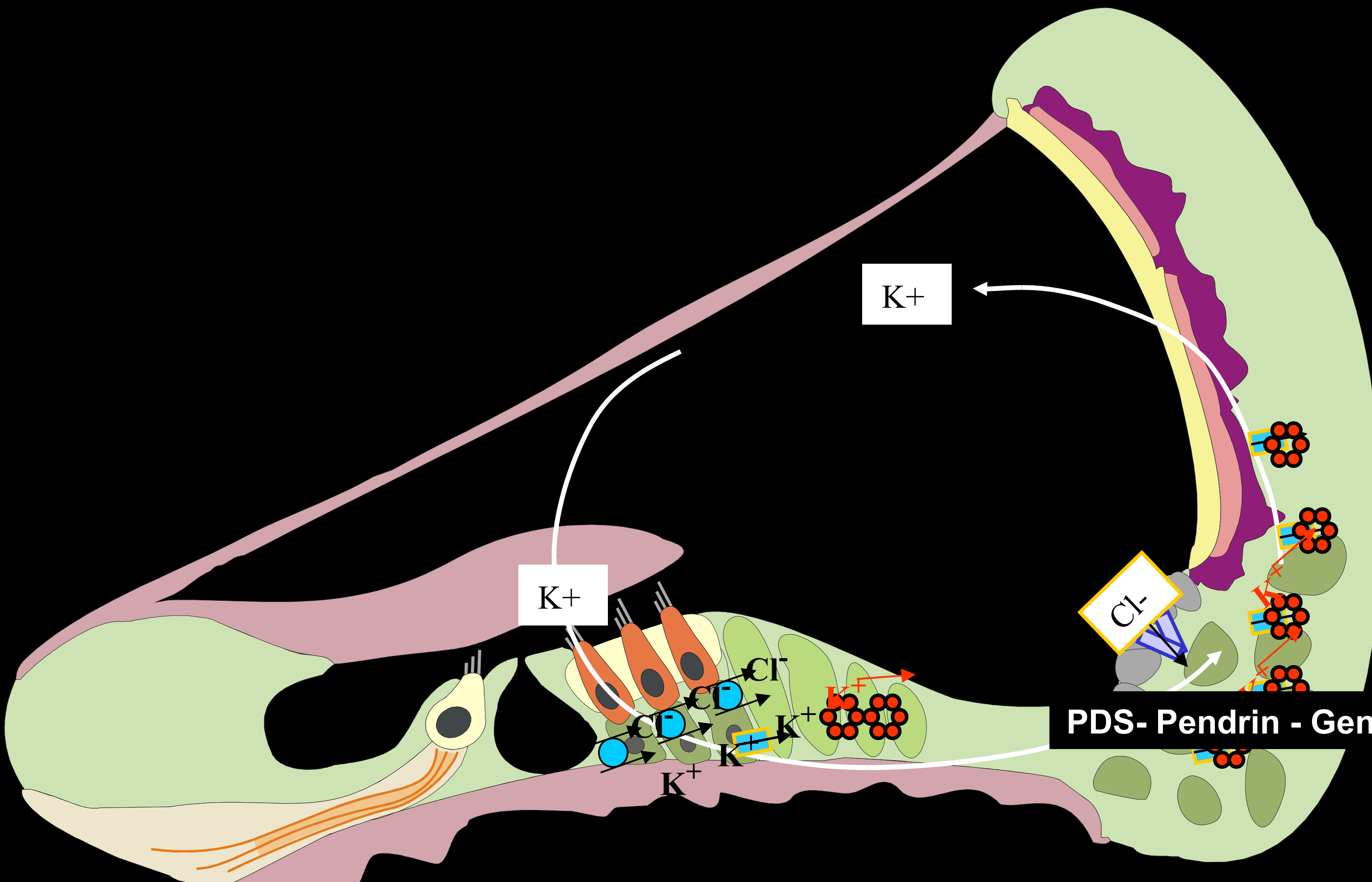


Abbildung 3

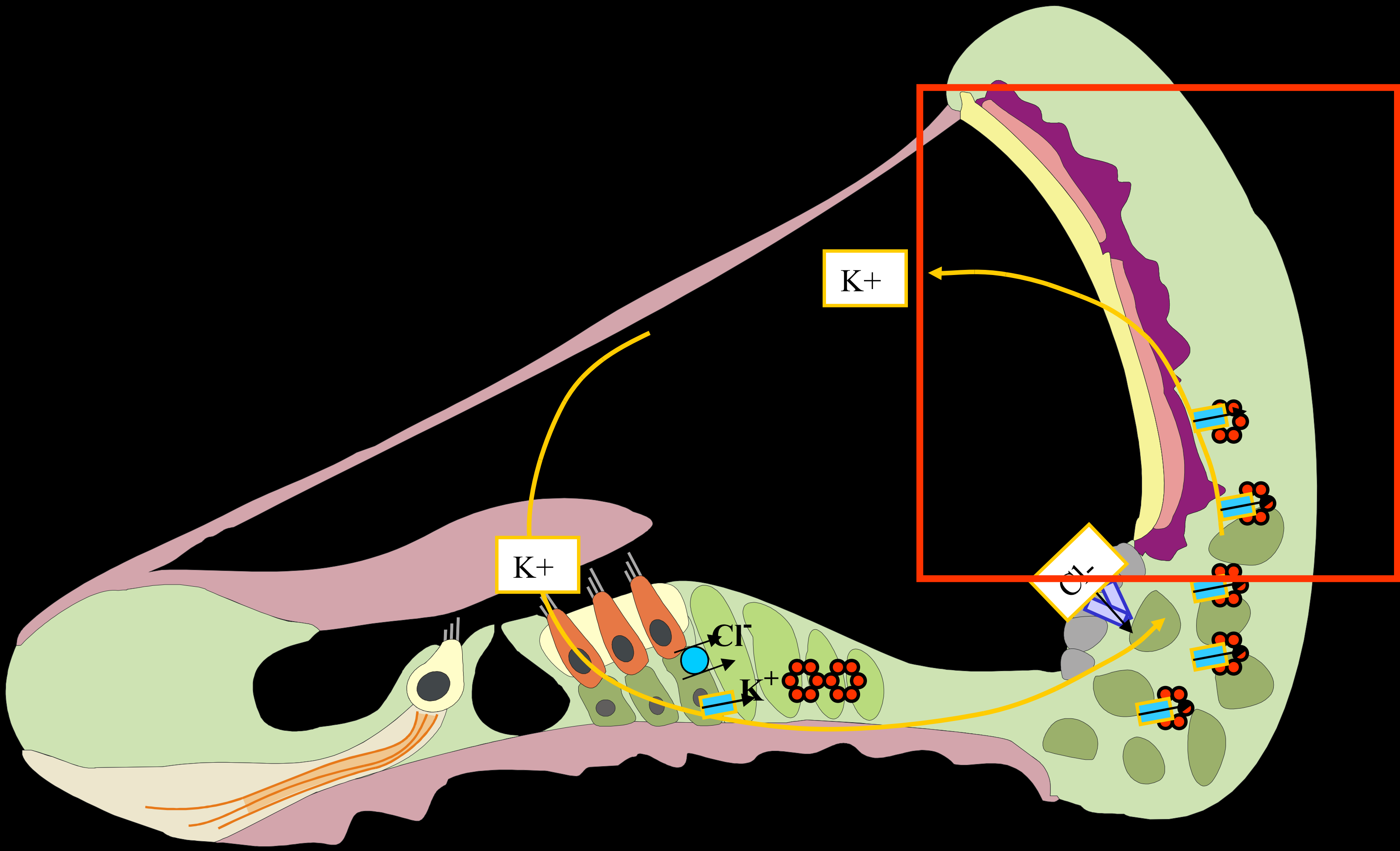
Nach Kemperman, MH, Figure 1(Kemperman, 2002): Aufbau eines Connexons aus mehreren Connexinen



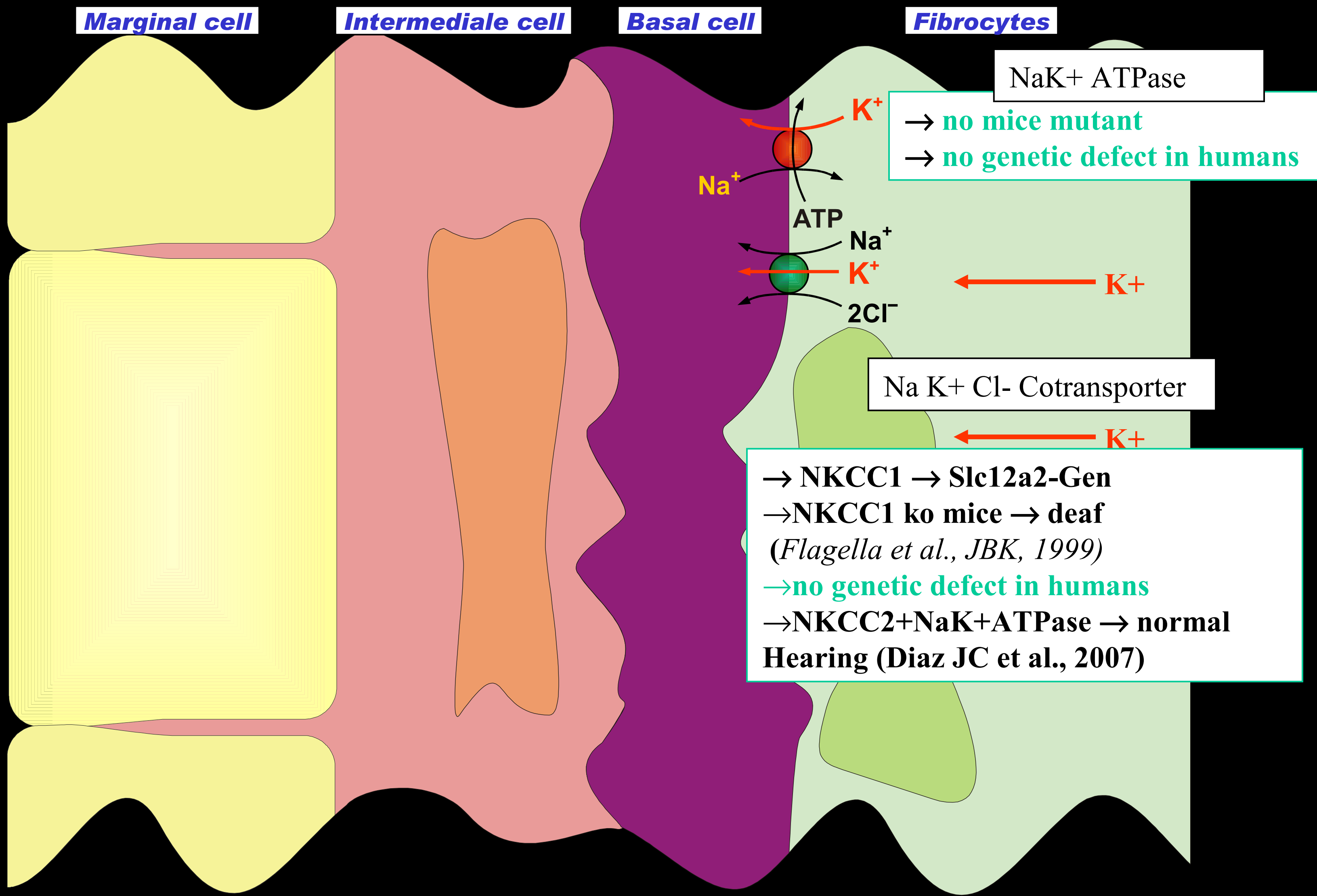
PDS → Pendred-Syndrom → Anion/Jodid Transport → Homeostase? Chromosome 7q31 → DFNA4 locus

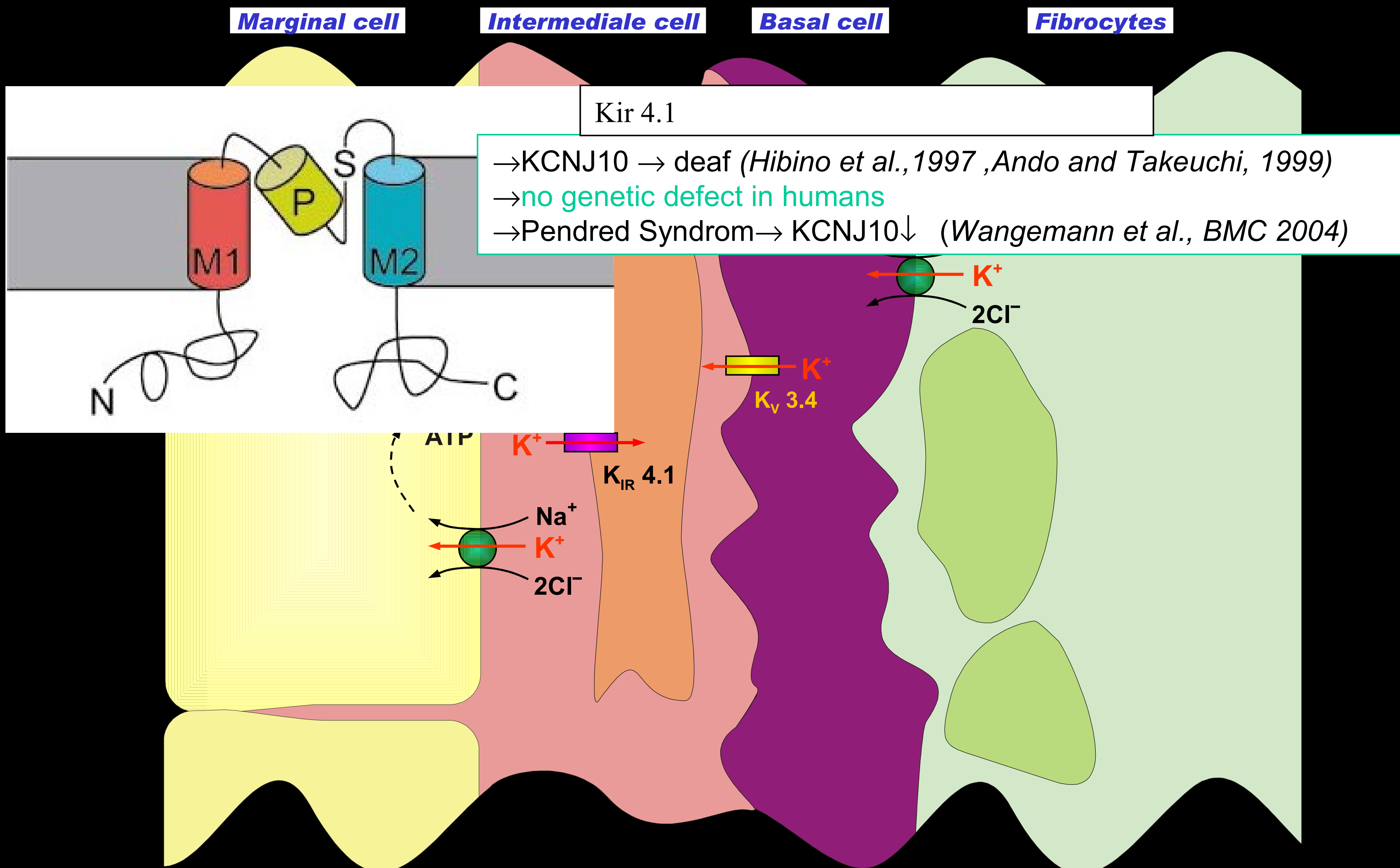
→ *autosomal recessive deafness in patients with Pendred Syndrom (Everett, 1997; Li et al., 1998)*

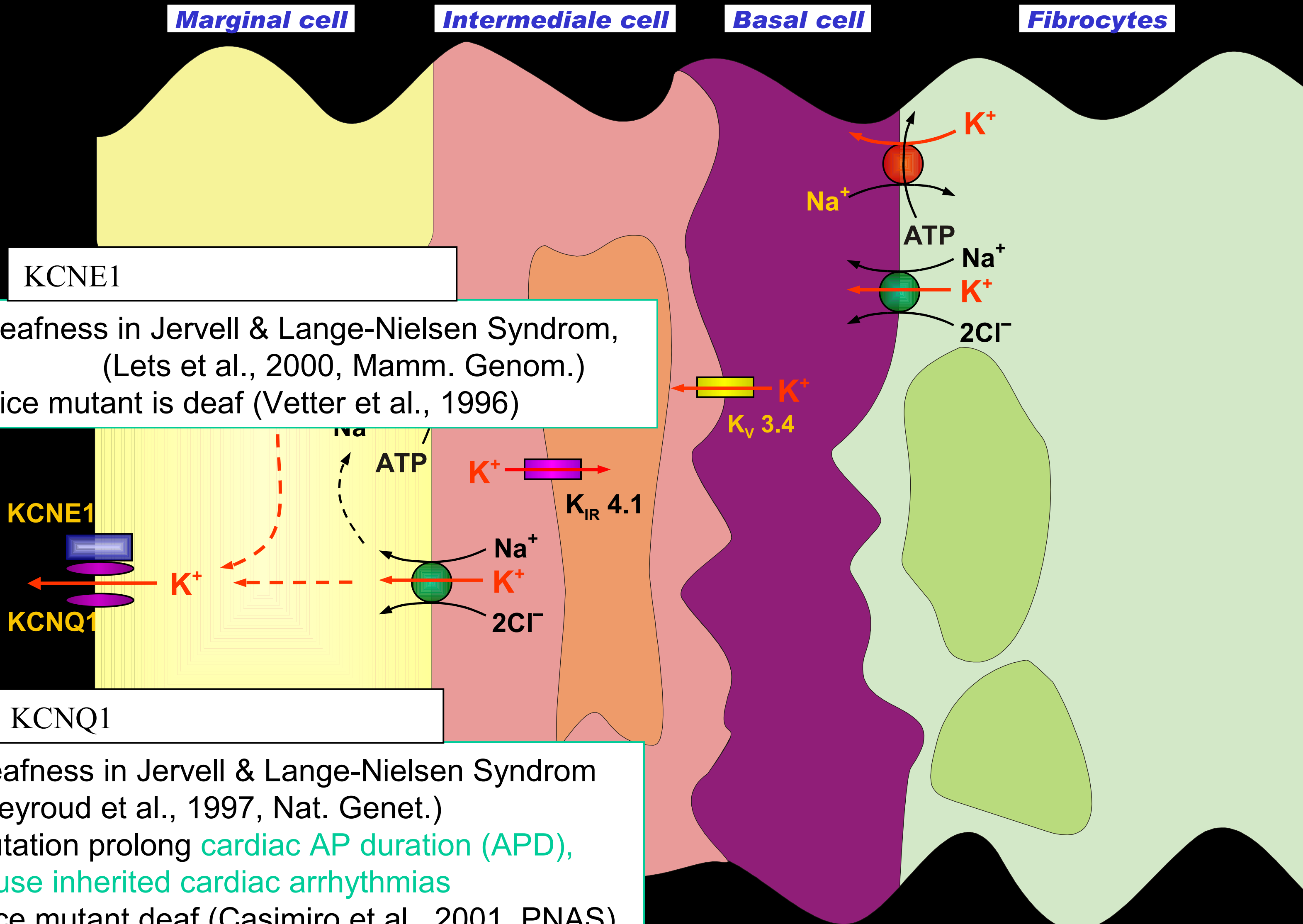
→*Mice mutants*→ *Deaf* → *(Everett et al., 2001; Novartis, 2006)*



K+
K+
Cl-
K+
Cl-


Marginal cell
Intermediale cell
Basal cell
Fibrocytes
NaK+ ATPase
→ no mice mutant
→ no genetic defect in humans
K+
Na+
ATP
Na+
K+
2Cl−
K+
Na K+ Cl- Cotransporter
K+
→ NKCC1 → Slc12a2-Gen
→NKCC1 ko mice → deaf
(Flagella et al., JBK, 1999)
→no genetic defect in humans
→NKCC2+NaK+ATPase → normal
Hearing (Diaz JC et al., 2007)


Marginal cell
Intermediale cell
Basal cell
Fibrocytes
Kir 4.1
→KCNJ10 → deaf (Hibino et al.,1997 ,Ando and Takeuchi, 1999)
→no genetic defect in humans
→Pendred Syndrom→ KCNJ10↓ (Wangemann et al., BMC 2004)
M1
P
S
M2
N
C
ATP
Na+
K+
2Cl−
K_{IR} 4.1
K_V 3.4



KCNE1

→ deafness in Jervell & Lange-Nielsen Syndrom, (Lets et al., 2000, Mamm. Genom.)

→Mice mutant is deaf (Vetter et al., 1996)

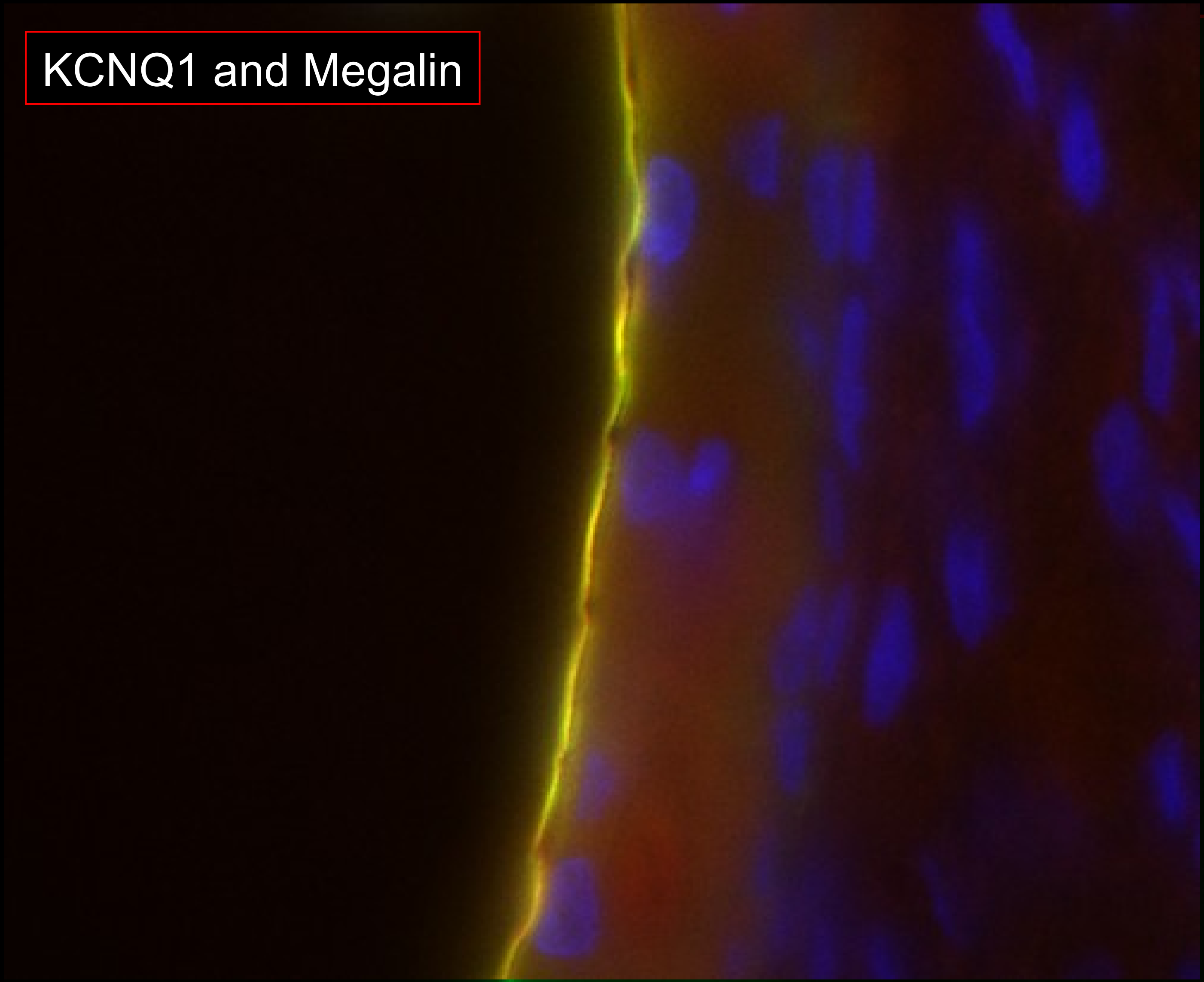
KCNQ1

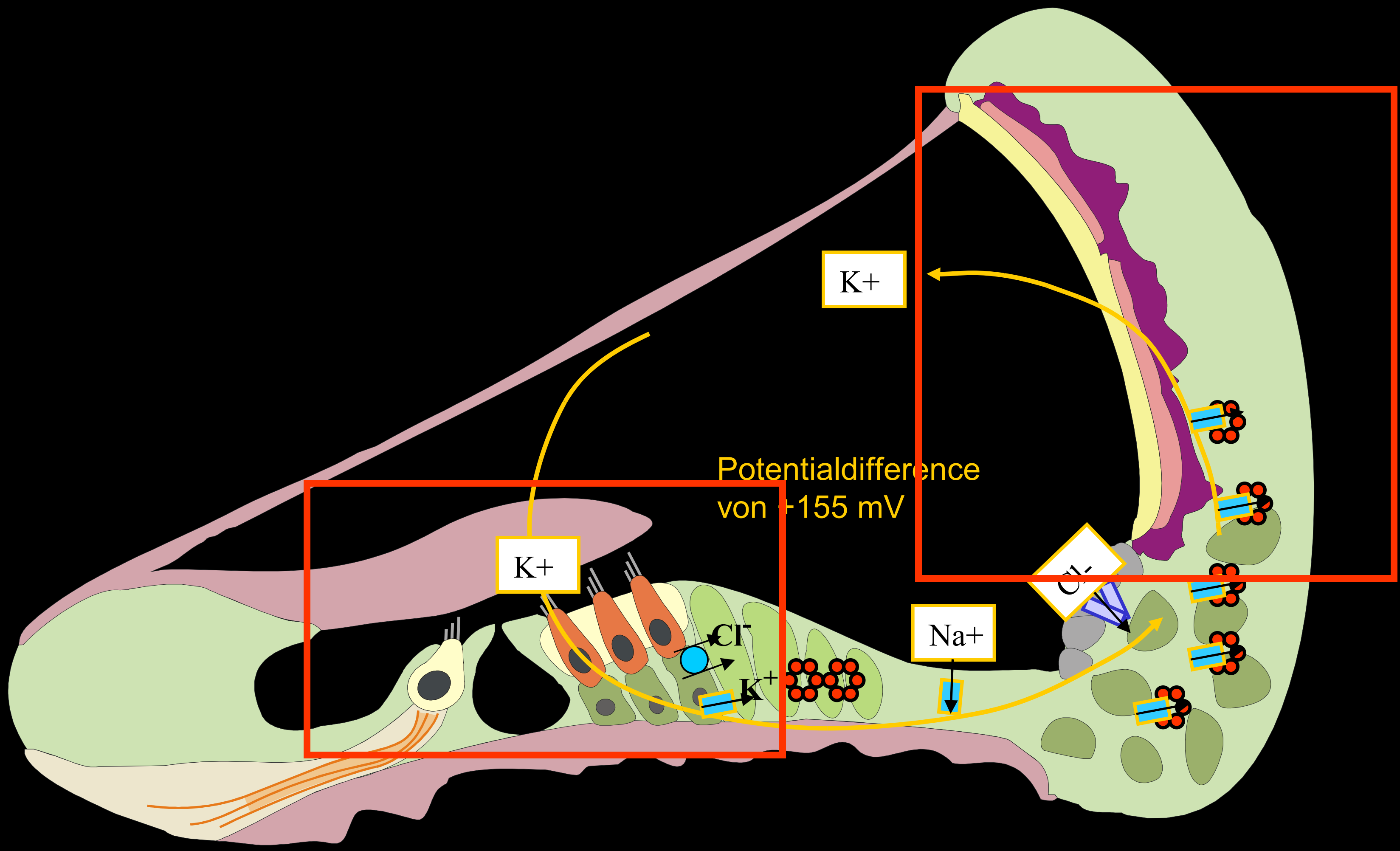
→ deafness in Jervell & Lange-Nielsen Syndrom (Neyroud et al., 1997, Nat. Genet.)

→Mutation prolong cardiac AP duration (APD),

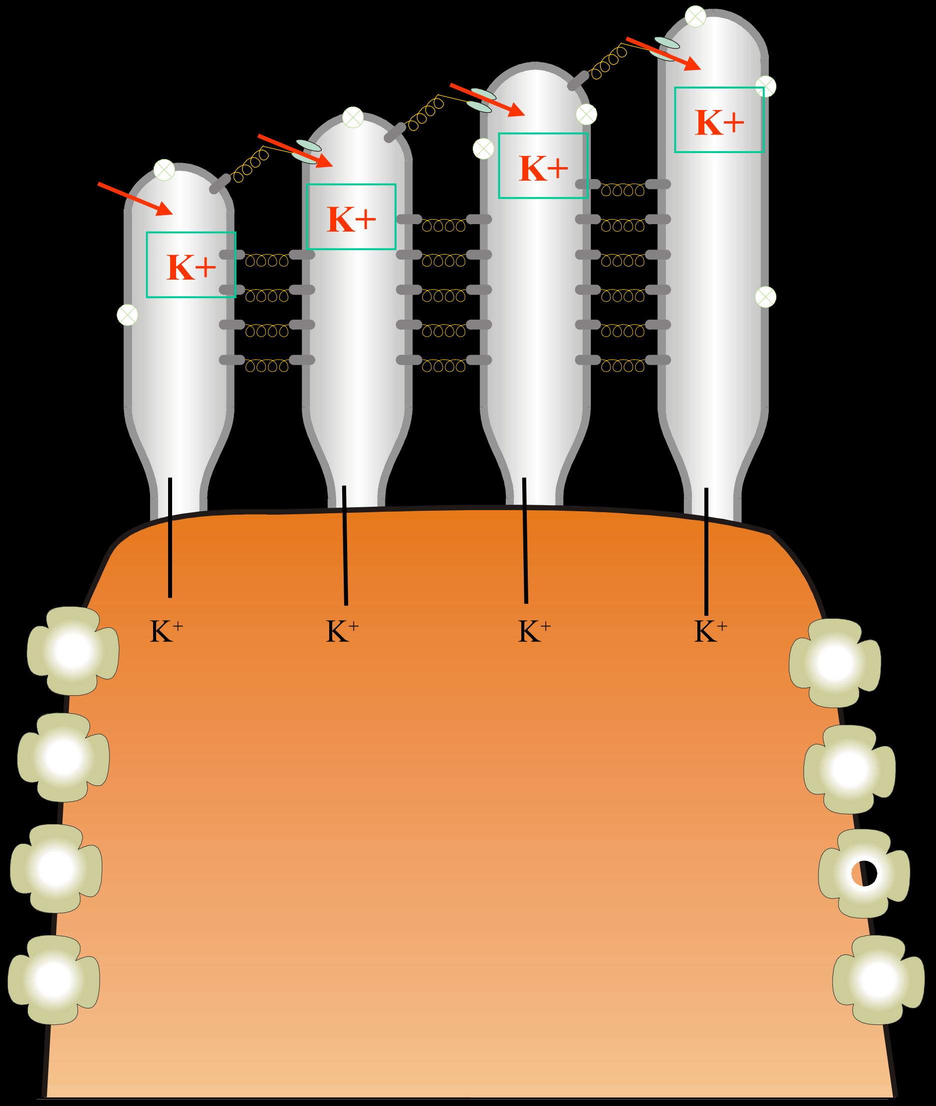
→cause inherited cardiac arrhythmias

→Mice mutant deaf (Casimiro et al., 2001, PNAS)


KCNQ1 and Megalin


K+
Potentialdifference
von +155 mV
K+
Cl-
K+
Cl-
Na+

Mechanotransducer has not been found yet!

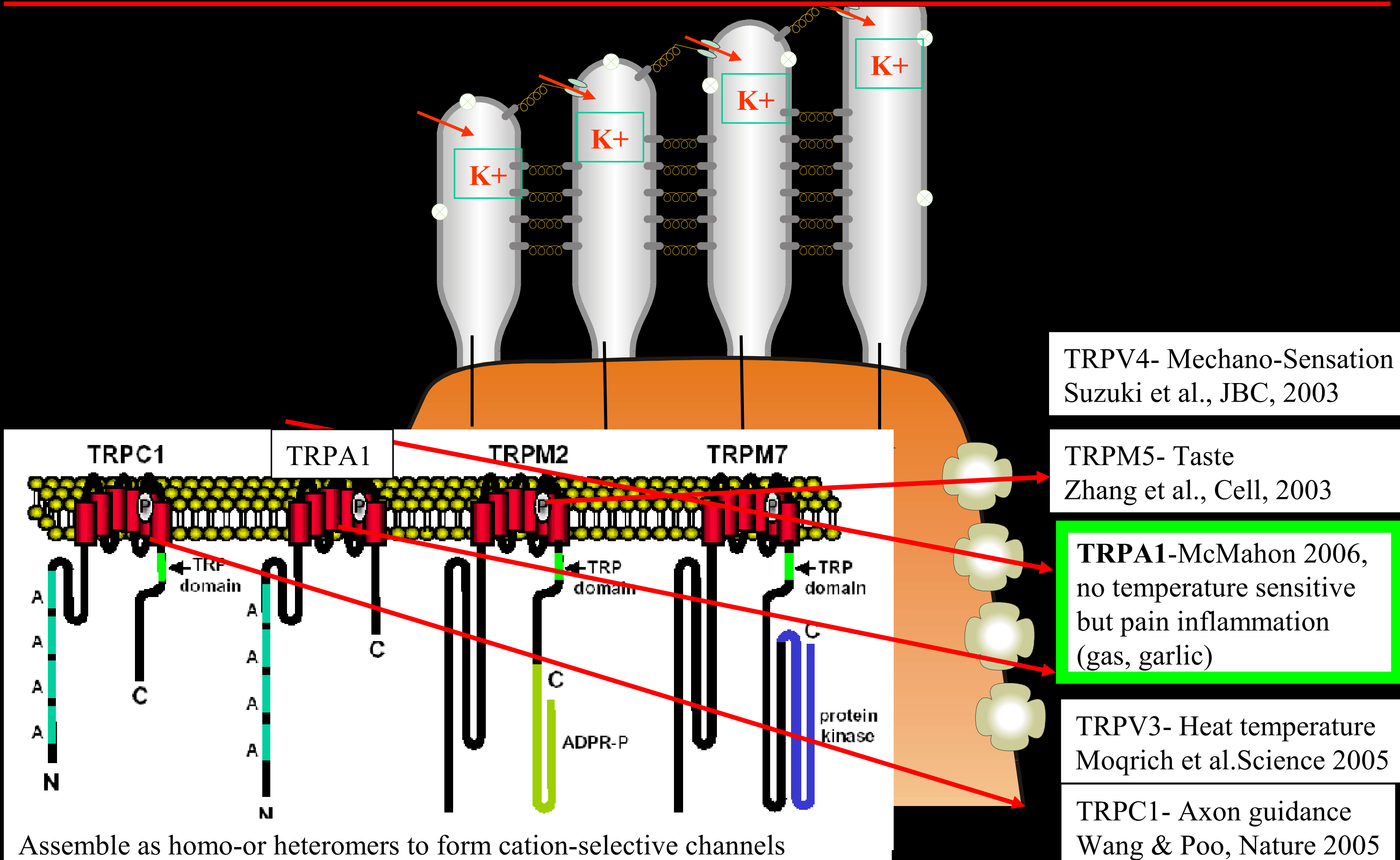


NOMPC/TRPN →Zebrafish (*Sidi et al., 2003, Science 301, 96-98.)*

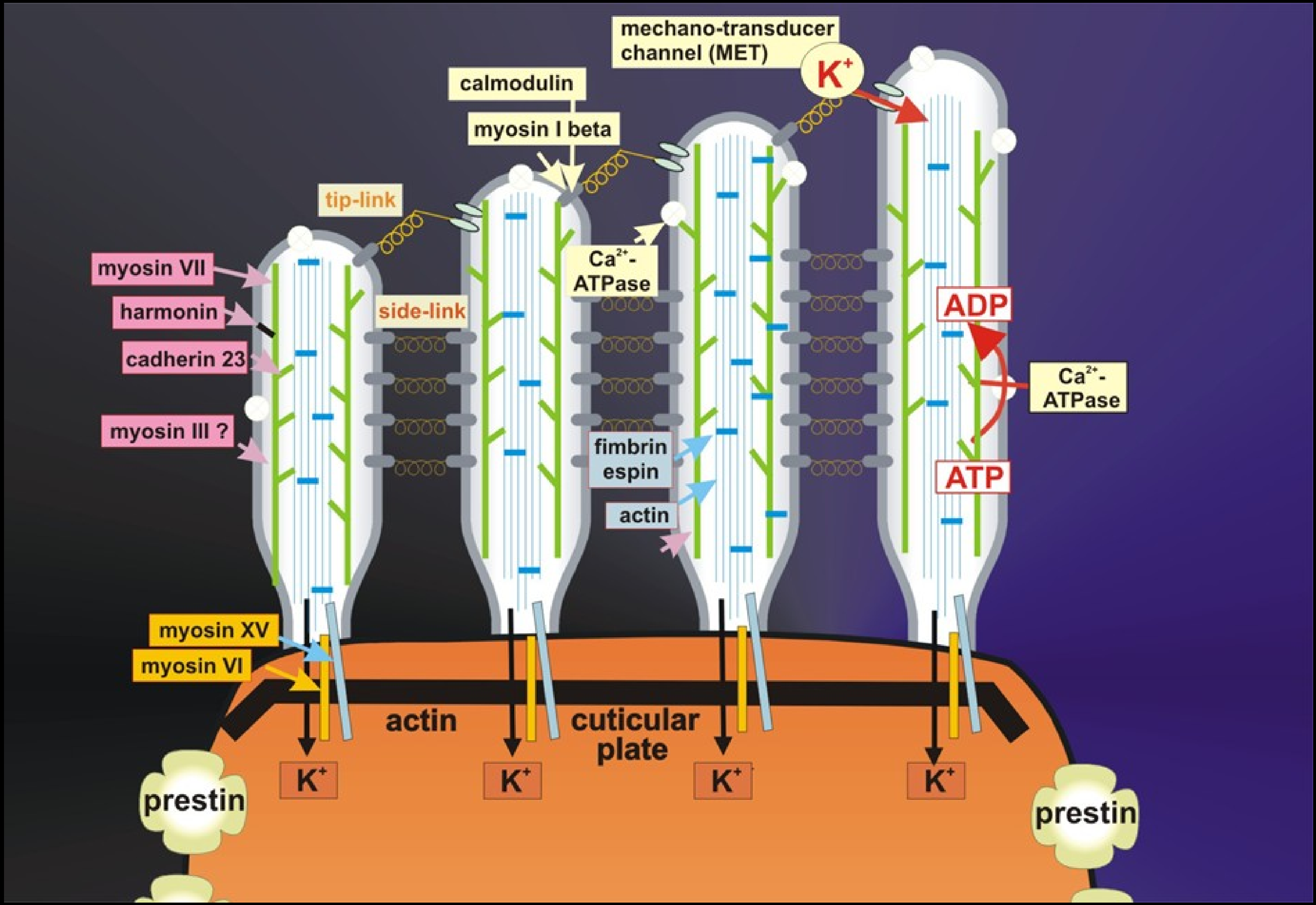
Mucolipin-3/TRP → Mice (*DiPalma et al., 2002, PNAS, 99, 14613-14815*)

TRPA1 (ANKTM1) → Mice (Corey et al., 2004, Nature)

TRPA1 → Zebrafish Chemosensor not Mechantransducer!! (Prober et al., J Neurosc 2008)

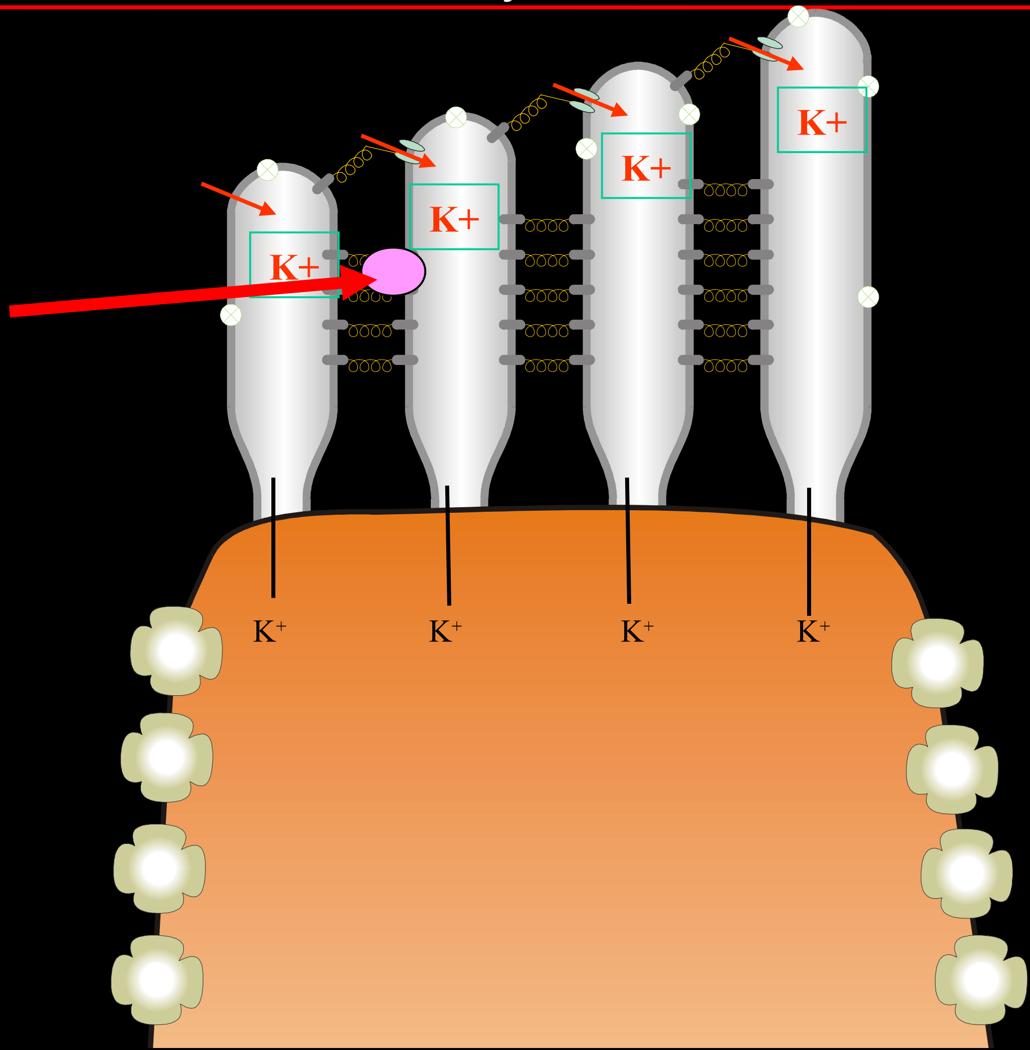


USHER Proteins Induce Hearing Loss and Blindness



Cadherin 23 → 20% of Usher 1D have Cad23 mutations
→ Usher 1D = RP early aldolence

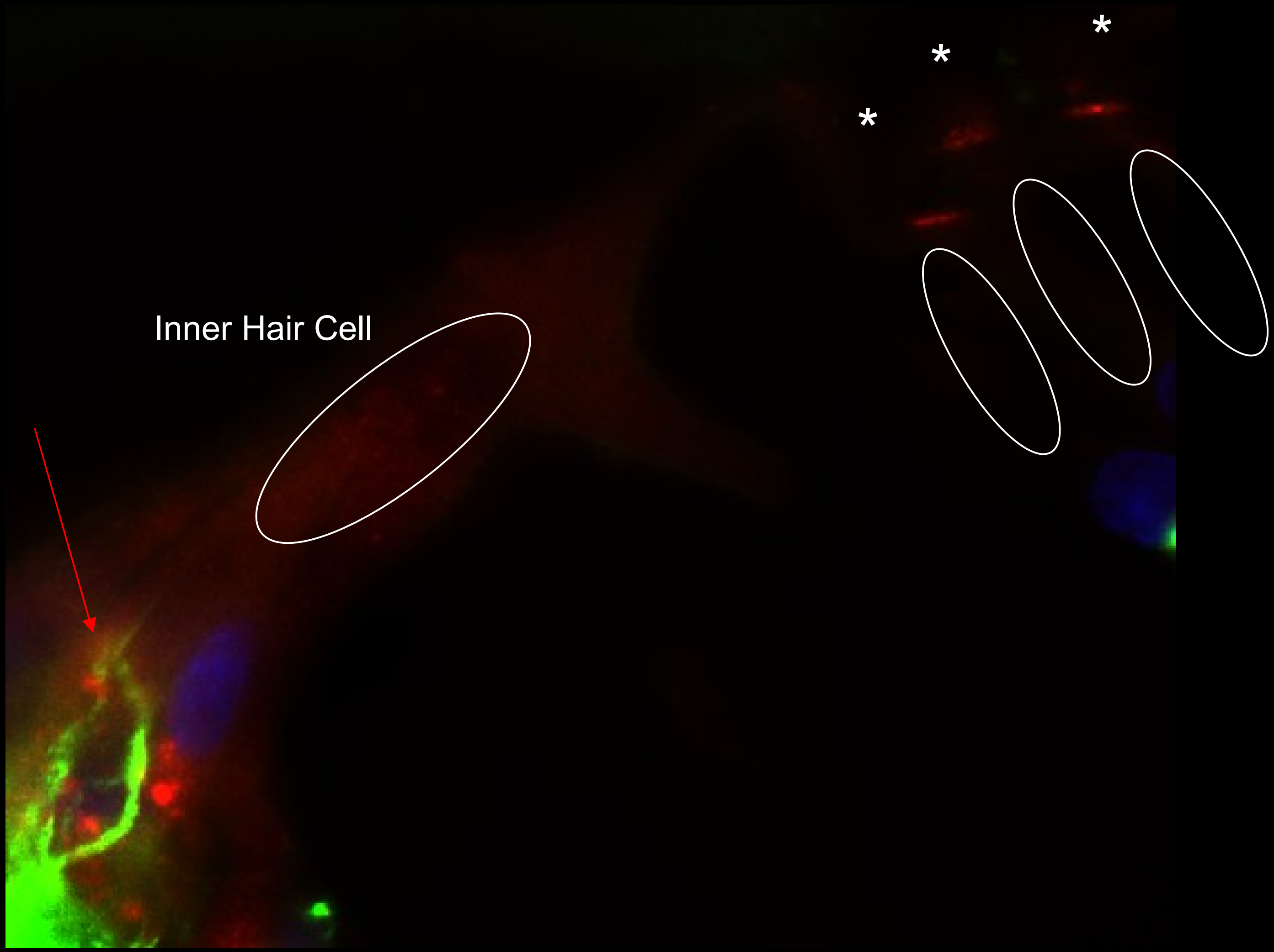
Cadh23
Pro-Cadh15



→(CDH23) and Procadherin 15 (PCH15) interact through N-terminal part and Ca^{2+} dependent (Mueller et al., Kachar, 2008)

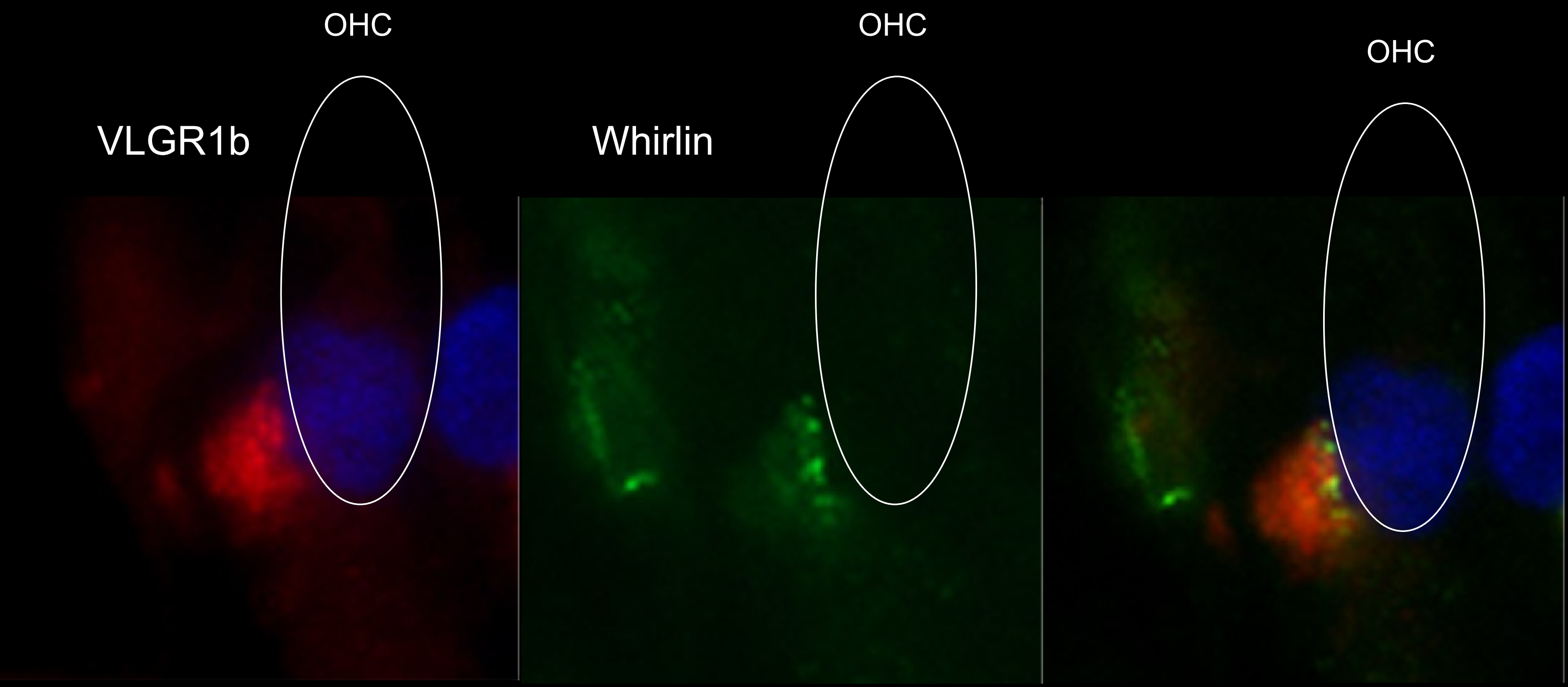
→ Oshima et al., Kimberling, 2008

USH2C (VLGR1b) in IHC Synapse



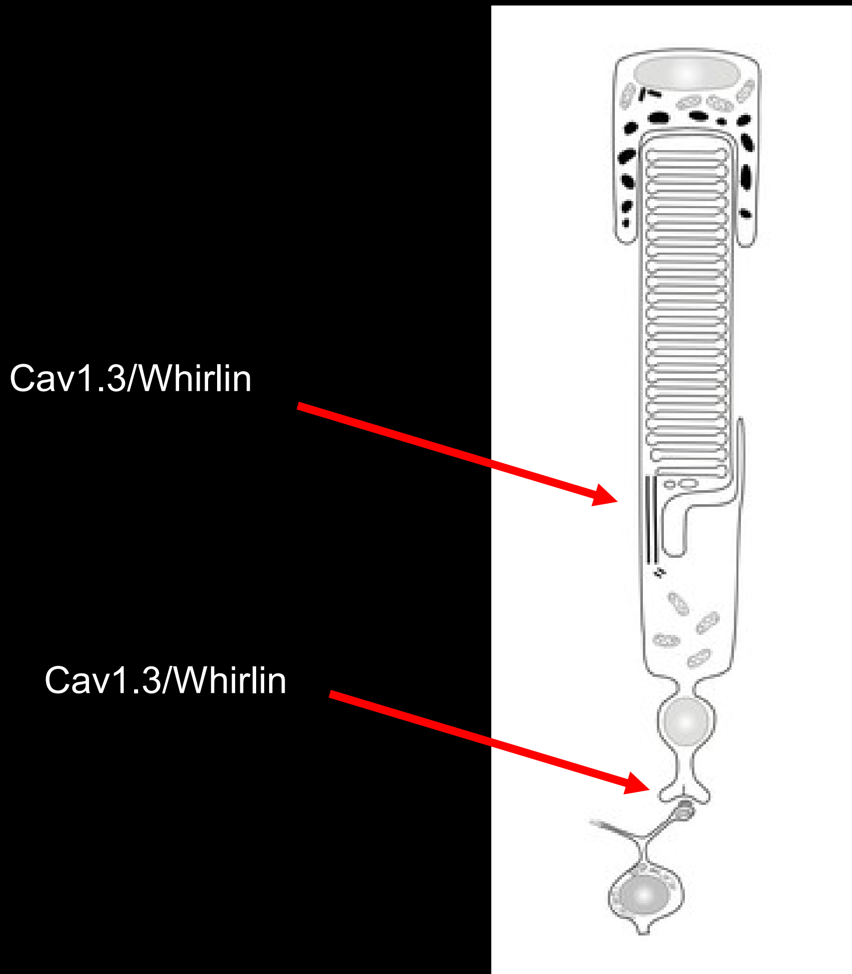
Reiner et al., Zimmermann, Knipper, Wolfrum (2006) Hum Mol Genet

USH2D (Whirlin) and USH2C (VLGR1b) in OHC Synapse



Van Wijk et al., Knipper, Kremer (2006) Hum Mol Genet

USH2D (Whirlin) colocalizes with Cav1.3 in photoreceptor cells

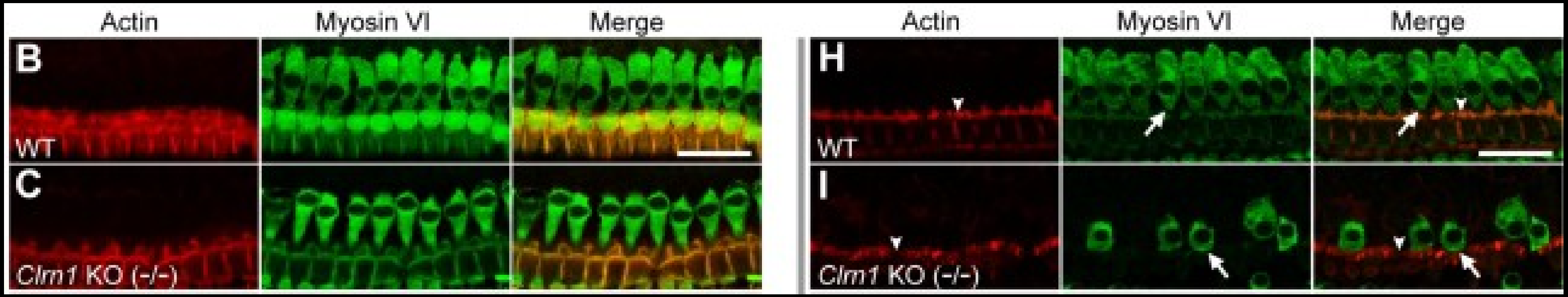


Invest Ophthalmol Vis Sci. 2009 ; ***Whirlin associates with the Cav1.3 ({alpha}1D) channels in photoreceptors, defining a novel member of the Usher protein network.***
Kersten et al. Kremer H.

USH3A (Clarin) → blindness in humans due to Müller Cell Dysfunction
→ progressiv hearing loss → Ribbons?

P15

P120

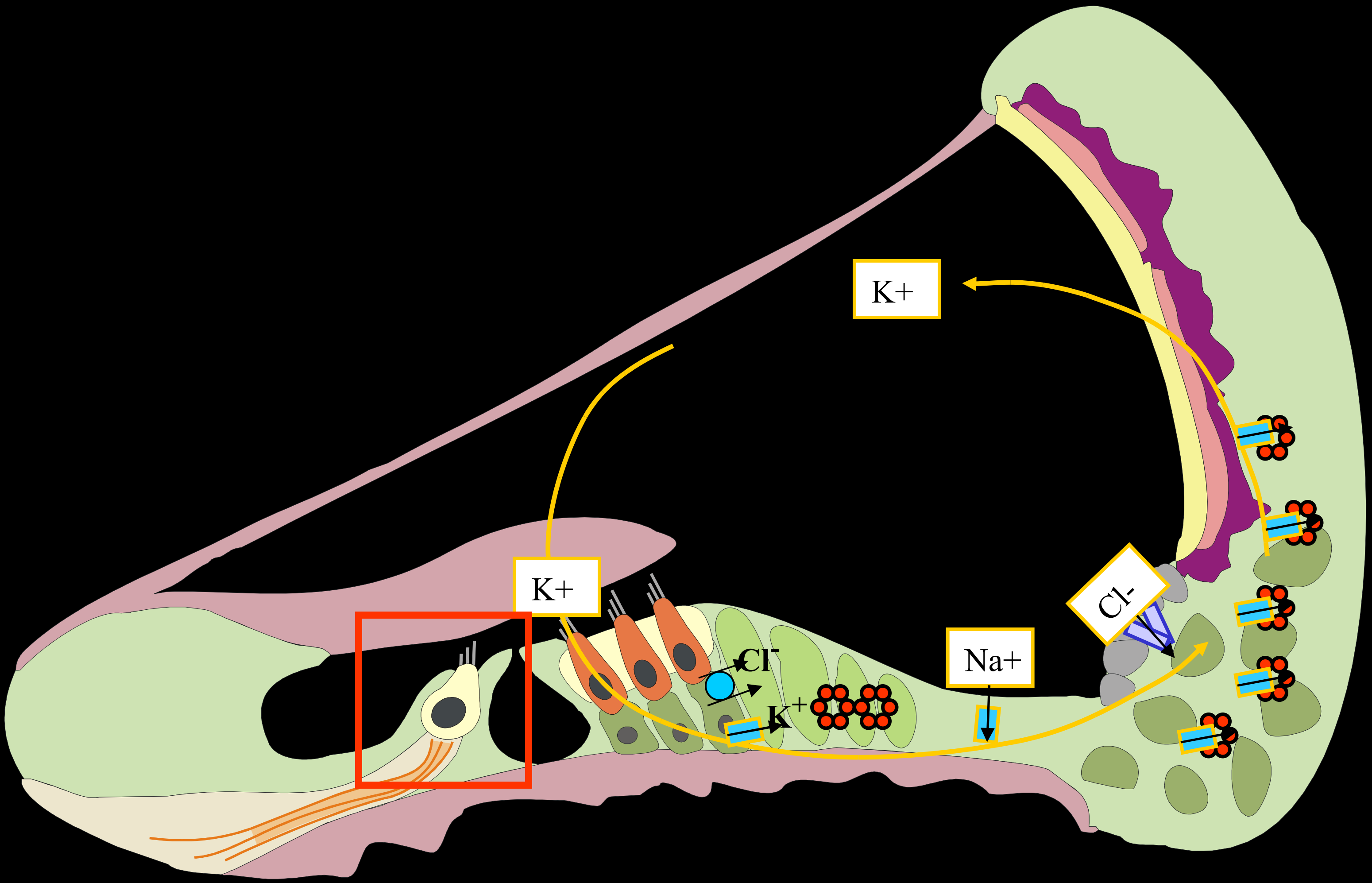


Zallocchi M, Meehan DT, Delimont D, Askew C, Garige S, Gratton MA, Rothermund-Franklin CA, Cosgrove D. Hear Res. 2009

CLRN1 is nonessential in the mouse retina but is required for cochlear hair cell development.
Geller SF, et al. Flannery JG.PLoS Genet. 2009.

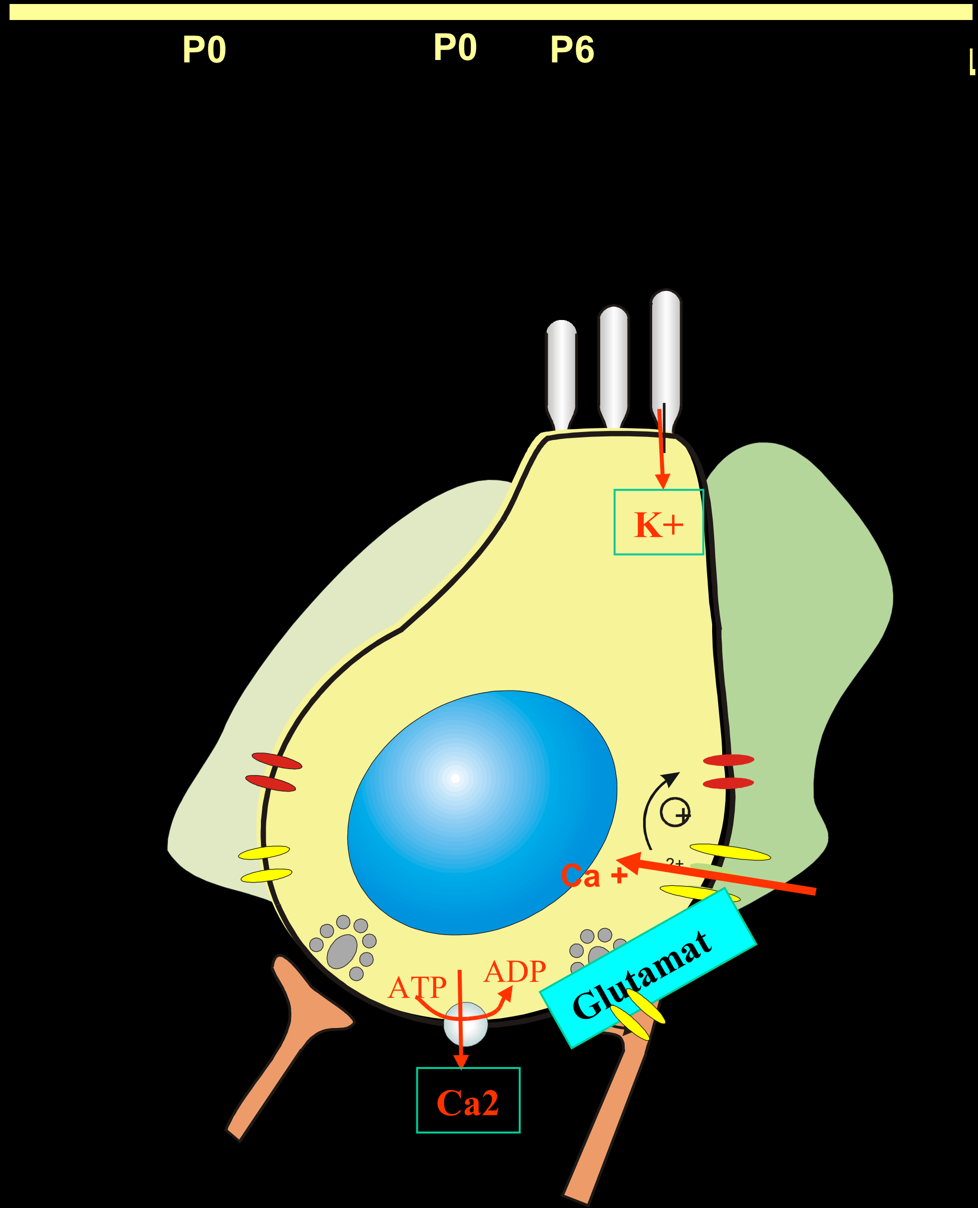
Summary I

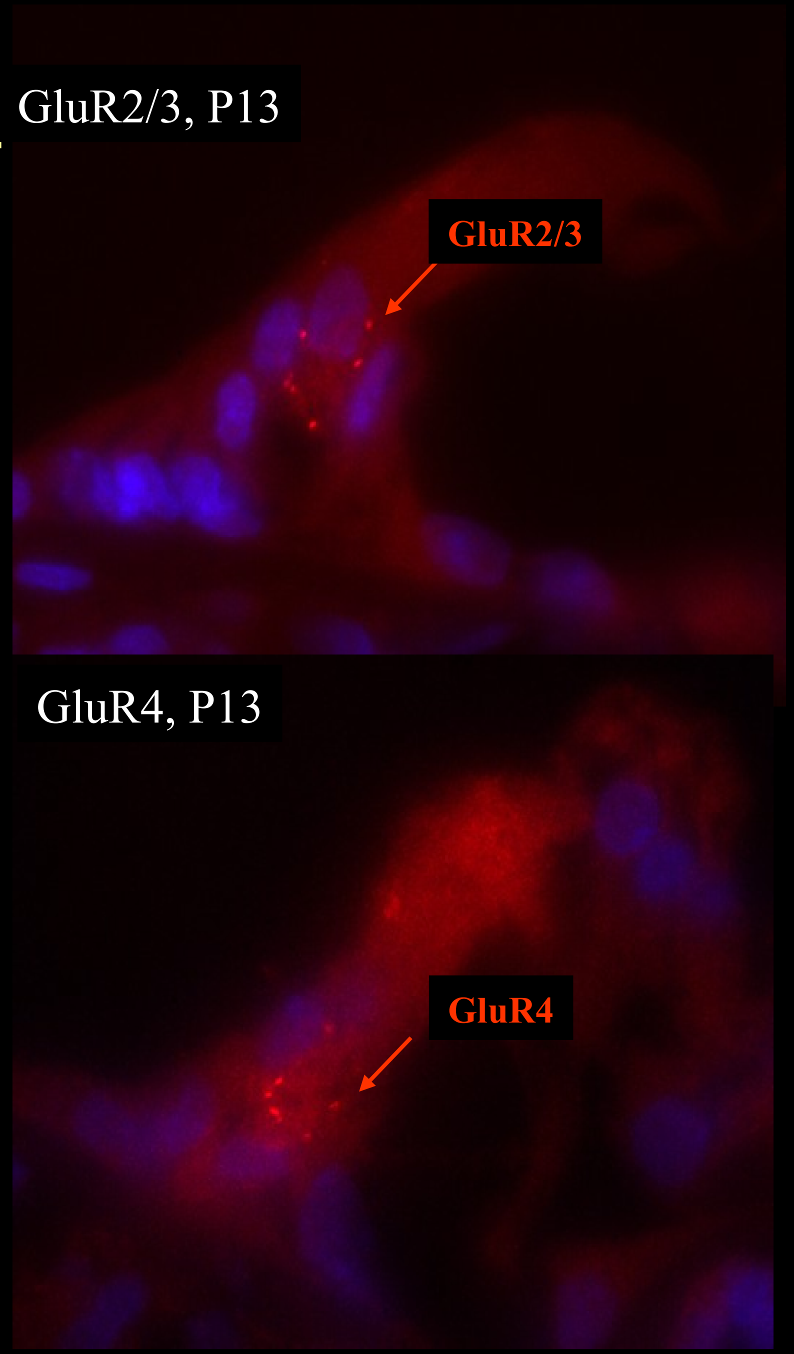
- Multiple Genes participate to the generation of the EP and cause deafness
- GJB 26, 30, 31 = 49 % of heriditary deafness
- PDS = Pendred Syndrom → Deafness (Cl- Transport, Kir4.1)
- KCNQ1/ KCNE1= Jervell-Lange-Nielsen Syndrom, progressiv Hearing loss
- TRP-protein = candidate Mechanotransducer= not found
- Cadherin 23 = 20% of Usher 1D
- USHC (VLGR1), USHD (Wirlin) and USH3A in Synapses or Ribbons

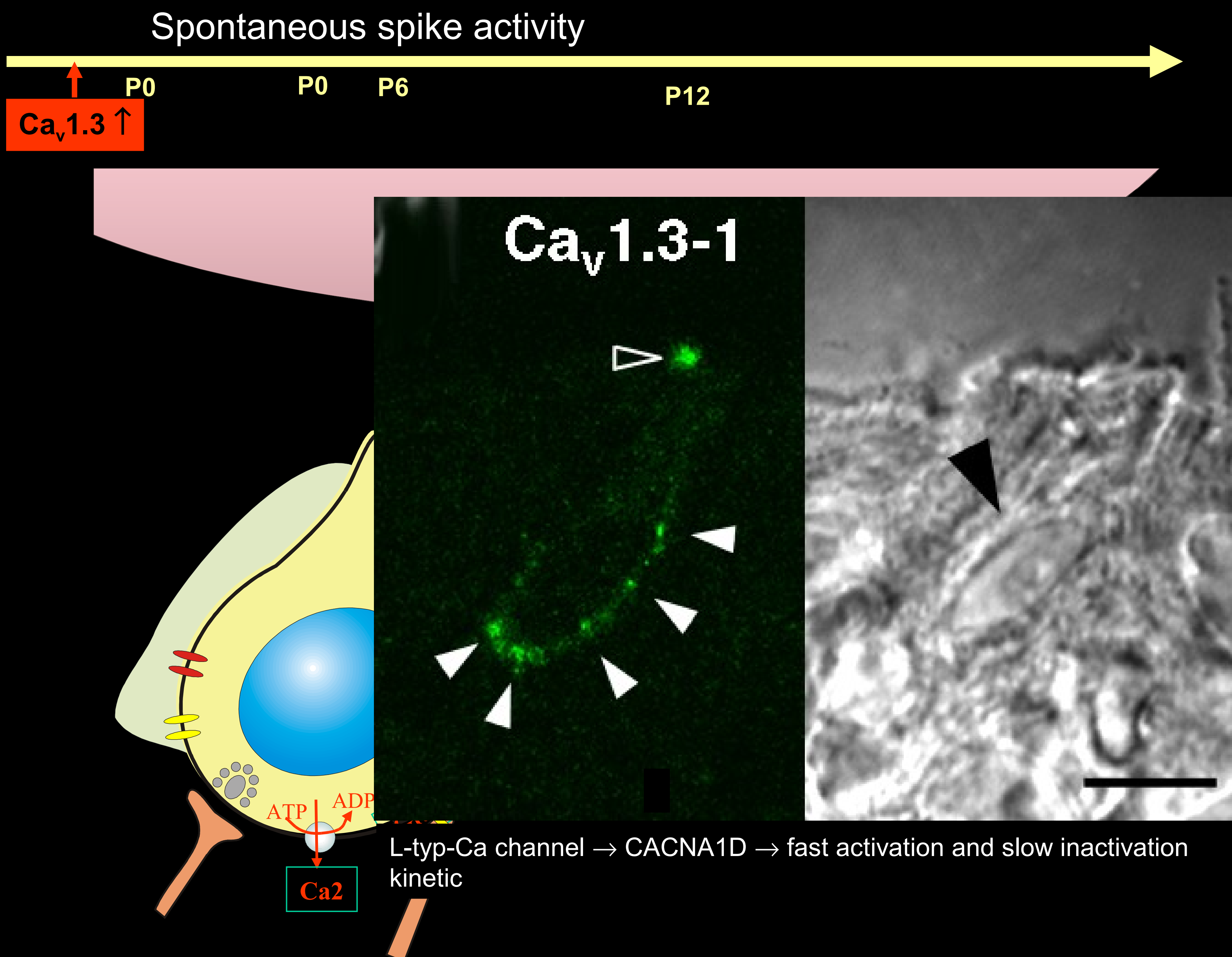


K+
K+
Cl-
K+
Na+
Cl-

Spontaneous spike activity

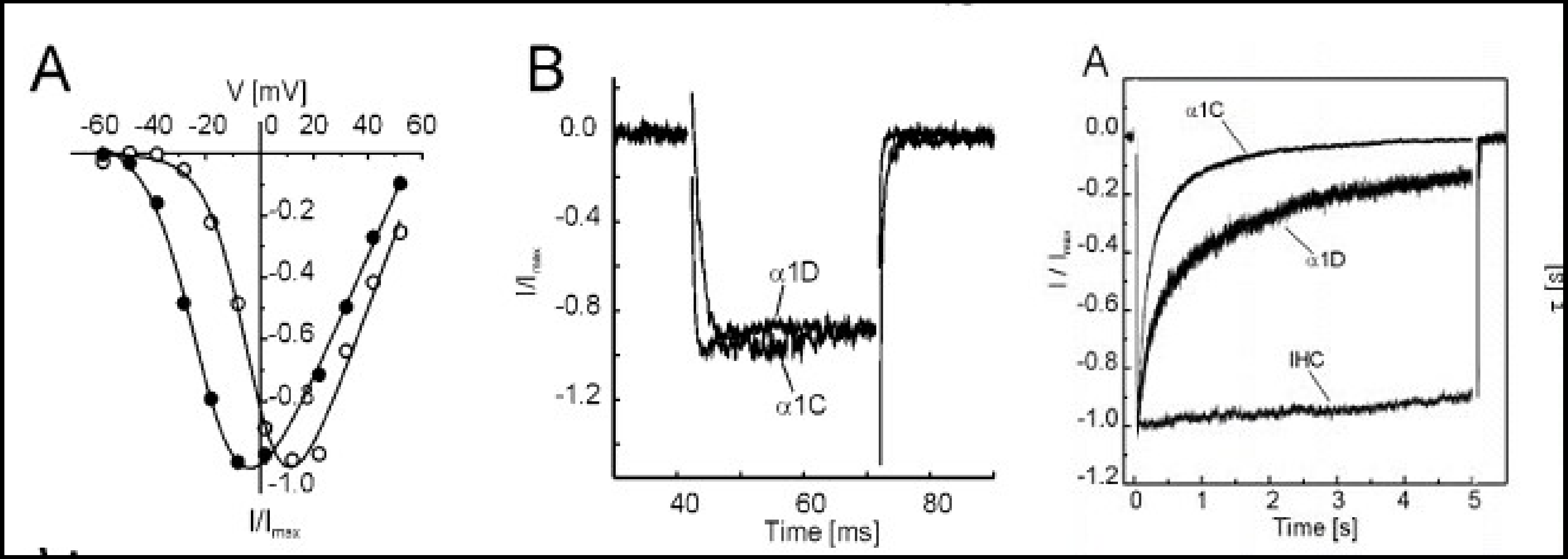






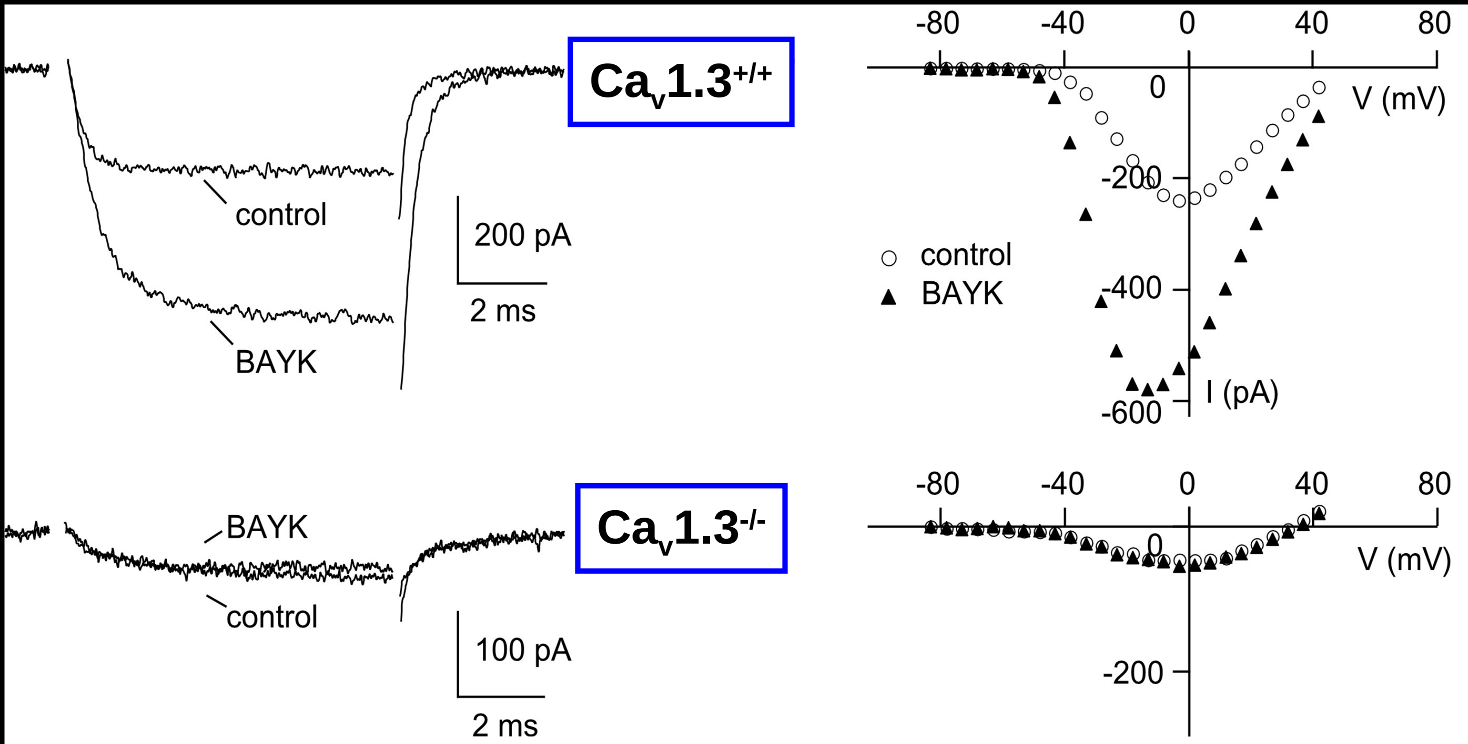
L-typ-Ca channel → CACNA1D → fast activation and slow inactivation kinetic

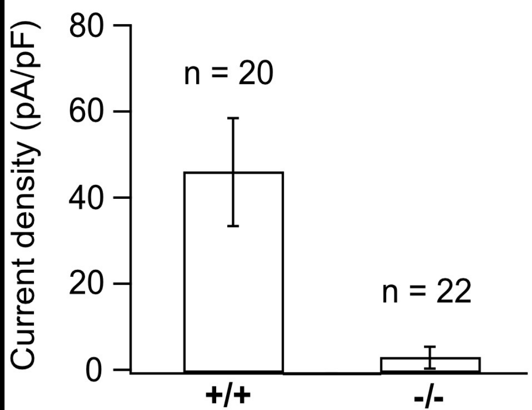
Cav1.3 exhibit negativ & fast activation and slow inactivation kinetic



Koschak et al., Striessnig, 2001, JBC

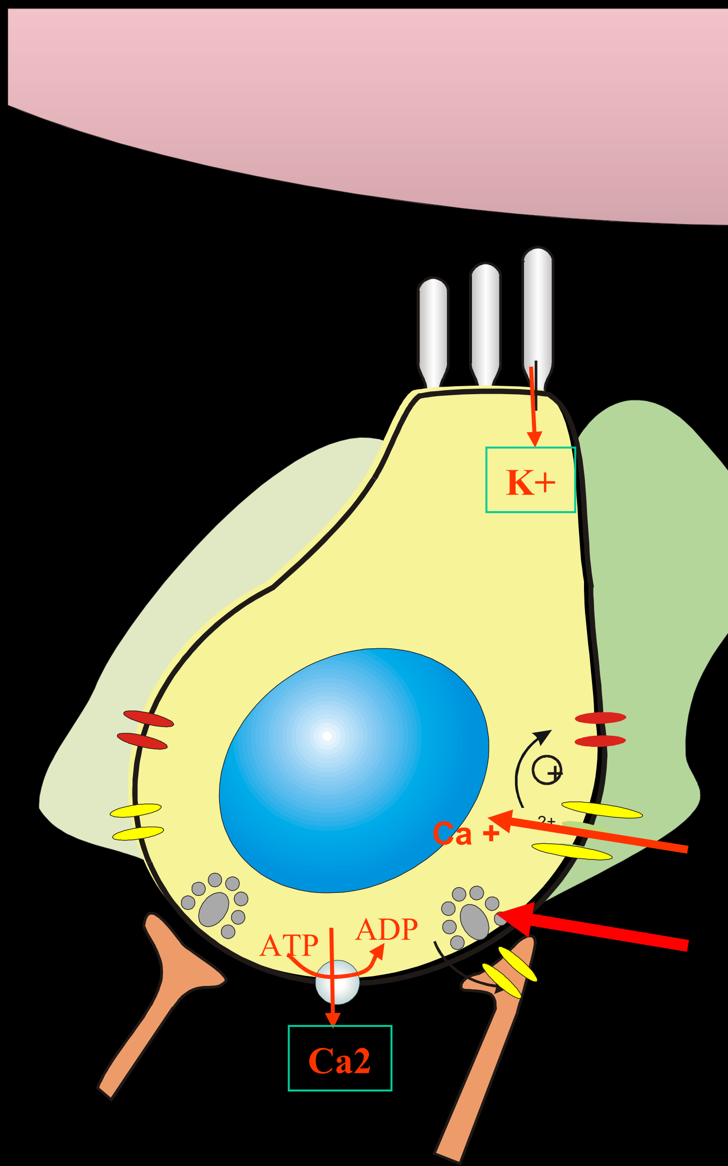
Cav1.3 Knockout Mice Mutants are Deaf



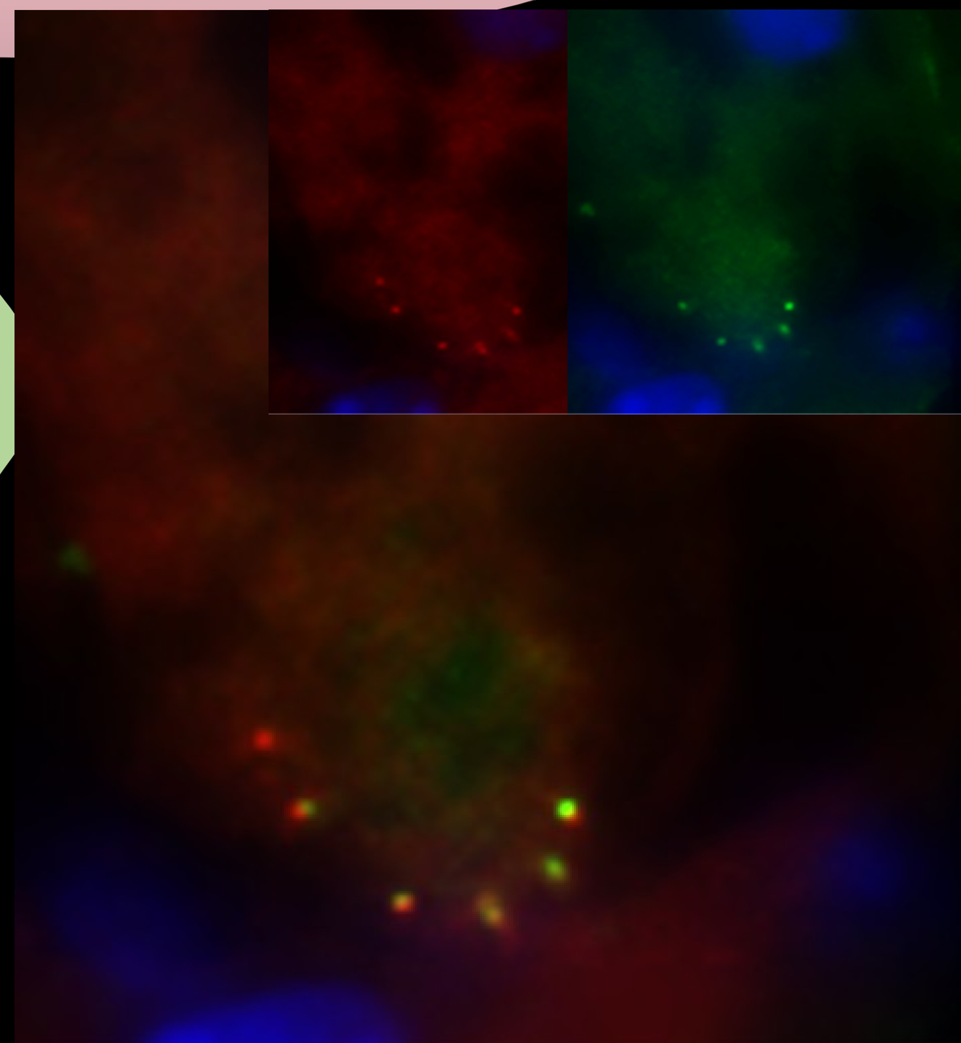


>90 % of Ca^{2+} currents in IHCs flow through Ca$_v$1.3 channels

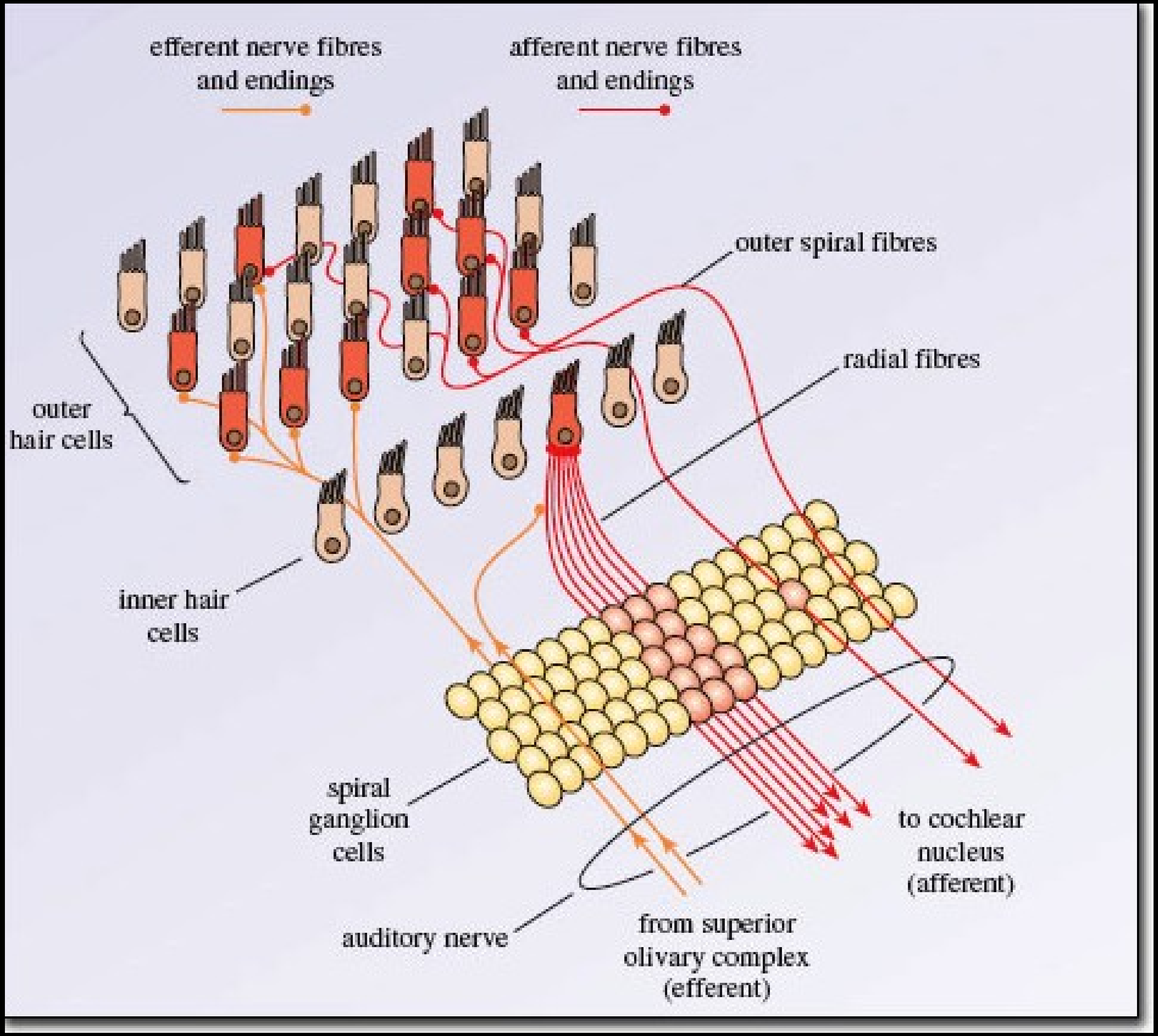
Platzer, Engel et al., Striessnig, Cell 2000

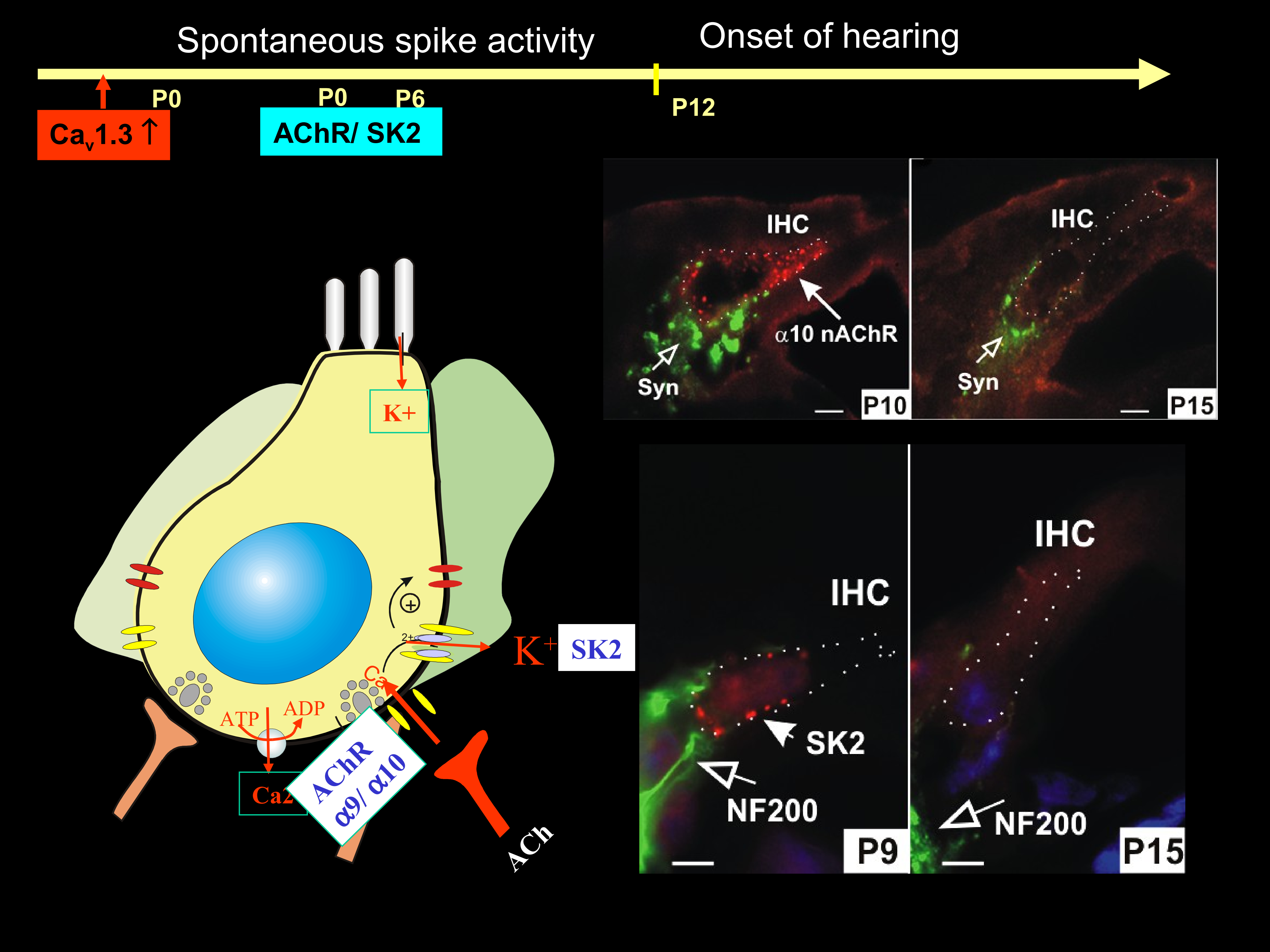


Cav1.3/ Ribbon (CtBP2)

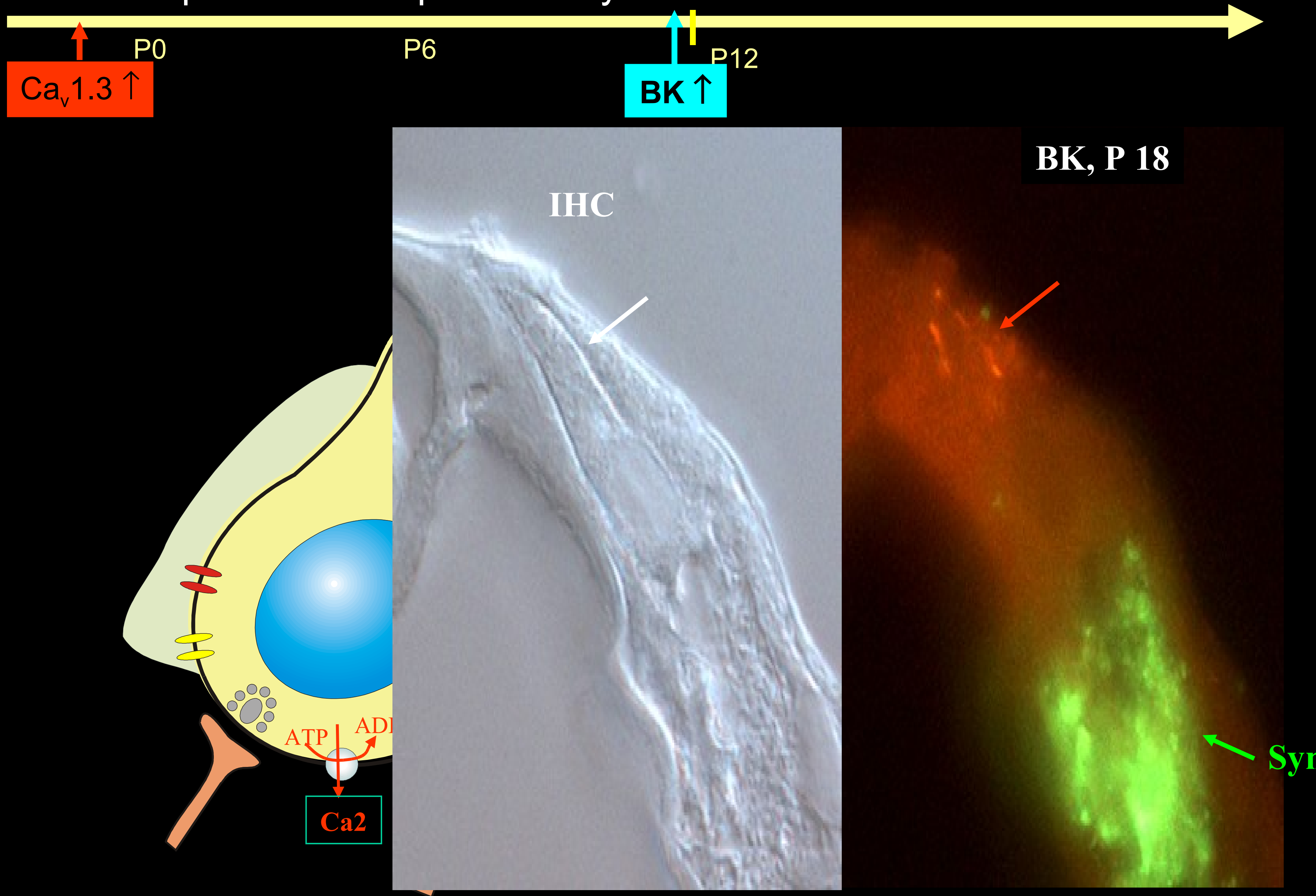


Around 8-30 ribbons correlate to 8-30 fibers that innervate a single IHC




Spontaneous spike activity
Onset of hearing
P0
Cav1.3 ↑
P0
P6
AChR/ SK2
P12
IHC
α10 nAChR
Syn
P10
IHC
Syn
P15
K+
ATP
ADP
Ca2
AChR
α9/ α10
K+
SK2
ACh
IHC
SK2
NF200
P9
IHC
NF200
P15

Spontaneous spike activity



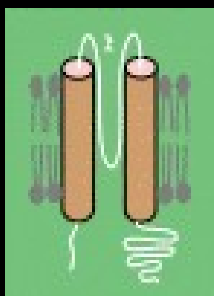
Onset of hearing

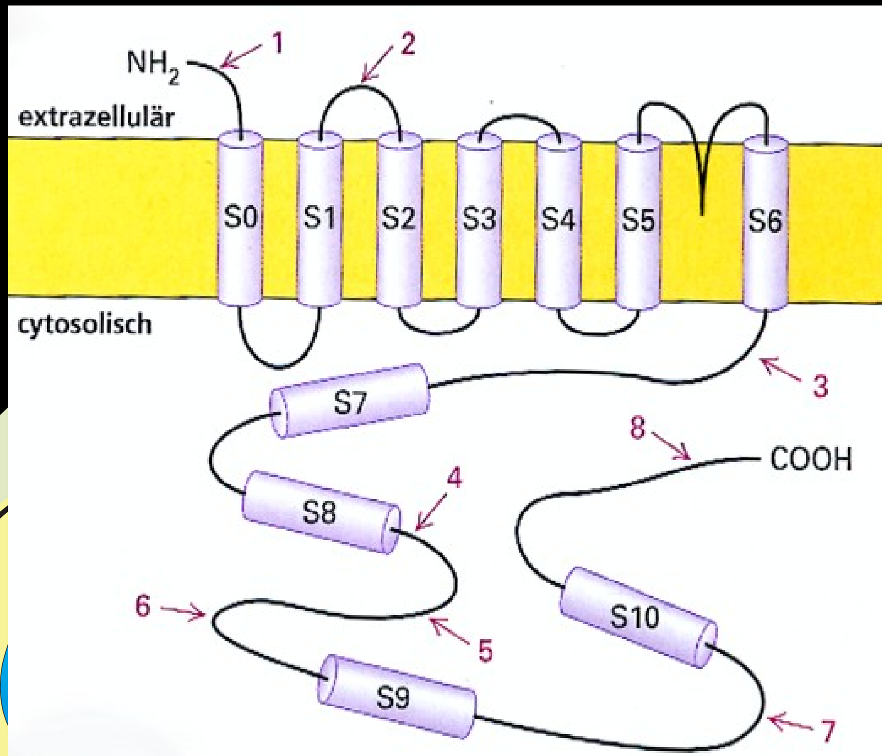
P0 P6 P12

$Ca_v 1.3$ ↑

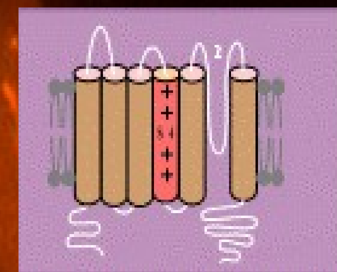
BK ↑

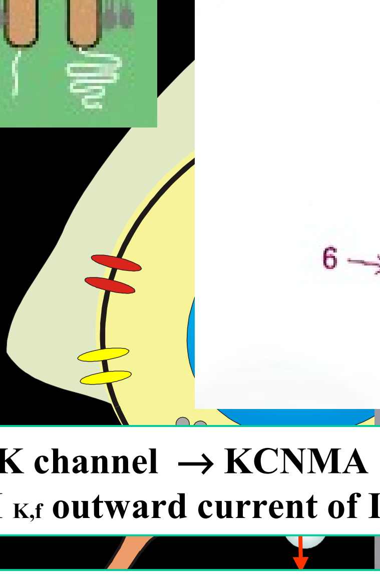
´BK ß subunit





´BK slo alpha subunits

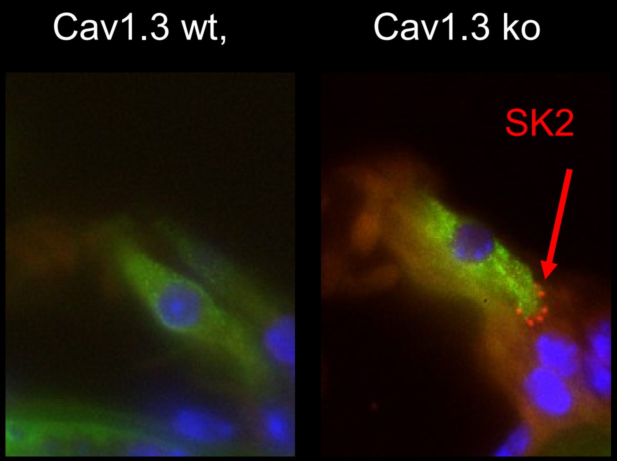


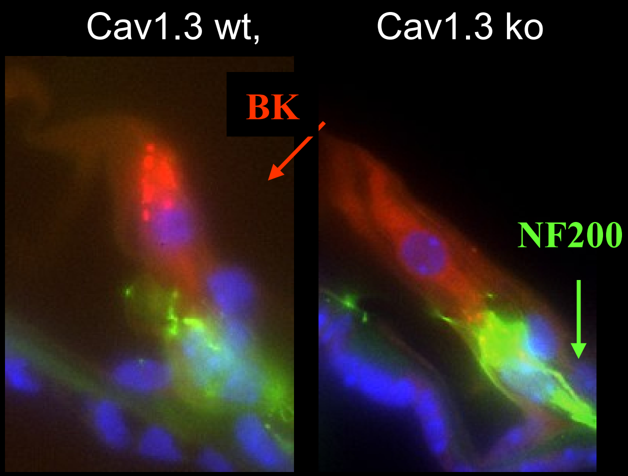


Ca2+ BK channel → KCNMA 1 (slo α or BK or maxi K channel Gen)→
→ fast $I_{K,f}$ outward current of IHCs → causes transmitter release to be phase-locked (Kros et al., 1998)

→ multiply splicing
→ forms heterooligomeric complexes with KCNMB1-B4 → sloß
→ electrical tuning in lower vertebrates

Maturation of IHCs (normal hearing) requires BK ↑ SK2 ↓

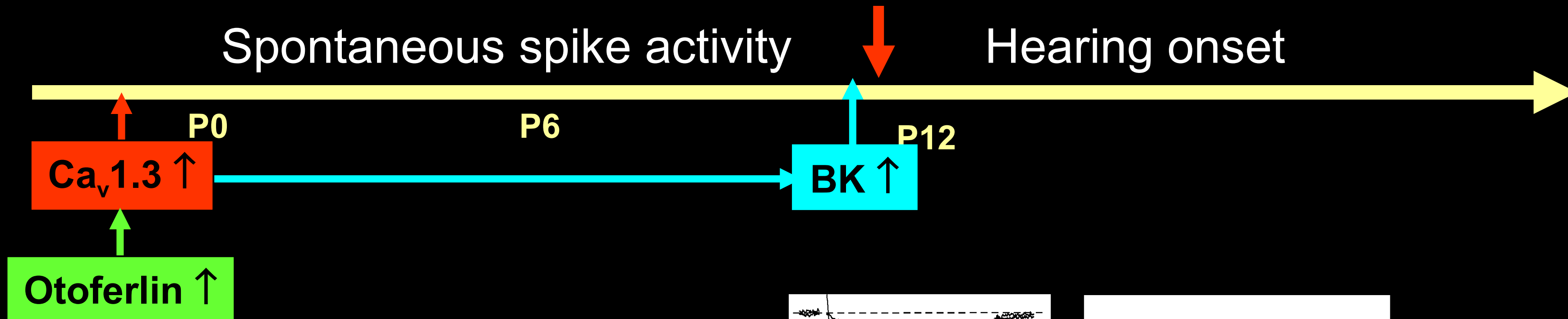


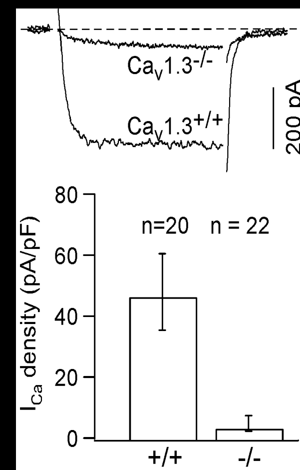


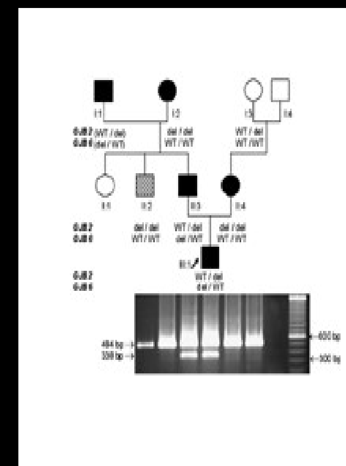
Cav1.3 ↑
↓
Spontaneous and Depolarization-Induced Ca^{2+} Action Potentials
↓
Normal Synapse Formation in the Central Auditory System

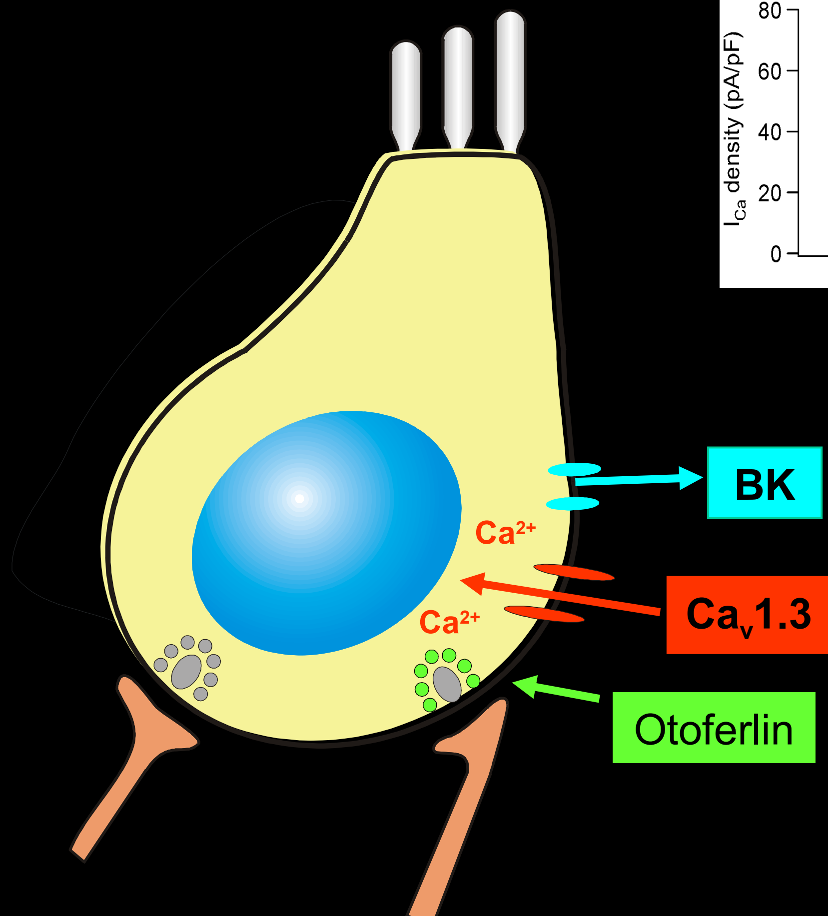
→ **BK ↑ SK2 ↓** →

Stimulated Graded Receptor Potentials
↓
Normal Hearing









Progredient Hearing Loss in BK Channel Deficient Mice (Rüttiger et al., Knipper (2004) PNAS

Congenital Deafness in Cav1.3 Deficient Mice
Platzer, Engel et al. (2000) Cell

Otoferlin Missense Mutation in Deaf Patients
Mirghomizadeh et al., Pfister, Blin (2002) Neurobiol Disease

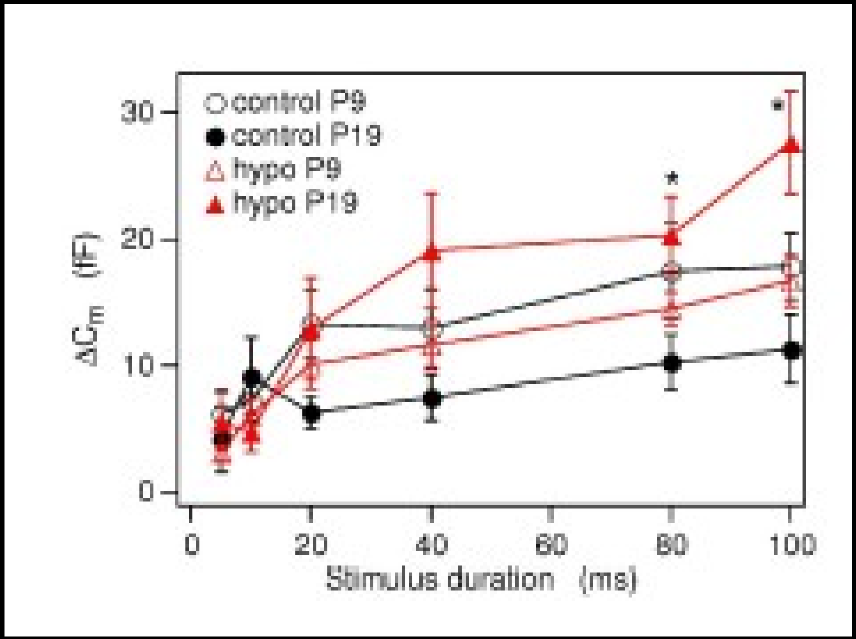
Otoferlin, a protein essential for exocytosis at the auditory ribbon synapse

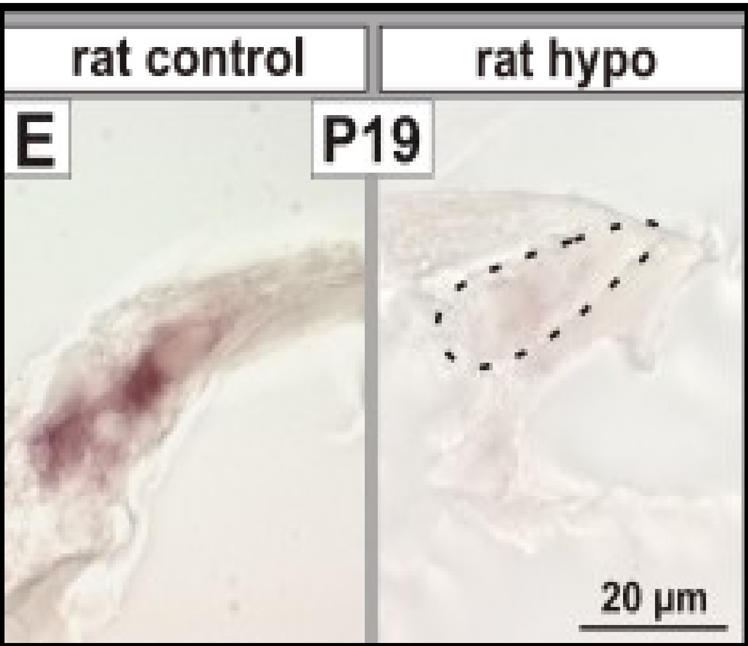
Cell, 2006 Oct 20;127(2):277-89.

Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse.

- **Roux I, Safieddine S, Nouvian R,Grati M, Simmler MC,**
- **Bahloul A, Perfettini I, Le Gall M, Rostaing P, Hamard G, Triller A,**
- **Avan P,Moser T, Petit C.**

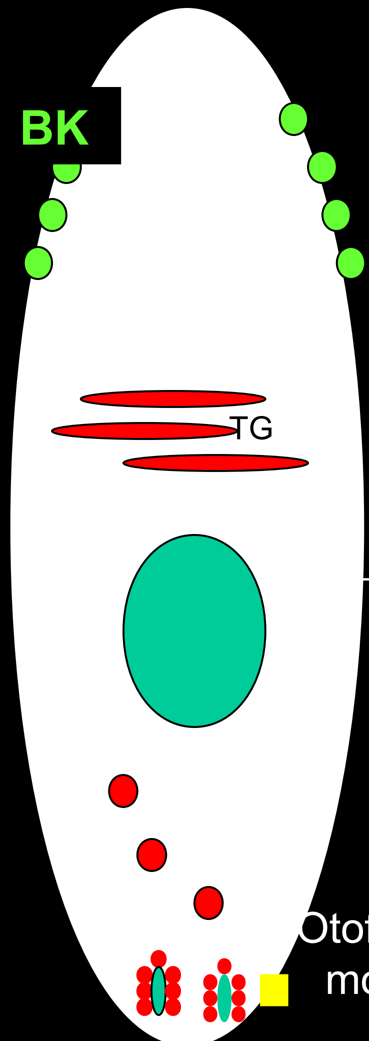
In Contrast: Without Otoferlin exocytosis can still occur in IHCs





Brandt et al., Engel, 2007, J Neurosci
Heidrych et al. Knipper, 2009, Hum Genet

Otoferlin: Candidate gene for replenishment of vesicles/priming acting with MyosinVI



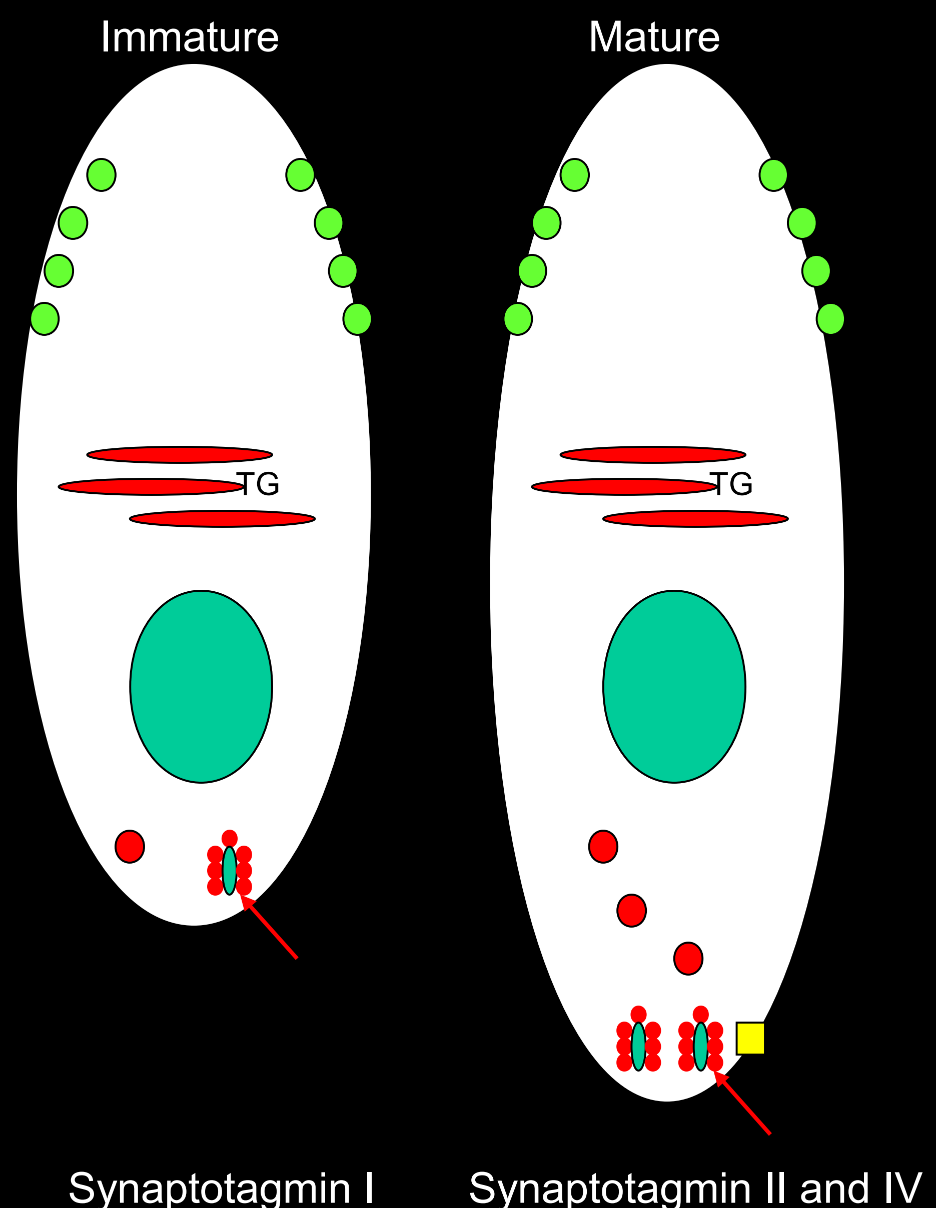
Myosin VI independent of otoferlin
$\rightarrow$ Targeting of BK to TG

Otoferlin & Myosin VI (SI-NI)
$\rightarrow$Targeting of endocytotic compartments to TG

Otoferlin independent of myosin VI $\rightarrow$ Positioning of molecular primed vesicles to ribbons: Exocytosis

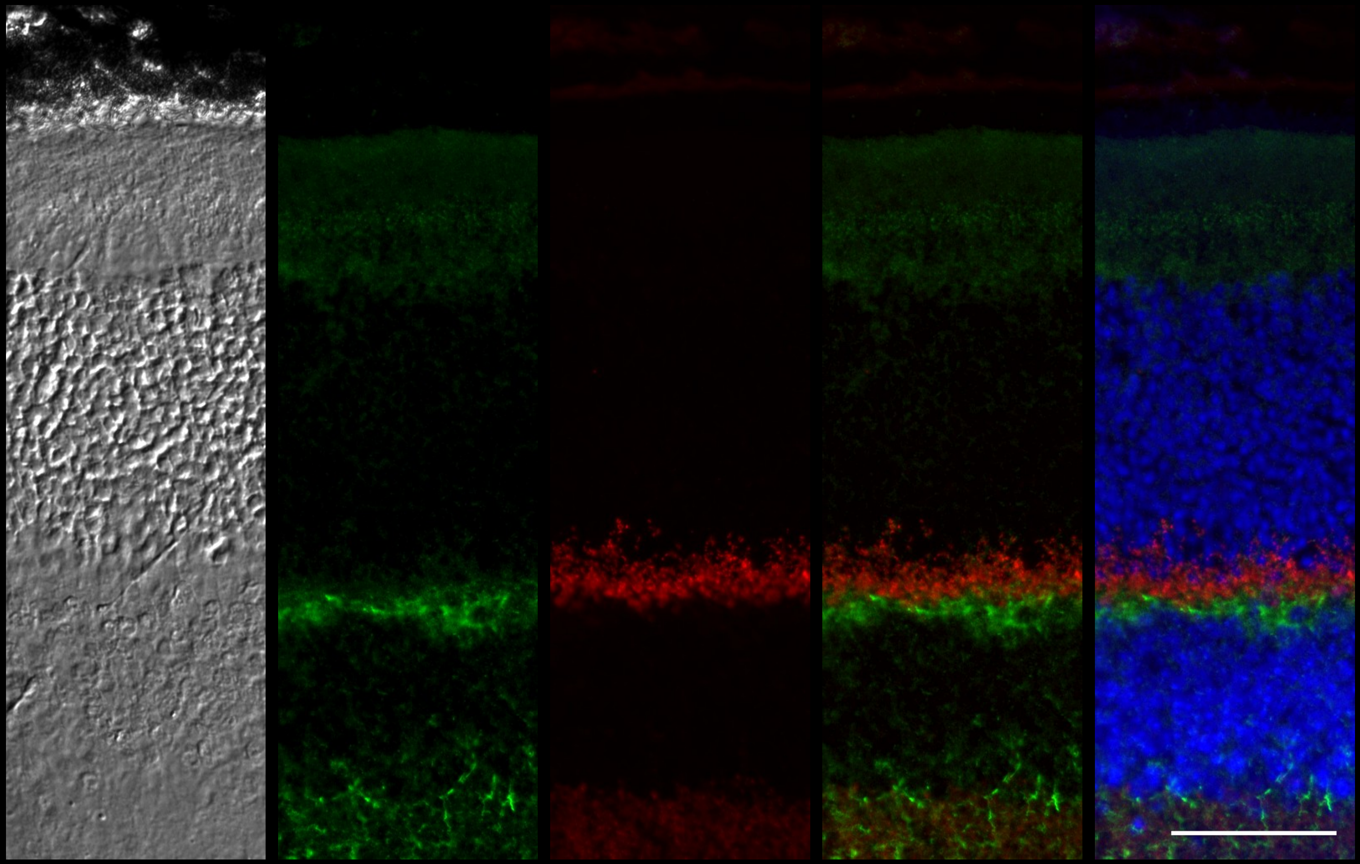
Heidrych et al. Knipper, 2009, Hum Genet

Synaptotagmin I, II and IV are expressed in IHCs



Johnson, Franz et al., Knipper, Marcotti, Nat Neurosc 2009

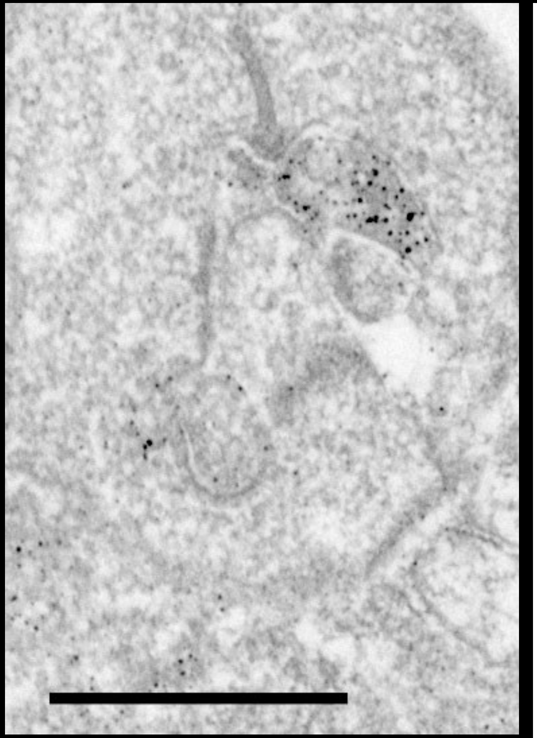
Otoferlin: in the Retina

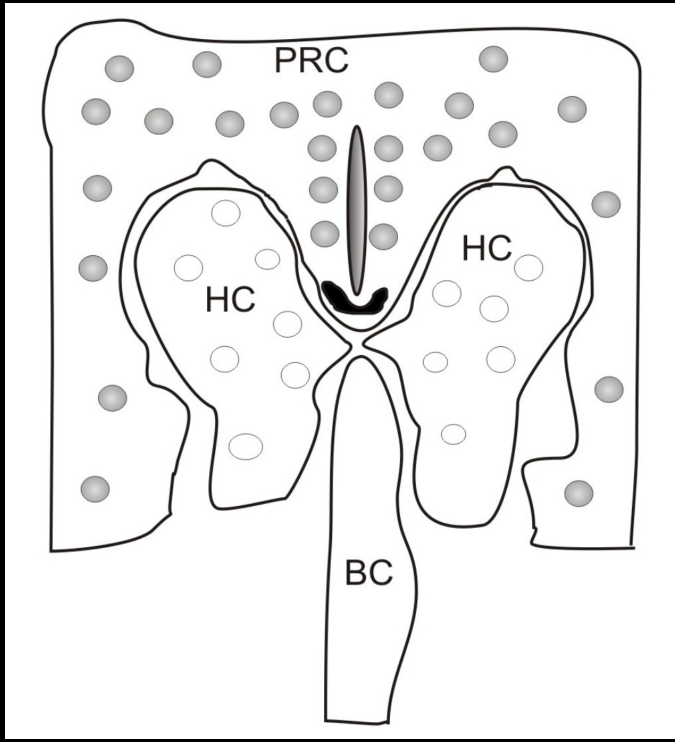


DIC otoferlin Ribeye otoferlin Ribeye otoferlin Ribeye DAPI

Wolfrum, Franz et al. Knipper, in preparation

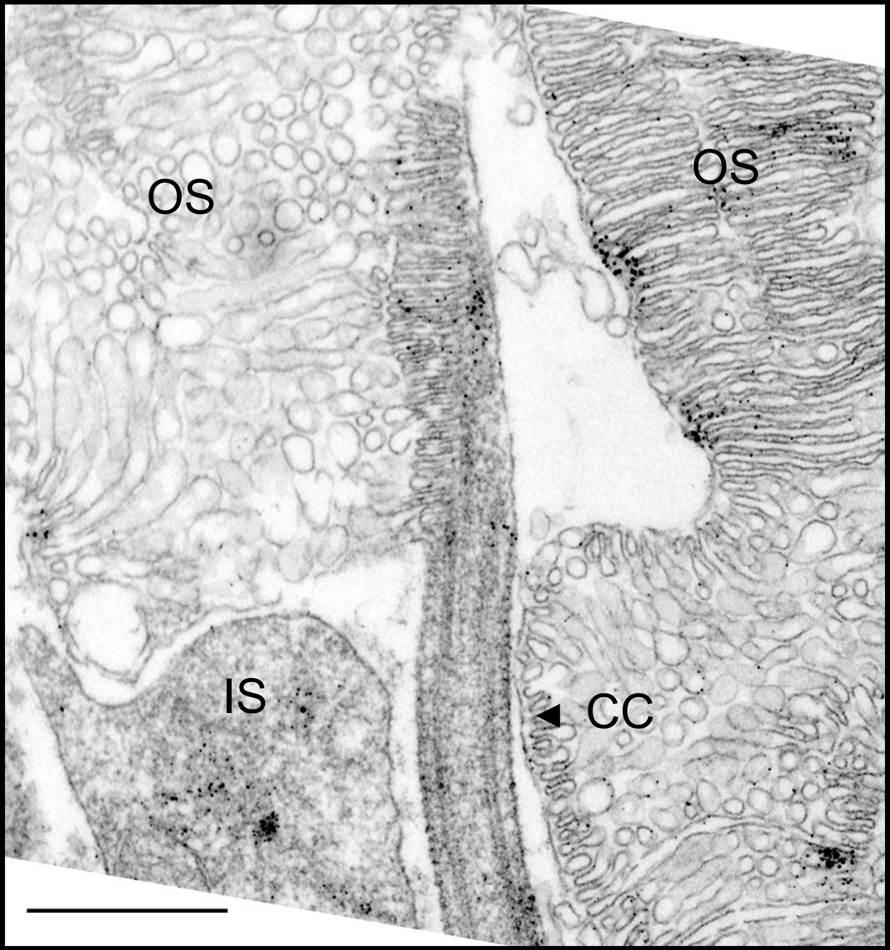
Otoferlin: in the Retina





Wolfrum, Franz et al. Knipper, in preparation

Otoferlin: in the Retina

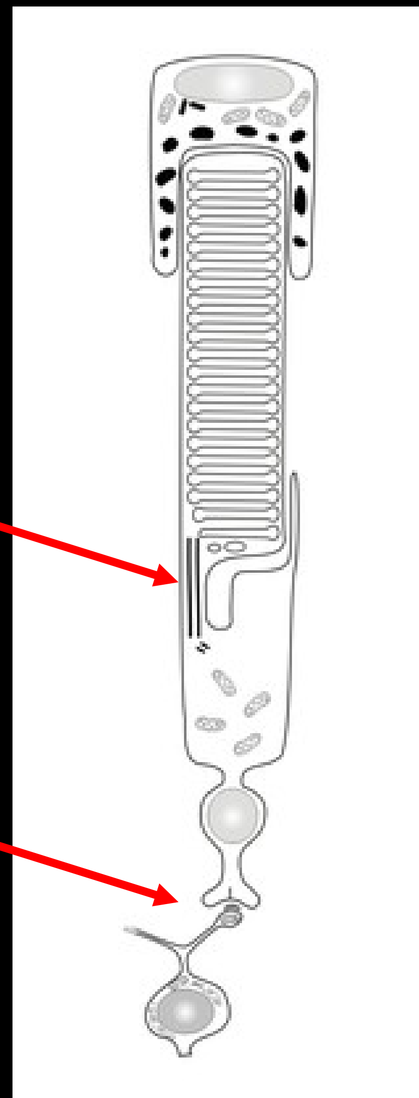


Wolfrum, Franz et al. Knipper, in preparation

Otoferlin: in the Retina

Otoferlin

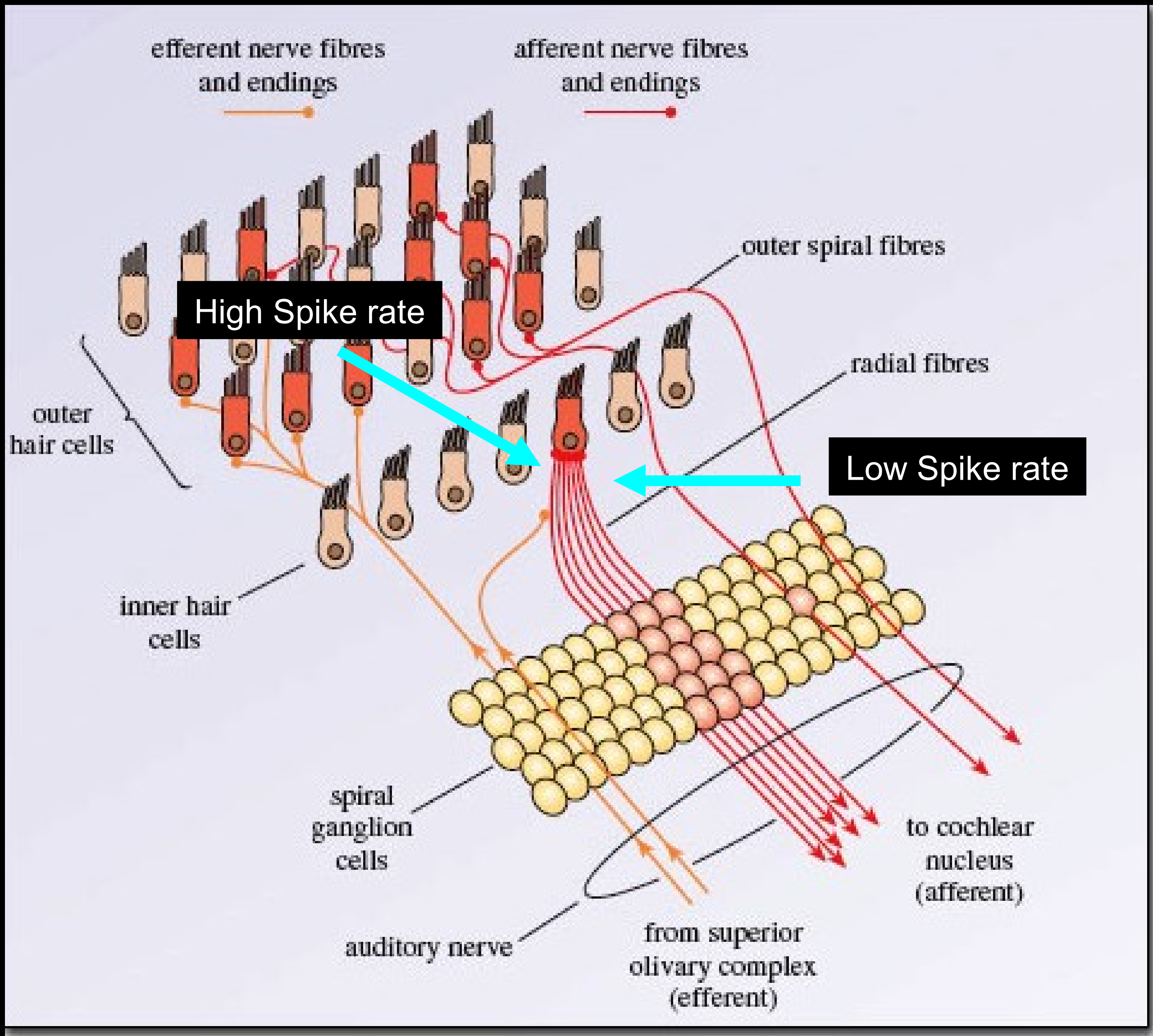
Otoferlin



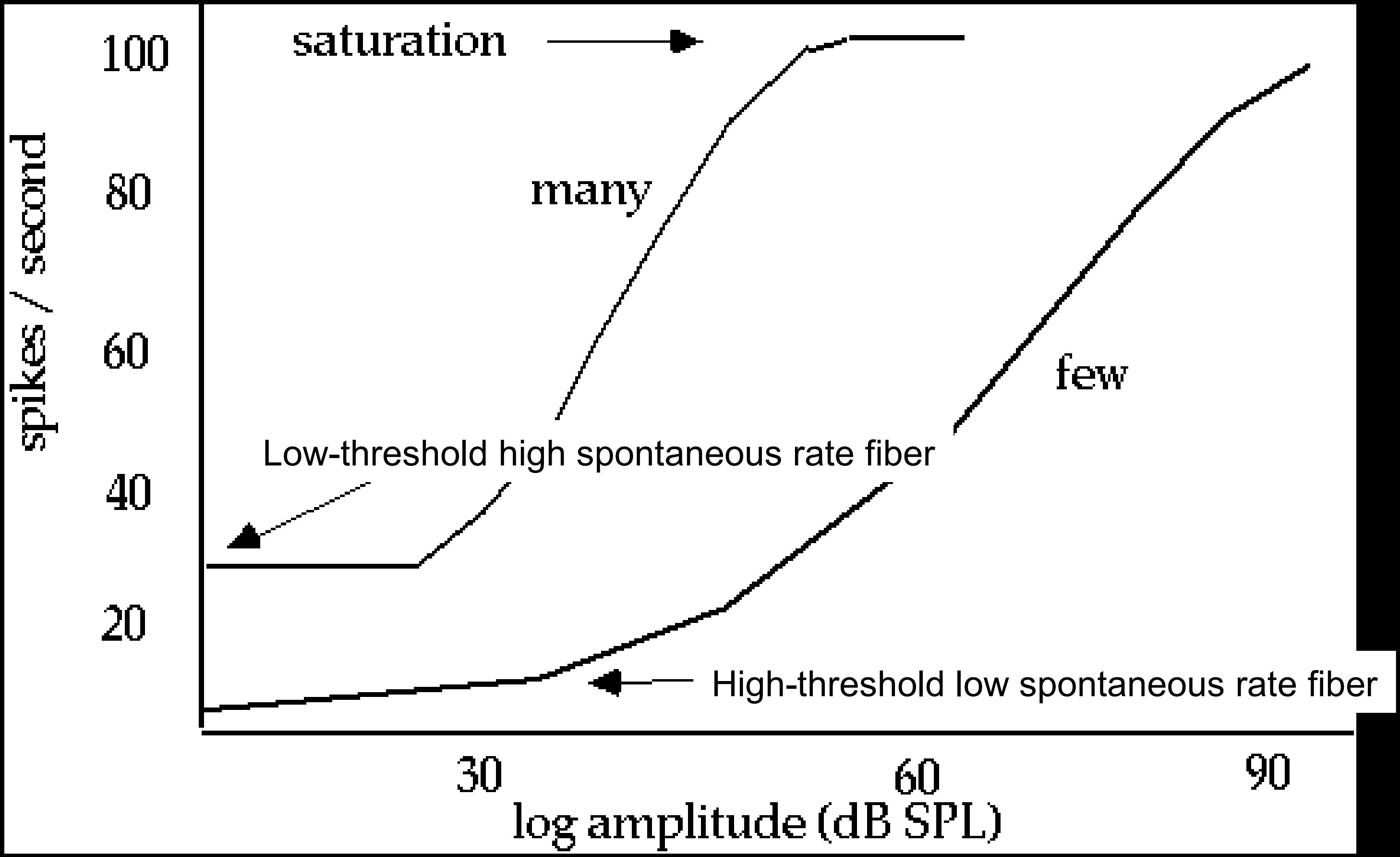
Preliminary data:
In preparation

Wolfrum, Franz et al. Knipper, in preparation

Spontaneous Spike Rate of AN fibers differs



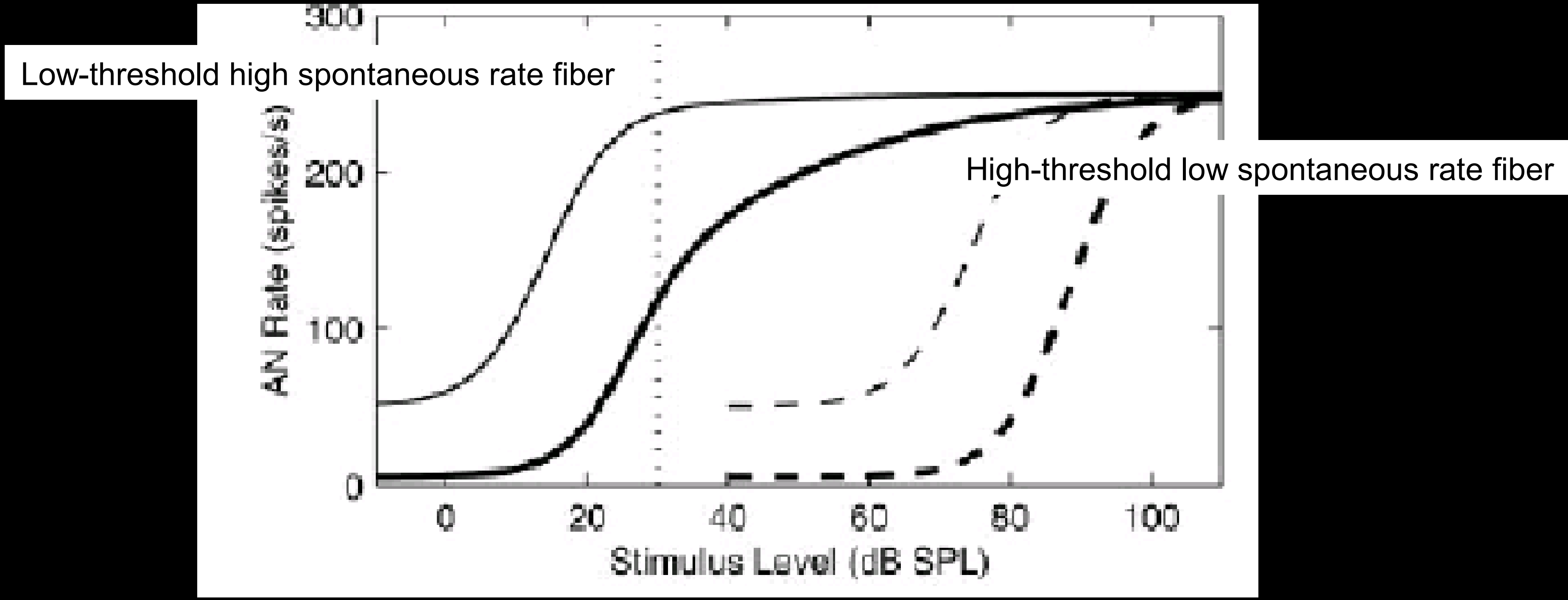
The sensitivity of AN fibers for SPL- levels decreases with spike rate



Low spike rate = High Sound Threshold = unsensitive
High spike rate = Low Sound Threshold = sensitive

Heinz and Young 2004

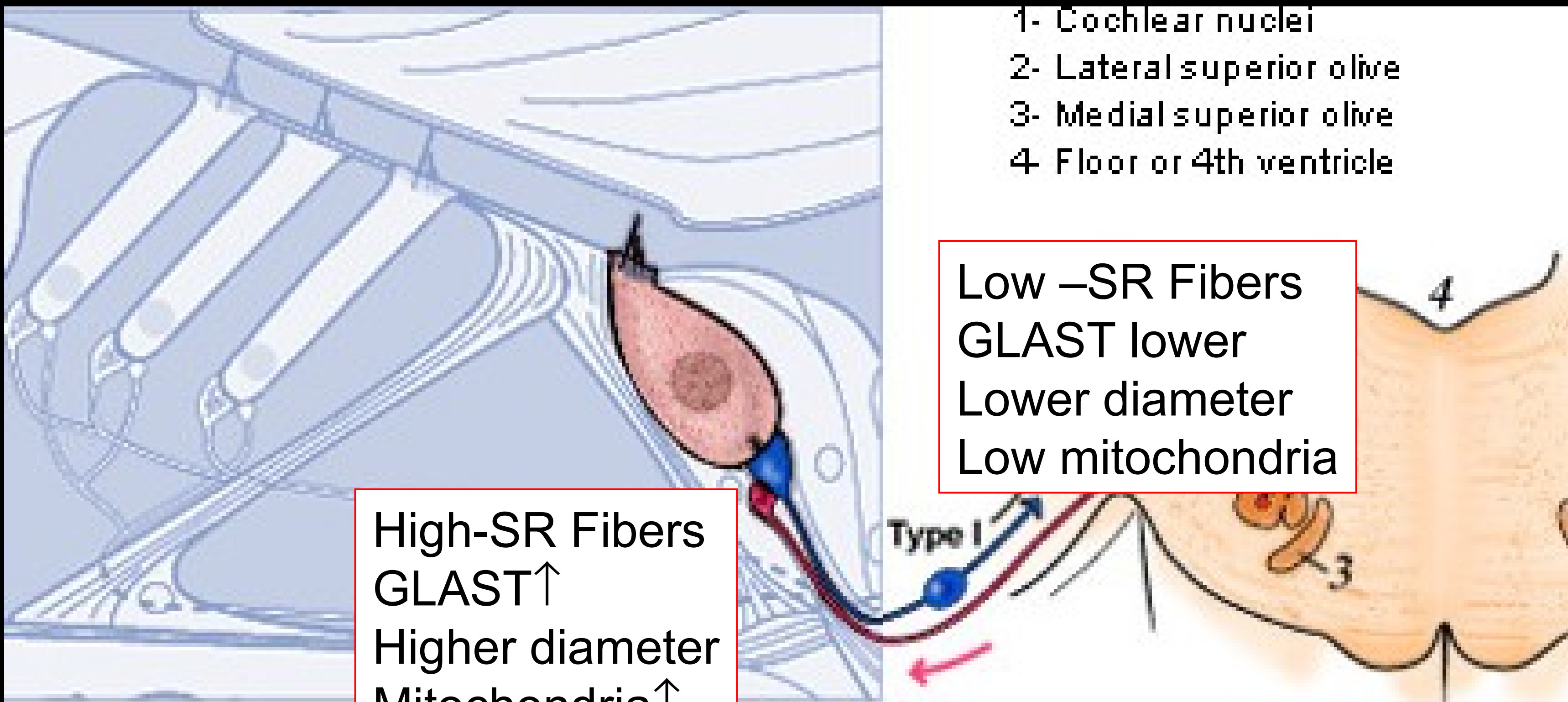
Middle – low SR fibers but not High SR participate to Loudness Sensation



Low spike rate = High Sound Threshold = unsensitive
High spike rate = Low Sound Threshold = sensitive

Heinz and Young 2004

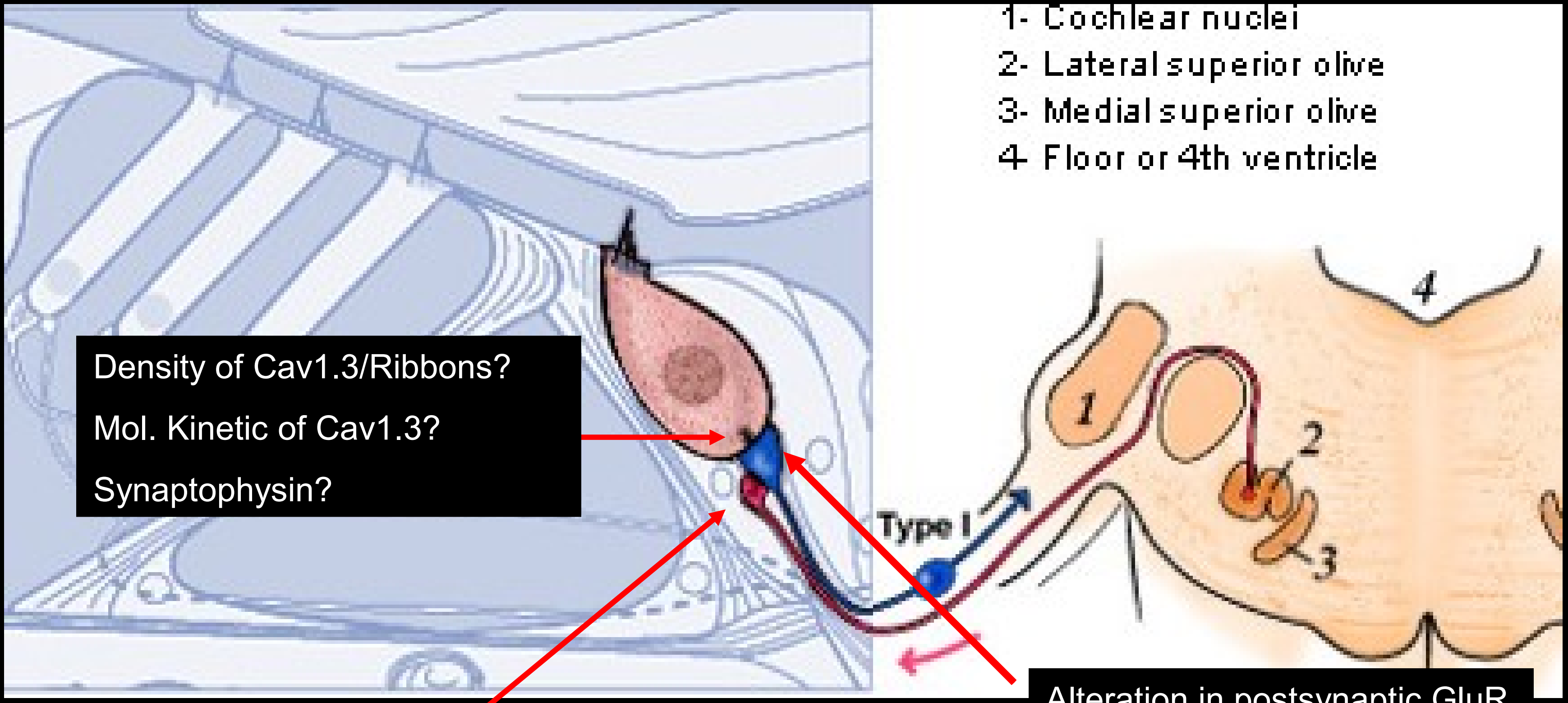
Middle / Low SR- = Modiolar Side; High – SR = Pillar Side



Low –SR Fibers
GLAST lower
Lower diameter
Low mitochondria

High-SR Fibers
GLAST↑
Higher diameter
Mitochondria↑

What defines Spike Rate is elusive (Pre-Postsynapse or Efferents?)



Summary II

- Glutamate Receptors GluR2/3 and GluR4 trigger postsynaptic TM release at the base of IHCs
- Cav1.3 triggers spontaneous TM release prior to hearing function
- Cav1.3 carries 90% of Ca-current in IHCs
- Cav1.3 is characterized by neg. fast activation and slow inactivation kinetic
- Cav1.3 triggers maturation of inner hair cells by BK ↑ and SK2 ↓
- ~8-20 Ribbons (Ribeye positive) correlate 1:1 to 8-20 auditory fibers
- Auditory fibers exhibit different spike rates (5-100)
- Auditory fibers exhibit different sensitivity for sound thresholds (different dynamic ranges)
- Low spikeing are insensitive (ca 40 dB), low GLAST, low mitochondria
- High spikeing are sensitive (ca 10 dB), high GLAST, high mitochondria