Pathology of the Ear: Genetics

- N. Blin
  Inst. of HumanGenetics

Genetics

- From Greek „geneá“ or „geneticos“ (***origin***)
- Science of heredity and variation including structure and function of hereditary material and its fate through generations
- term introduced by William Bateson in 1906

- Alkmaion (500 BC): offspring by mixing paternal and maternal „semen“ (that is produced in the brain, migrating to gonads)
- Hippon, Anaxagoras (400 BC): only male contribution
- Anaxagoras: „preformation hypothesis“

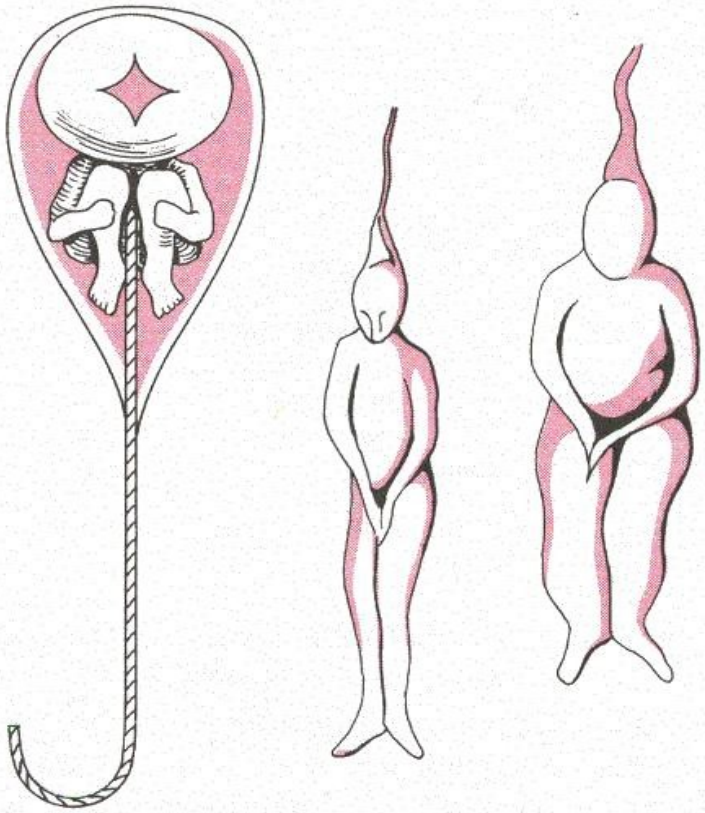


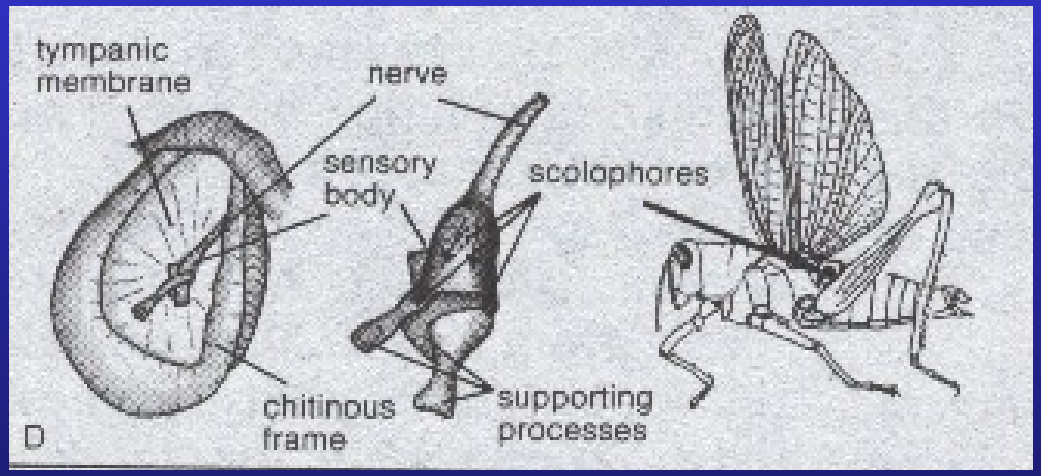
Abb. 1–1 „Homunculi", die man im menschlichen Sperma zu sehen glaubte (nach SINGER; Zeichnungen aus dem 17. Jahrhundert von HARTSOEKER und DELENPATIUS)

- Aristoteles (300 BC): „epigenesis“ successive formation of organs by contribution of „non-material“ principles in blood

Genetics of the ear

Hearing in insects

- via legs (*Ensifera*; since Jurassic)
- via trachea (grass hoppers)
- via abdomen (praying mantis)



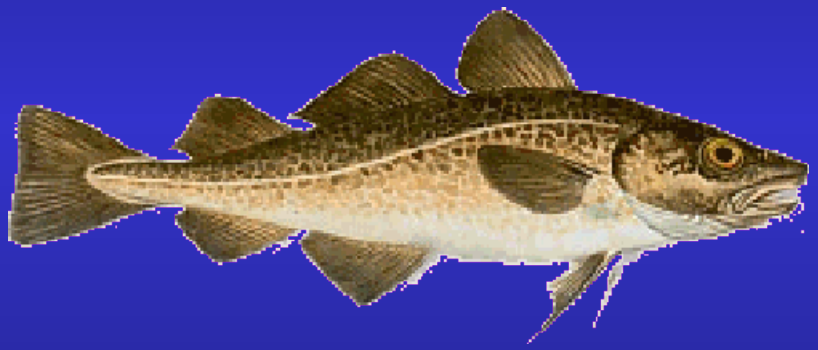
- In sound-producers:

  - detection of partners or competitors

- In mute insects:

  - detection of ultra-sound (predators)

Sound reception in fish



- Linea lateralis
  - perception of water pressure

  - usually horizontal, in exception vertical (Rondeletia)

Sound reception in fish

- Cyclostomes (lamprey) and elasmobranchs (sharks) with primitive labirynth (3 semicircular canals with statoliths)
- In teleosts, a true “inner ear” with macular otoliths is found

Danio rerio mutants

Bundle integrity	*mariner, sputnik, orbiter, satellite*
Mechanotransduction	*mercury, cosmos, asteroid, nebula*
Post-transduction	*gemini, astronaut, cosmonaut, spacelab,titan, luna, stardust, comet, pulsar,andromeda, milky way*
Degeneration	*skylab, raumschiff, meteor*

Danio rerio mutants

- Starmaker: Otopt
- Sputnik: CDH23
- Gemini: Cav1.3
- Satellite: Myo6

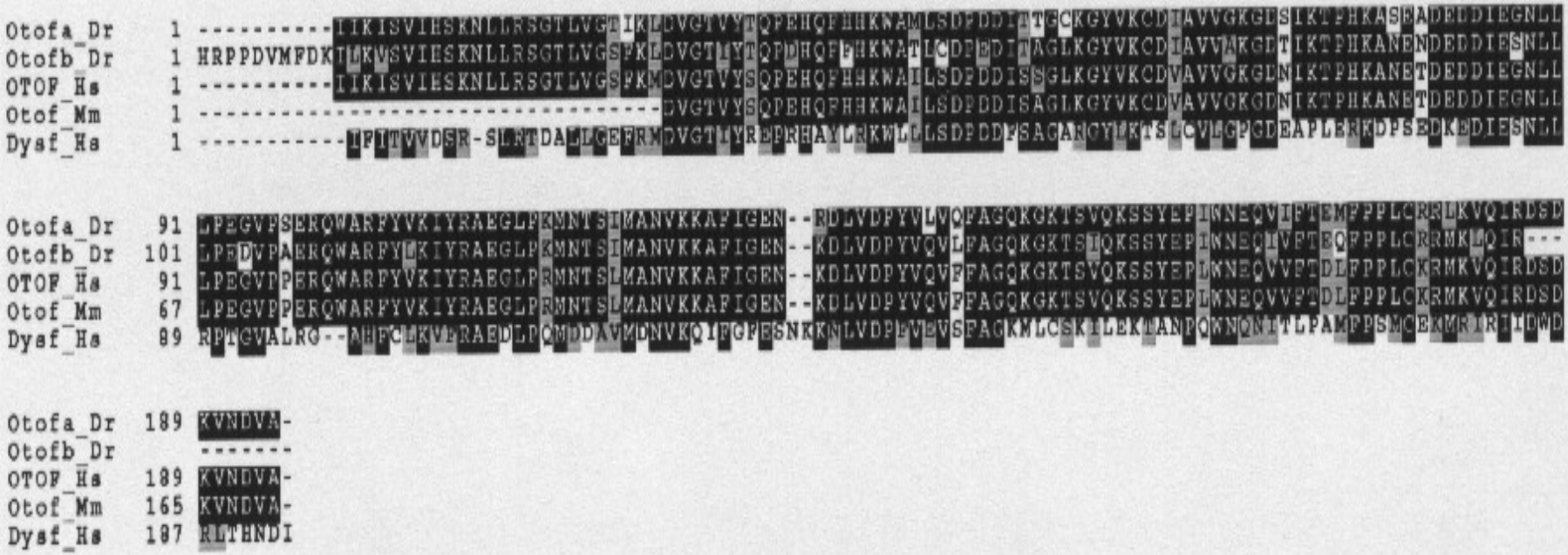
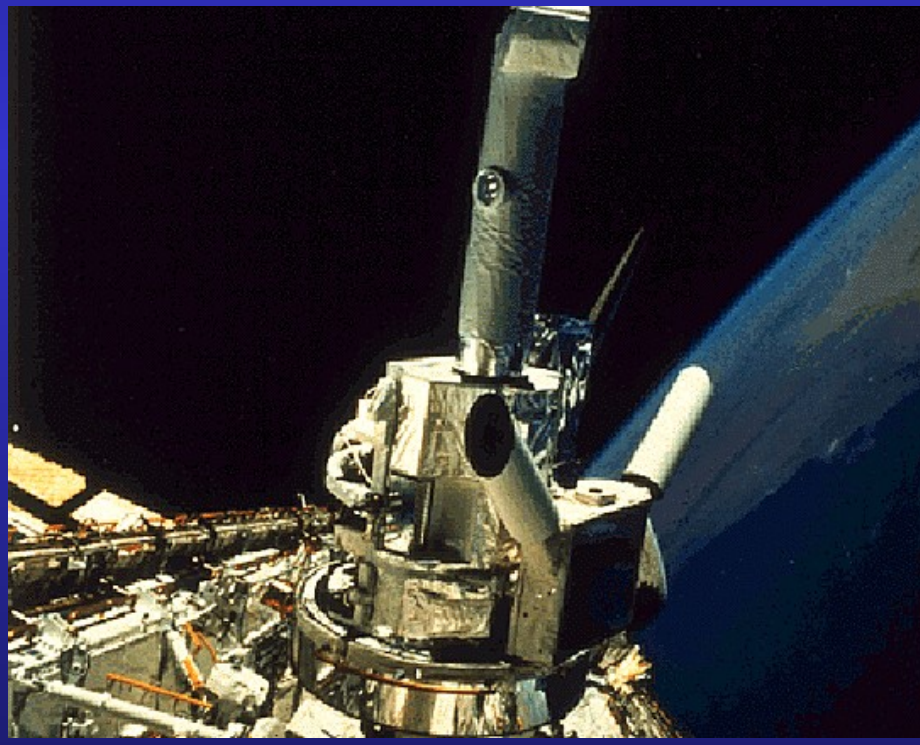


Figure 4 Alignment of a fragment of Otoferlin. Two orthologues of otoferlin were identified from zebrafish (Dr). Here an alignment of a part of the two zebrafish proteins as well as human (AA316-509) and mouse (AA315-508) is shown. For comparison a fragment of human dysferlin (Dysf, AA 282-474) is shown in the lower row.

Development of fish vestibular organ

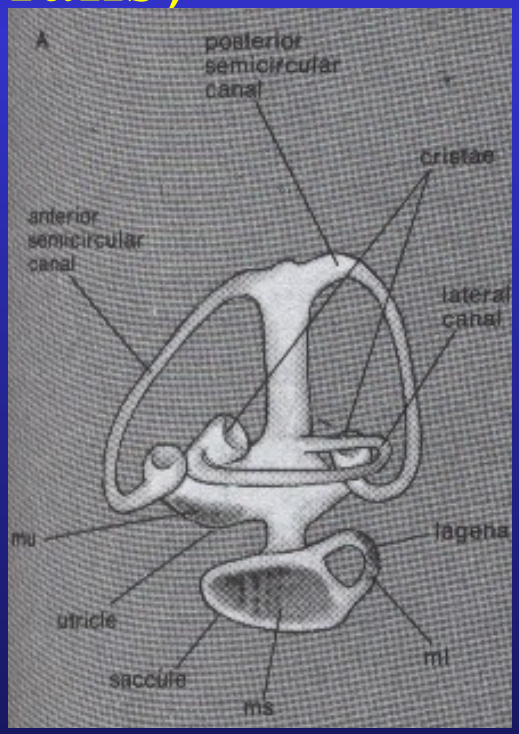
- Univ. Hohenheim (2003): 30 fish with Columbia, all destroyed

- Univ. Hohenheim (2009):
  26 fish larvae in space shuttle,
  about 180 rounds,
  11 recovered,
  will be investigated
  for formation of otoliths



Evolution in Vertebrates

- Fish: sacculus, utriculus, lagena (developed from linea lateralis)

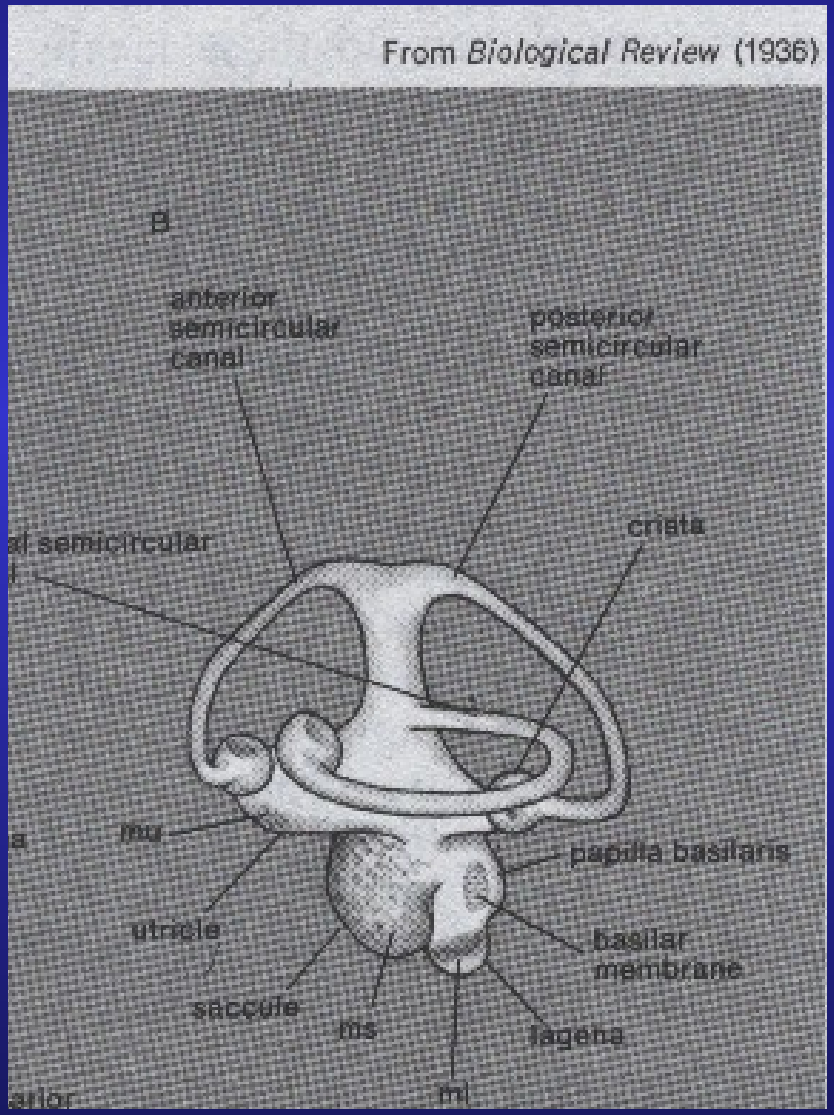


- Amphibians: like in fish, in frogs a middle ear with tympanum and a columnela is found

Evolution in Vertebrates

- Reptiles: lagena elongated (the later cochlea), tympanum not external

  In snakes, middle ear is lost
  Geccos with best tonal discrimination

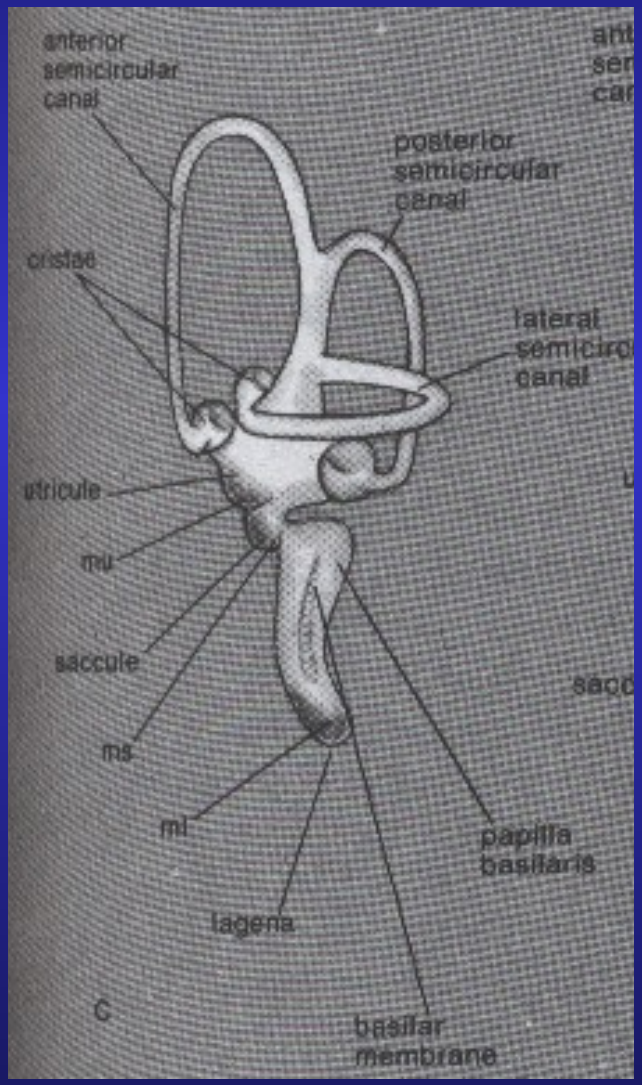


Hearing in Reptiles

- Turtles are considered mute (only rare sounds like hissing)
- ... are considered deaf, or with hearing of low quality

- Snakes are mute (only hissing)
- do not register air waves
- May use their tongue for chemo- and mechanoreception (incl. hearing?)

Evolution in Vertebrates



- Birds: reptilian ear but longer, more sensitive cochlea

Evolution in Vertebrates

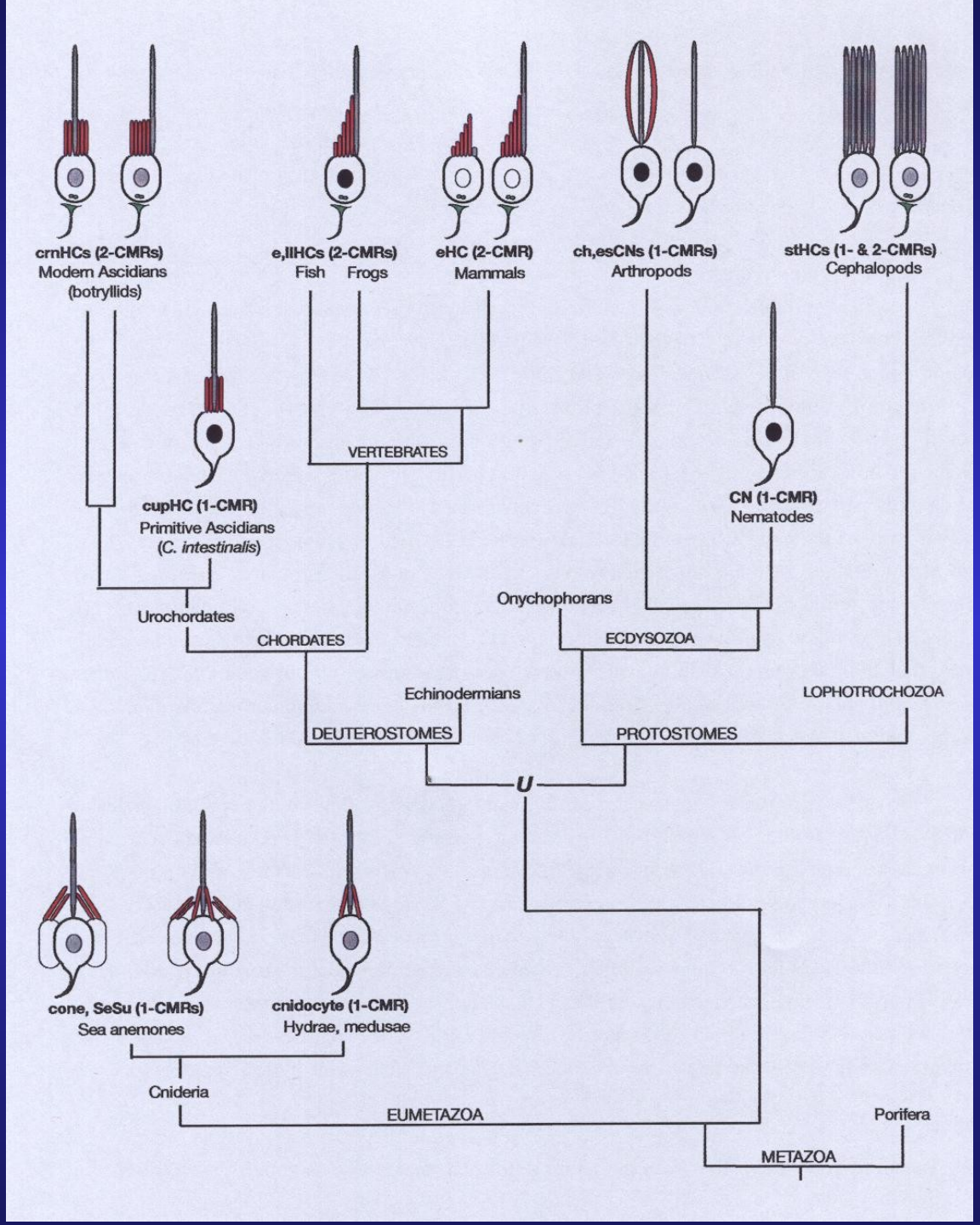
- Owls can localize prey without visual aid
- Some birds can use echolocation (*Colocallia, Steatornis*)

Auditory feed-back

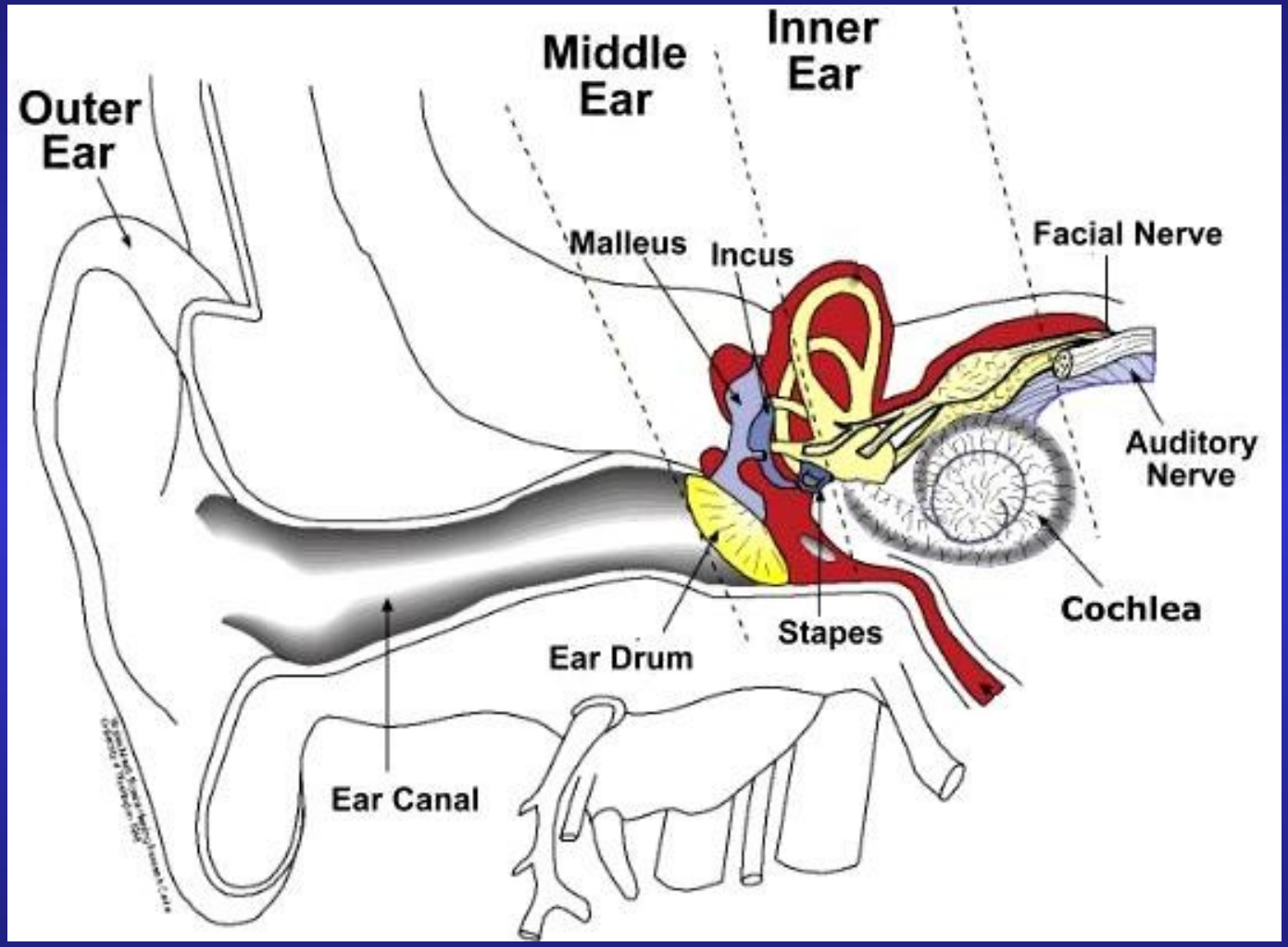
- Androgens control development of the vocal chords
- Females remain mute (:-)

Zebra finch

- Samuel Sidi PhD Thesis 2004



- **Clavelina lepadiformis**


Outer Ear
Middle Ear
Inner Ear
Malleus
Incus
Facial Nerve
Auditory Nerve
Cochlea
Stapes
Ear Drum
Ear Canal

Hereditary hearing impairment

Defects in 1/1000 newborn, >60% hereditary

A) Non-syndromic HHI

1. Dominant HHI (DFNA)
2. Recessive HHI (DFNB)
3. X-linked HHI (DFN)

B) Syndromic HHI

Known Loci & Genes

Loci / Genes	Inheritance
39 / 25	DFNA
60 / 28	DFNB
5 / 2	X-chromosomal
1(?)/ 0	Y-chromosomal

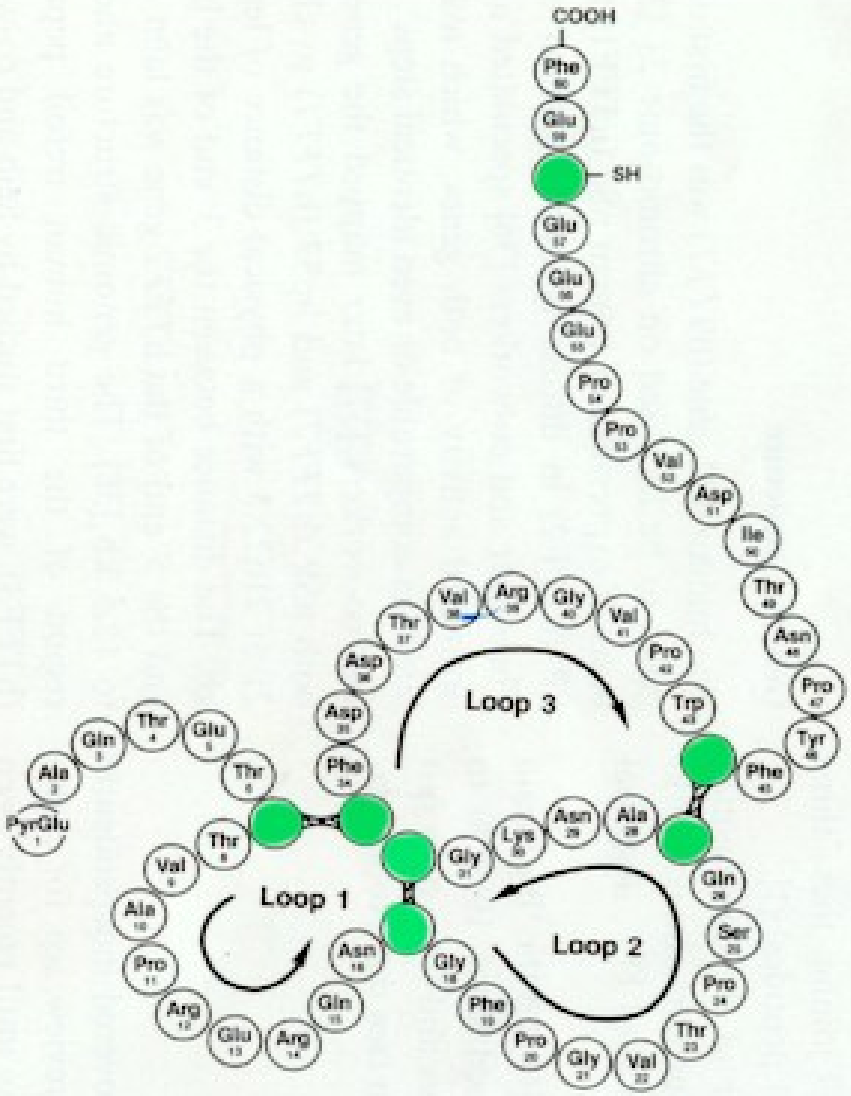
Hearing Impairment Homepage (2009): **46** genes

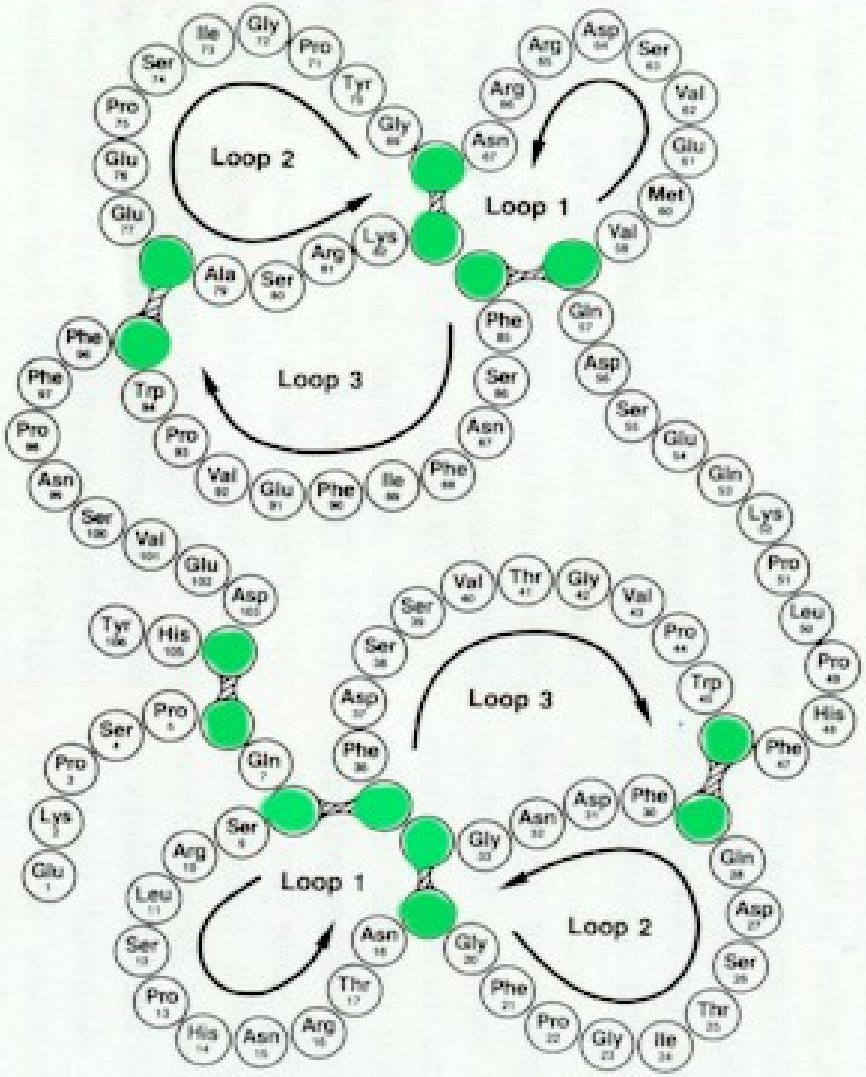
Genes in HHI

encoding products with functions in

- ion exchange processes
- cellular structures
- transcriptional & developmental regulation
- motor processes
- unknown
- cooperation with modifiers
- mitochondrial processes

Human patients:	linkage analysis, positional cloning, candidate genes, mutational screening, allelic distribution
Animal models:	
Xenopus	functional assays
Rodents	expression patterns, co-localization & interaction, knock-out/knock-in models, physiology, pathology
Cell lines	gene regulation (reporter assays, siRNA etc), co-localization & interaction





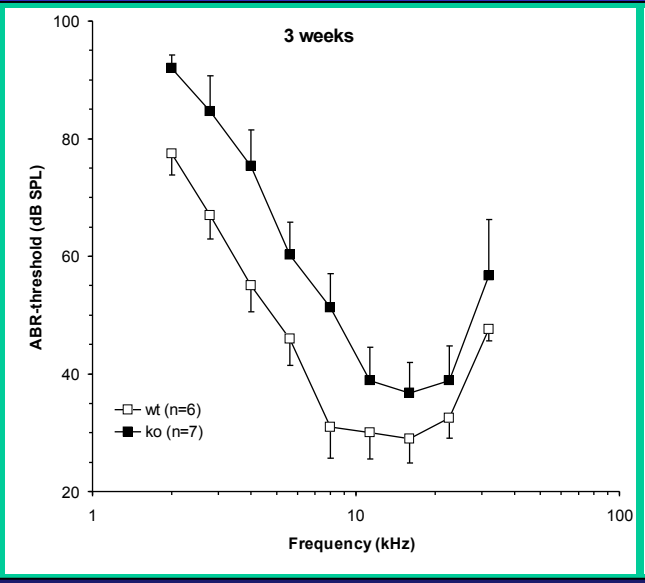
TFF1 (pS2)

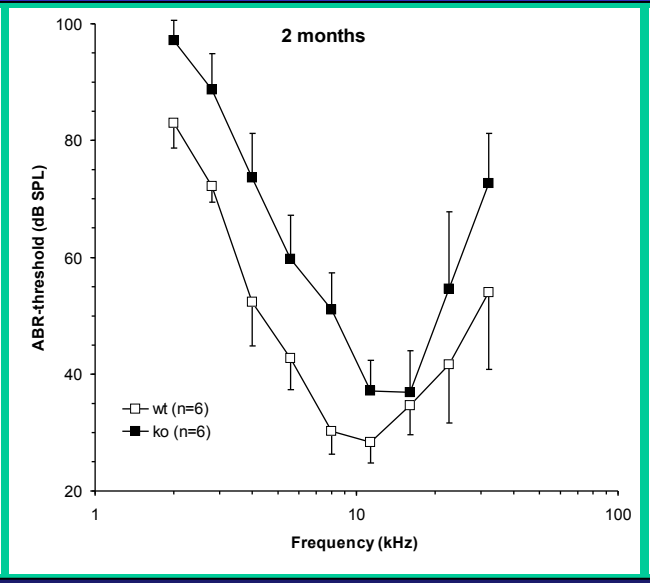
TFF2 (SP)

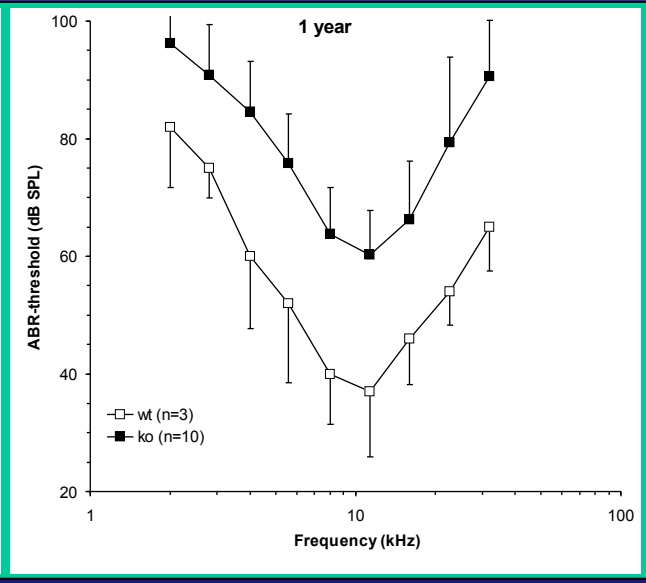
Auditory evoked brainstem responses (ABR):

- flat hearing loss below 10 kHz

- intensified during progressing age







Otoferlin

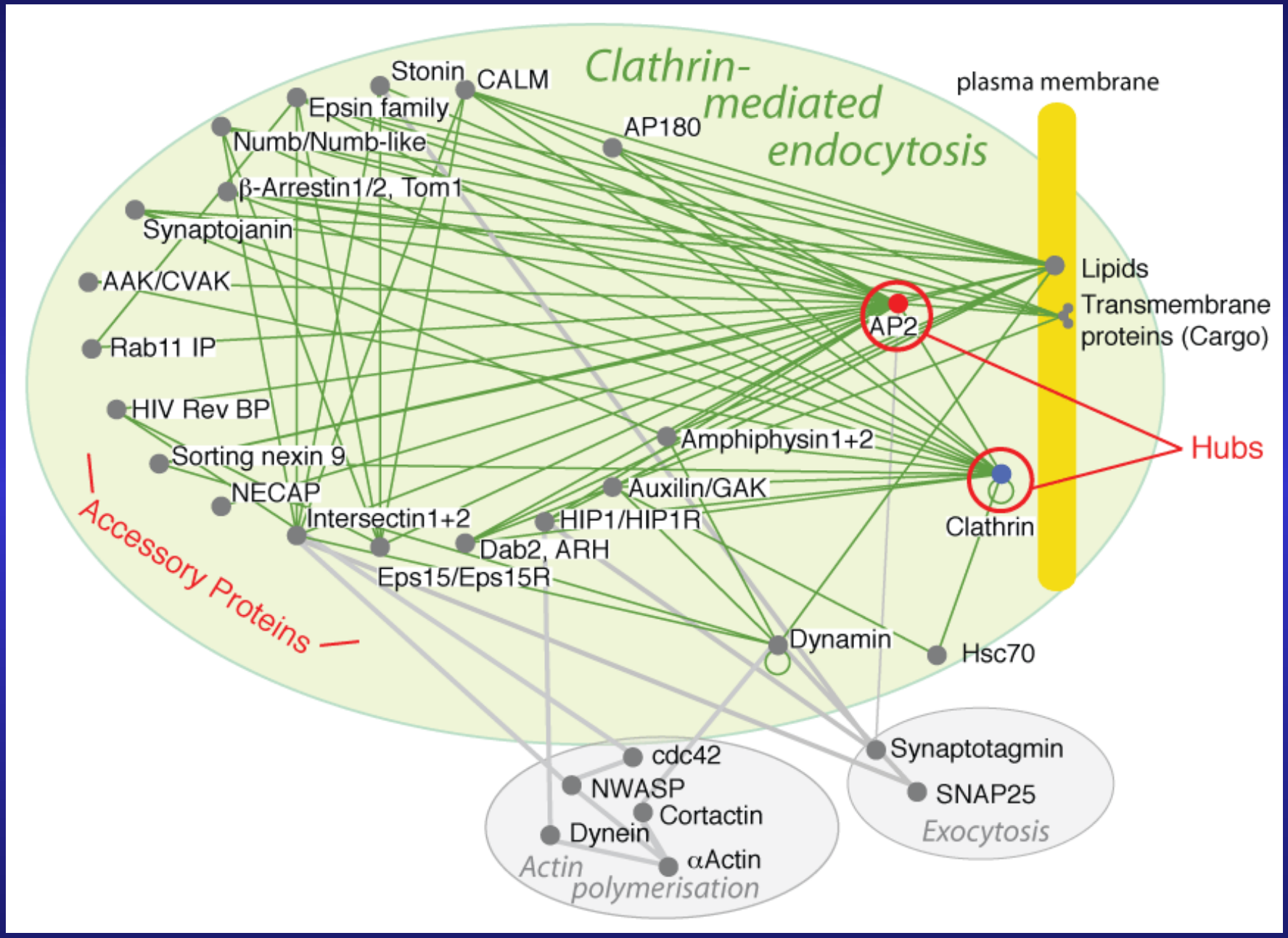
- first missense mutation
- first antibody
- intracranial expression patterns in rodents

Otoferlin

- in IHC/OHC <P12 mRNA & protein, all turns
- in IHC >P12 ongoing expression
- in OHC >P12 ongoing expression in apical turns (change from uniform to cell basis)
- shut-off in midbasal/basal turns (high freq.)

Otoferlin

- first missense mutation
- first antibody
- intracranial expression patterns in rodents
- first interacting partners
- cellular significance


Clathrin-mediated endocytosis
plasma membrane
Stonin
CALM
Epsin family
AP180
Numb/Numb-like
β-Arrestin1/2, Tom1
Synaptojanin
AAK/CVAK
Rab11 IP
HIV Rev BP
Sorting nexin 9
NECAP
Intersectin1+2
Eps15/Eps15R
Dab2, ARH
HIP1/HIP1R
Auxilin/GAK
Amphiphysin1+2
AP2
Lipids
Transmembrane proteins (Cargo)
Hubs
Clathrin
Accessory Proteins
Dynamin
Hsc70
cdc42
NWASP
Cortactin
Dynein
αActin
Actin polymerisation
Synaptotagmin
SNAP25
Exocytosis

Tools & Materials

- Protein interaction: Yeast-2-Hybrid, AP-MS

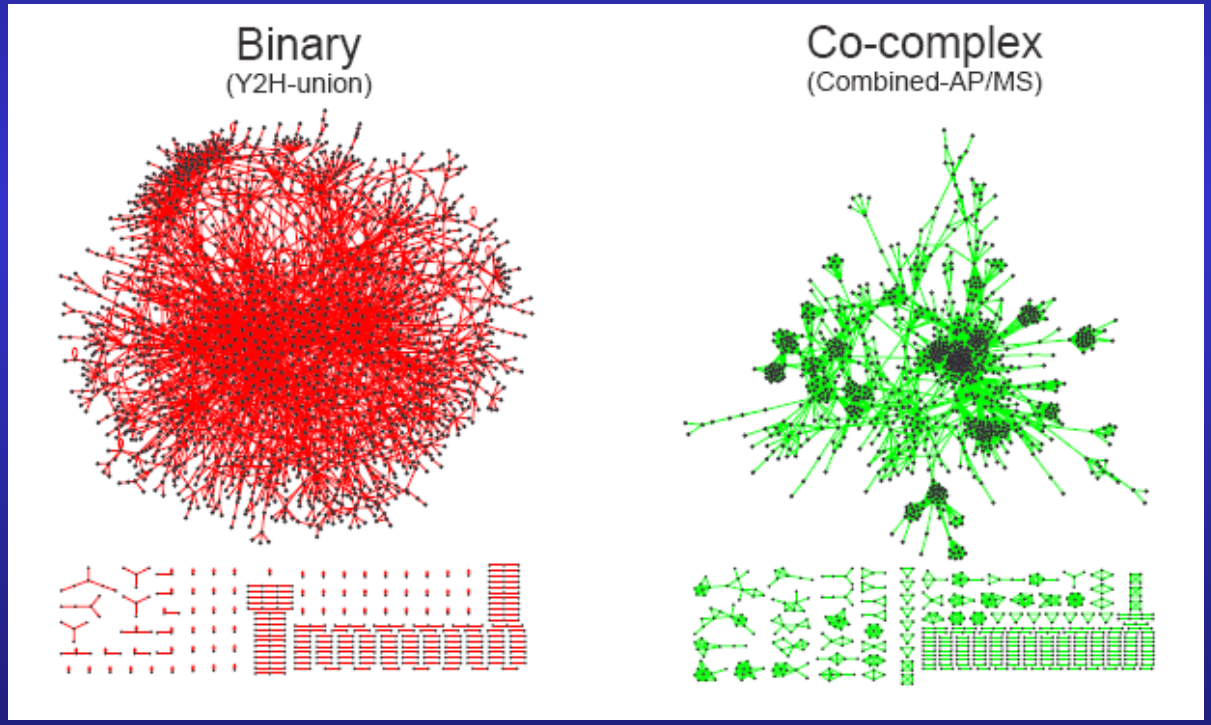
Tools & Materials

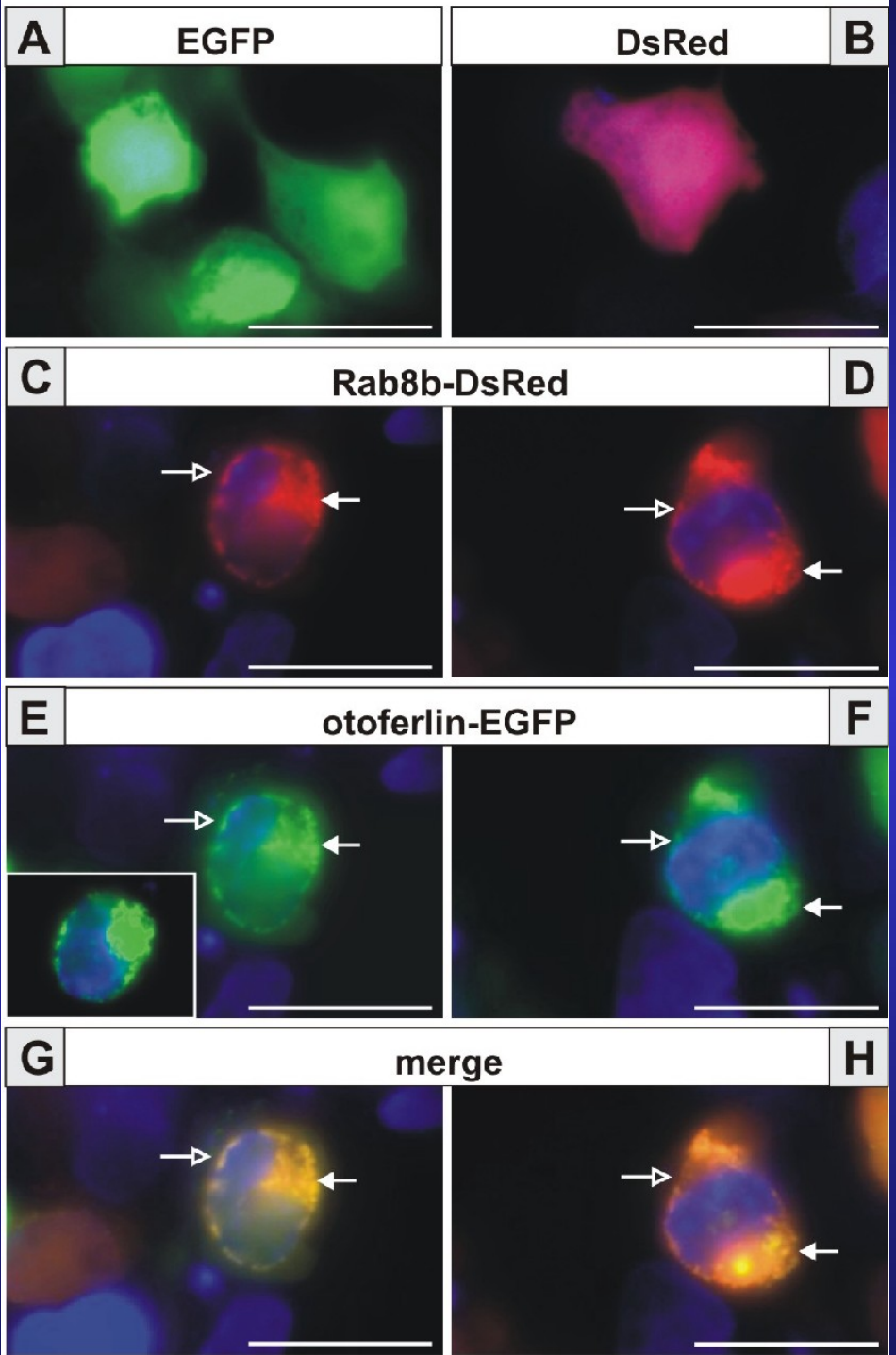
- Protein interaction: Yeast-2-Hybrid, AP-MS

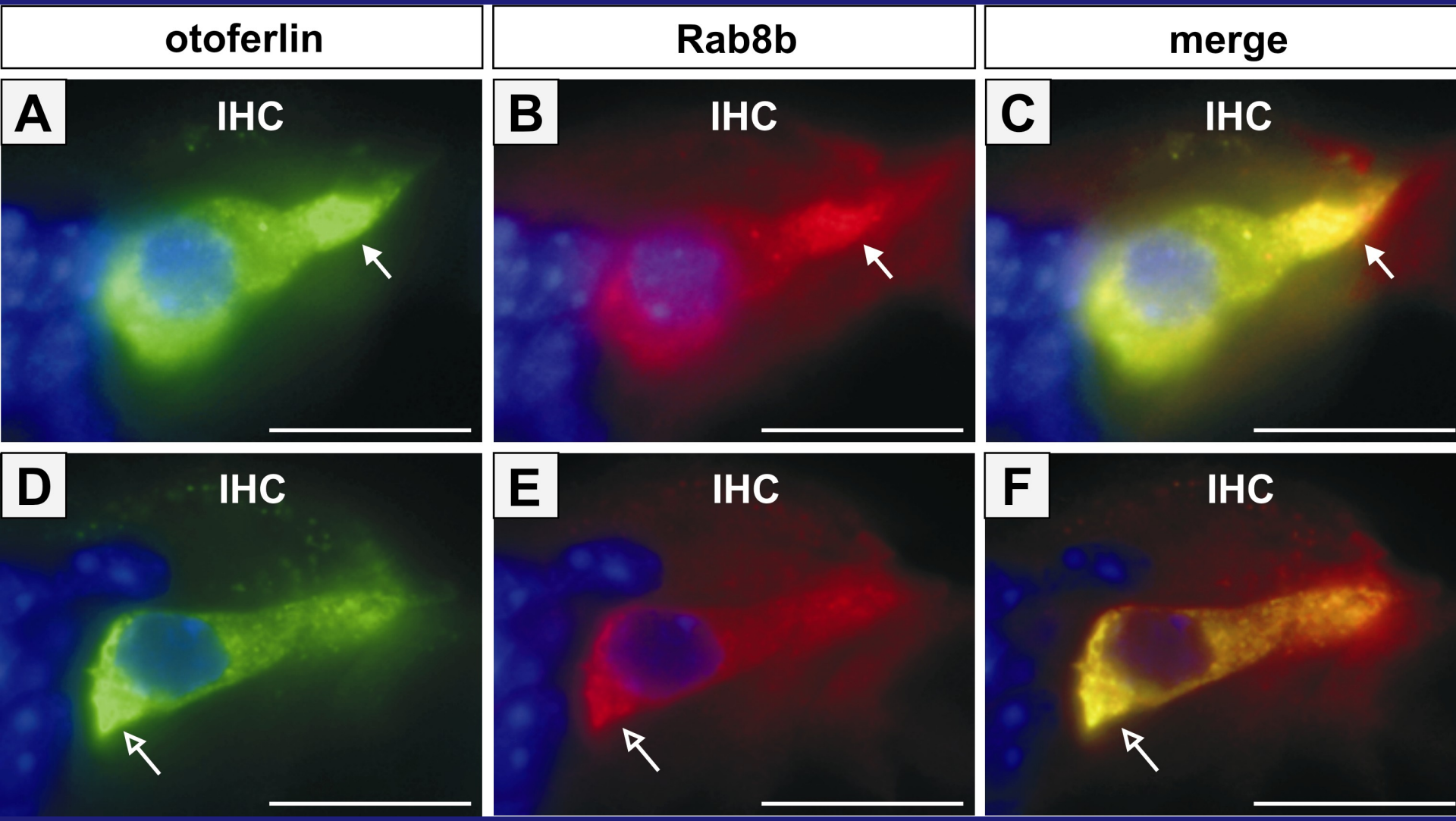
cDNA	protein
P3-P15	P20-P30

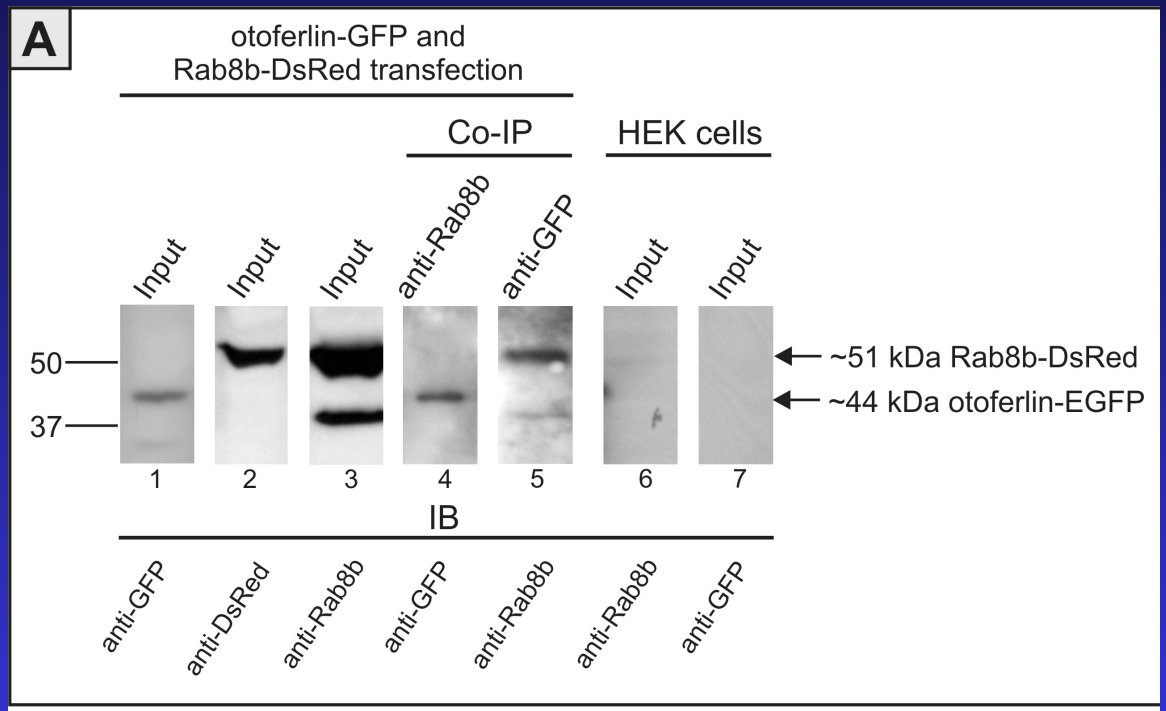
Tools & Materials

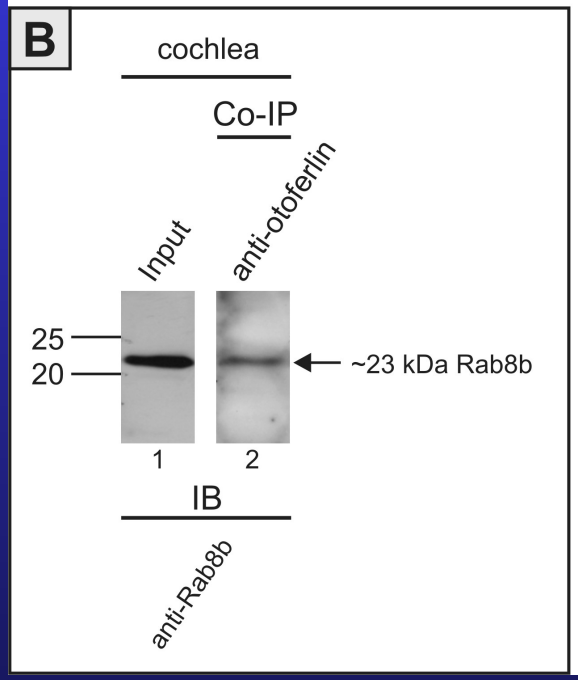
- Protein interaction: Yeast-2-Hybrid, AP-MS




A
EGFP
DsRed
B
C
Rab8b-DsRed
D
E
otoferlin-EGFP
F
G
merge
H


otoferlin
Rab8b
merge
A
IHC
B
IHC
C
IHC
D
IHC
E
IHC
F
IHC


A
otoferlin-GFP and
Rab8b-DsRed transfection
Co-IP
HEK cells
Input
Input
Input
anti-Rab8b
anti-GFP
Input
Input
50
37
~51 kDa Rab8b-DsRed
~44 kDa otoferlin-EGFP
1
2
3
4
5
6
7
IB
anti-GFP
anti-DsRed
anti-Rab8b
anti-GFP
anti-Rab8b
anti-Rab8b
anti-GFP


B
cochlea
Co-IP
Input
anti-otoferlin
25
20
~23 kDa Rab8b
1
2
IB
anti-Rab8b

Otoferlin Interactome

- Confirmed: Rab8, Myo6, AP2
- Investigated: Fkbp8, Rnd3, Tjp1, Coch5b2, Prot4.1G-like, GPSN2, Ckap4, Sim-c-c
- Additional candidates

Otoferlin Interactome

- Known partners (our investigations, literature)

OTOF

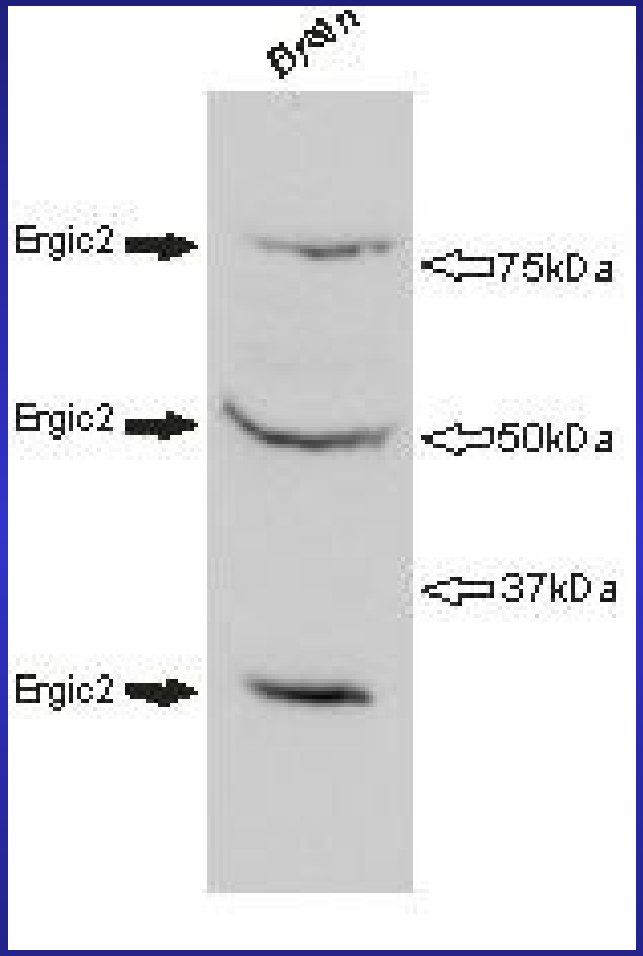
RAB8, MYO6, AP2, ERGIC2,
Syntaxin, SNAP25

Otoferlin Interactome

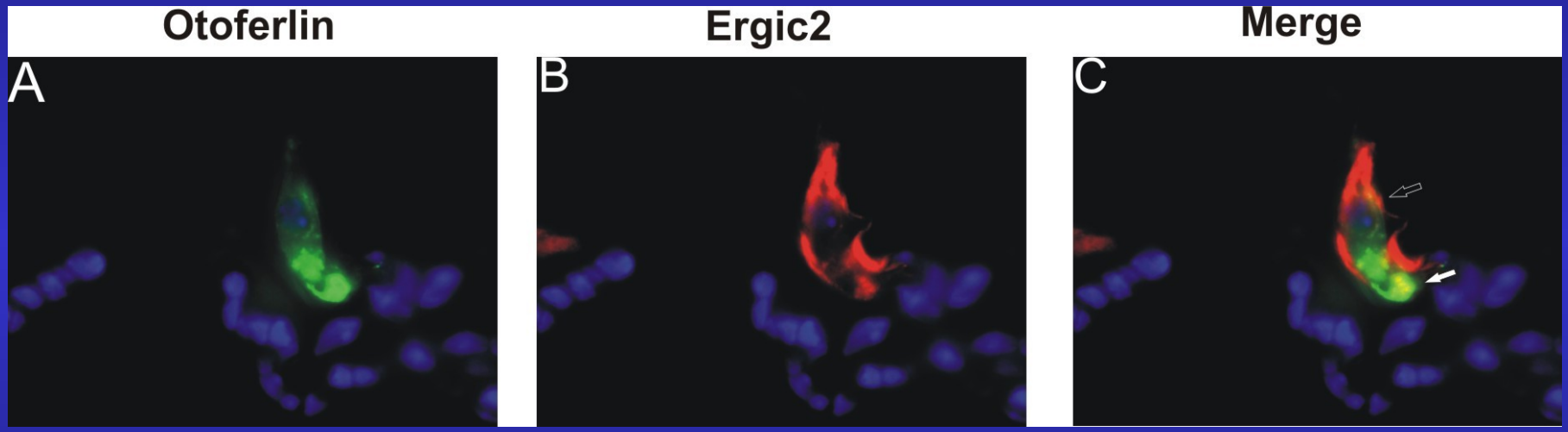
- Known partners (our investigations, literature)

OTOF

RAB8, MYO6, AP2, **ERGIC2**,
Syntaxin, SNAP25


Brain
Ergic2
Ergic2
Ergic2
75kDa
50kDa
37kDa

Co-localization of Ergic2 and OTOF in the IHC of the P17 mouse



Ergic2 shown in red (Cy3)
OTOF shown in green (Alexa green)
The co-localization is marked in yellow (c)
Nuclei were stained with DAPI (blue)

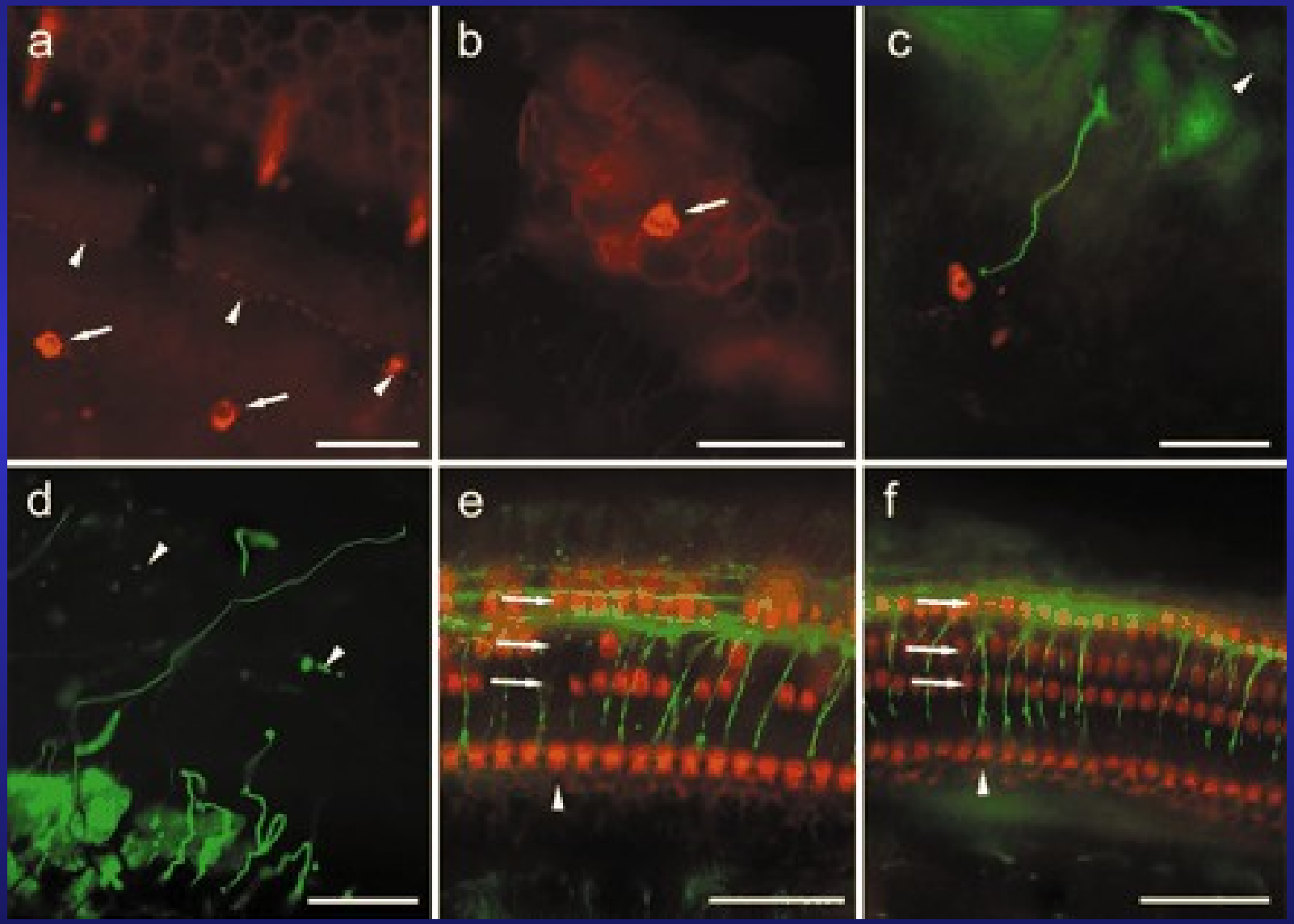
Progress in gene therapy?

Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, Brough DE, Raphael Y.

Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals.

Nat Med. 2005 Mar;11(3):271-6.

MyoVIIa



Progress in gene therapy

- Leber‘s amaurose
- ADA-SCID
- adenoleukodystrophy
- Wiskott Aldrich syndrome