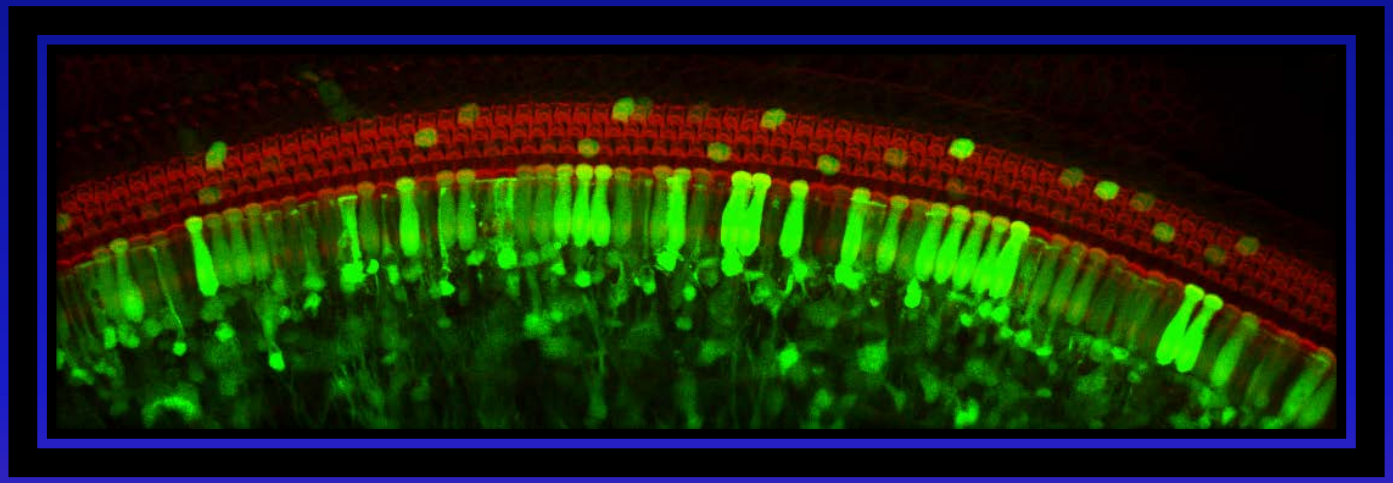
Usher Syndrome Research

Update to Families

Gwenaëlle Géléoc, PhD
Assistant Professor



BOSTON CHILDREN'S HOSPITAL

HARVARD MEDICAL SCHOOL

THE MANTON CENTER for Orphan Disease Research

Talk outline

I- What is Usher Syndrome?

II- Advances in understanding the patho-physiology of Usher Syndrome

III- Treatment: Strategies for restoring function

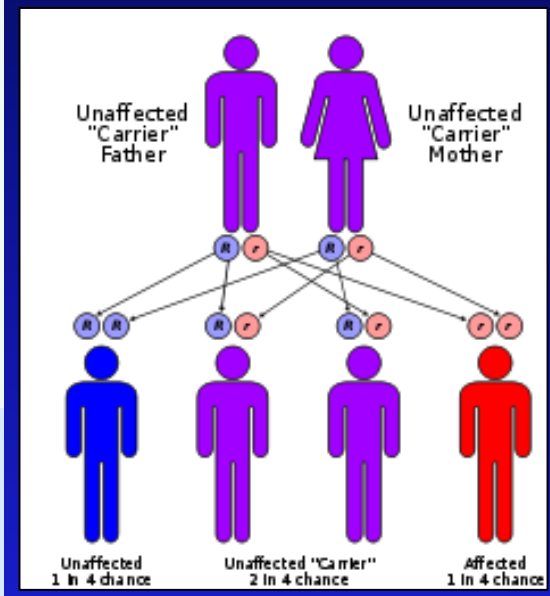
I- What is Usher Syndrome?

Charles Howard Usher
1865-1942
1914 Study on "the inheritance of retinitis pigmentosa"

- Usher syndrome causes hearing loss and retinitis pigmentosa (RP) which is responsible for night-blindness and progressive a loss of peripheral vision
- Many people with Usher also suffer from balance problems
- Usher Syndrome is the most common condition that affect hearing and vision
- 16-40,000 people with Usher syndrome in the US.
- Clinically and genetically heterogeneous
- Three clinical USH types: Type 1, Type 2 and Type 3 distinguished by their severity and age when signs and symptoms appear.

I- What is Usher Syndrome?

Locus	Location	Gene/protein	Function
USH1B	11q13.5	*MYO7A*/myosin VIIA	IE and R: transport
USH1C	11p15.1	*USH1C*/harmonin	IE and R: scaffolding
USH1D	10q22.1	*CDH23*/cadherin 23	IE: tip link formation; R: periciliary maintenance
USH1E	21q21	–/–	Unknown
USH1F	10q21.1	*PCDH15*/protocadherin 15	IE: tip link formation; R: periciliary maintenance
USH1G	17q25.1	*USH1G*/SANS	IE and R: scaffolding and protein trafficking
USH1H	15q22-23	–/–	Unknown
USH1J	15q24	CIB2/ Calcium And Integrin Binding Family Member 2	Calcium and Integrin-binding protein
USH2A	1q41	*USH2A*/usherin	IE: ankle links formation and cochlear development; R: periciliary maintenance
USH2C	5q14.3	*GPR98*/VLGR1	IE: ankle links formation Cochlear development; R: periciliary maintenance
USH2D	9q32-34	*DFNB31*/whirlin	IE: scaffolding and cochlear development; R: scaffolding0
USH2 Modifier	10q24	PDZD7	Homolog of Harmonin and Whirlin
USH3A	3q25.1	*USH3A*/clarin-1	IE and R: probable role in synapsis transport*
USH3B	20q	–/–	Unknown



11 different genes (15 genetic loci) have been linked to USH.

I- What is Usher Syndrome?

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Usher proteins function:

Transmembrane Adhesion

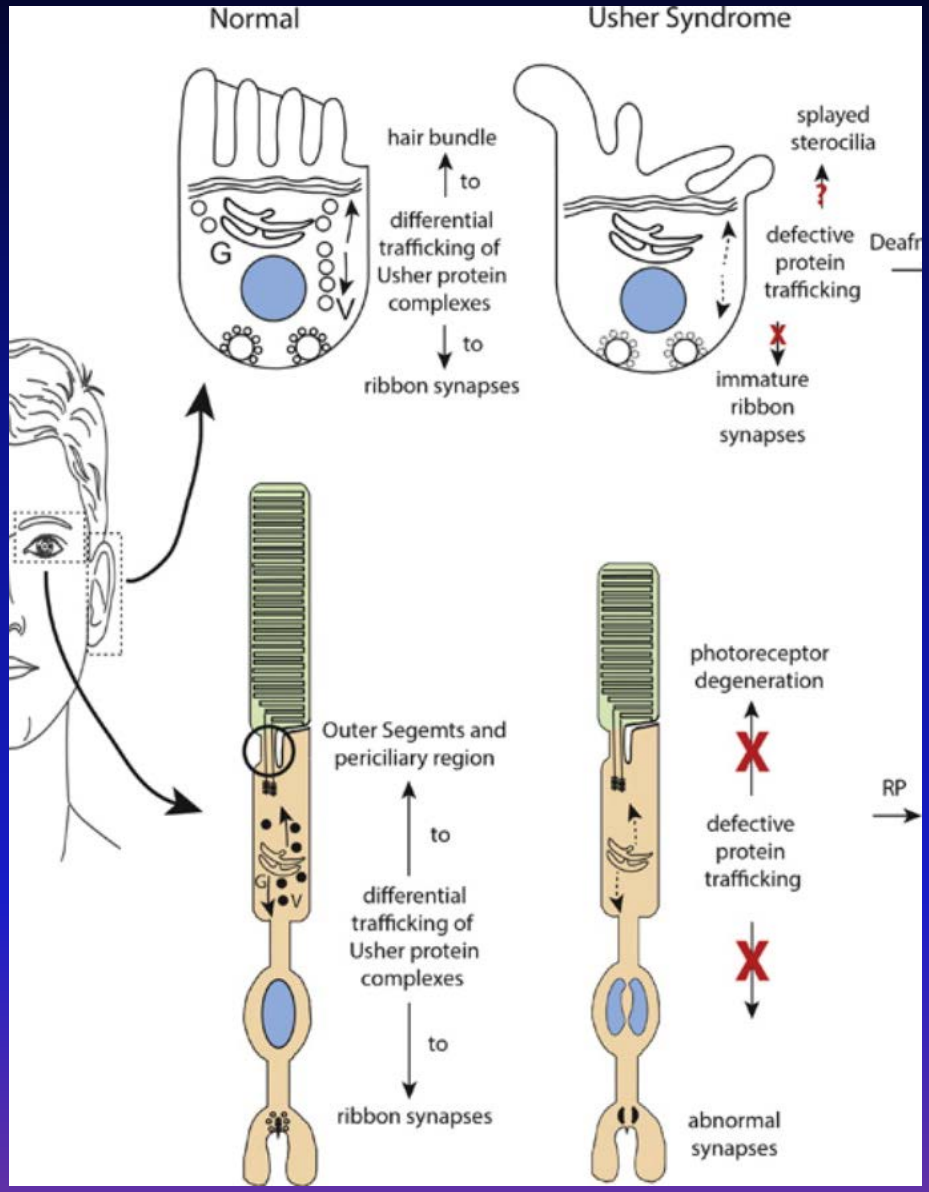
Signalling

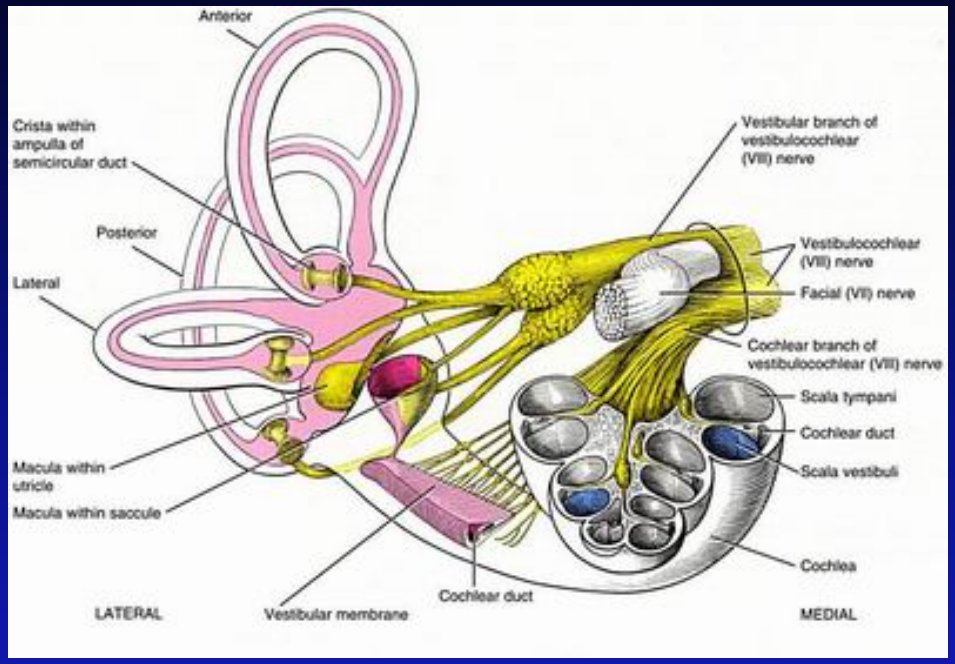
Scaffolding

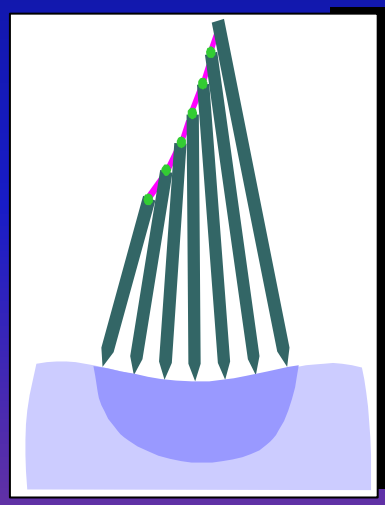
Transport

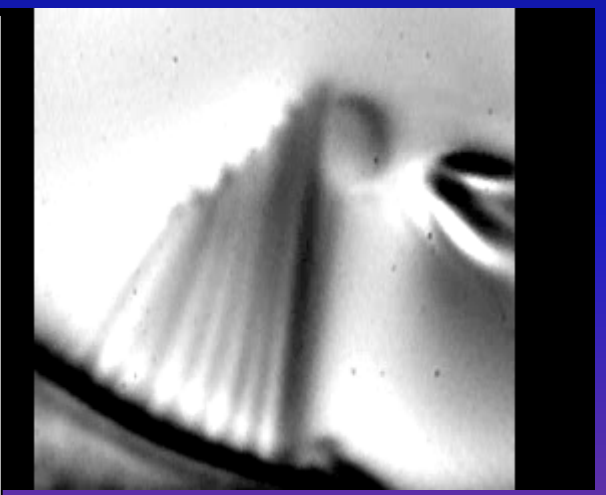
Development and synapse formation

I- What is Usher Syndrome?



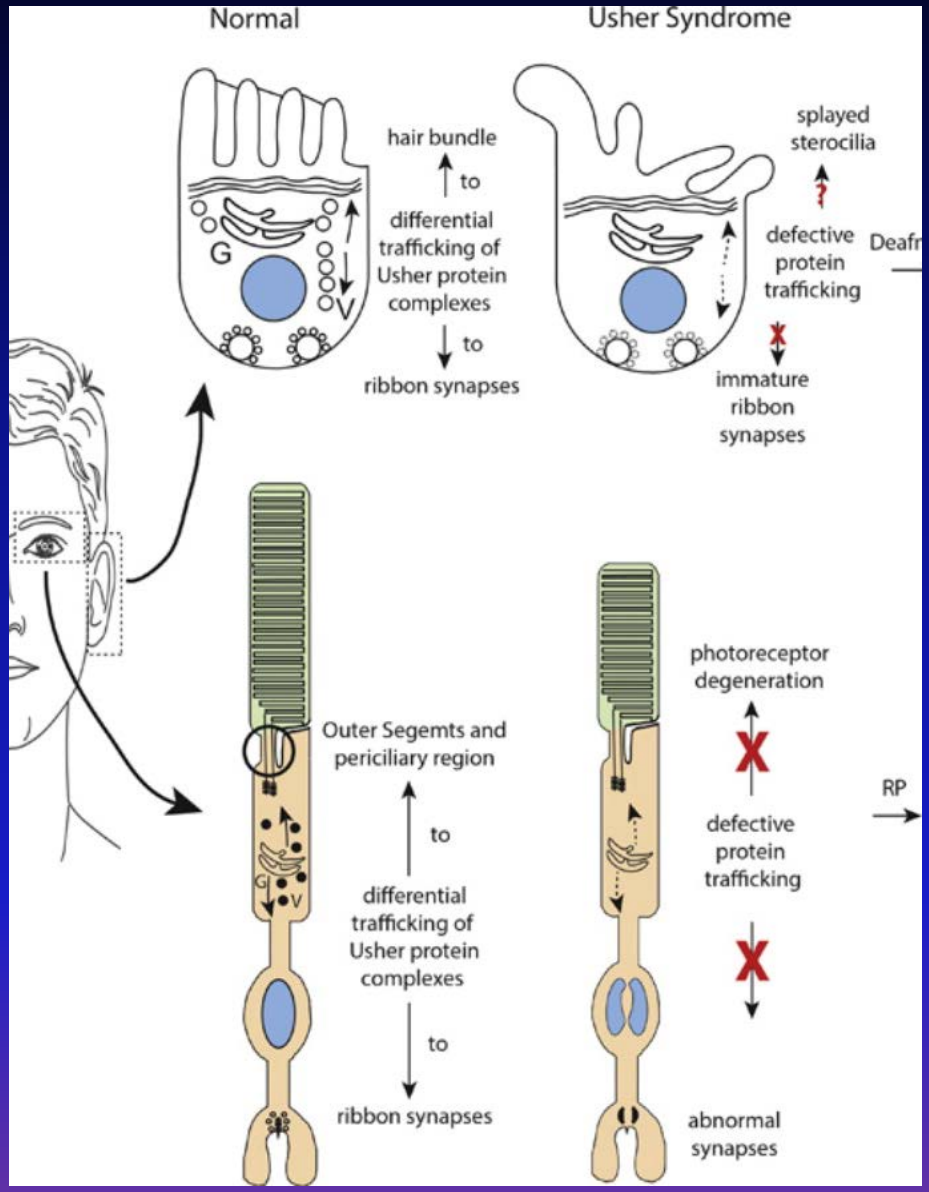






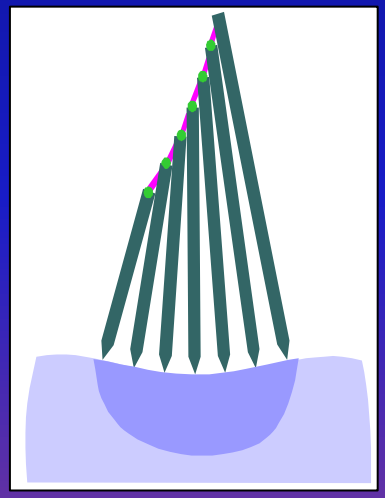
Cosgrove and Zallocchi, 2014, IJBCB

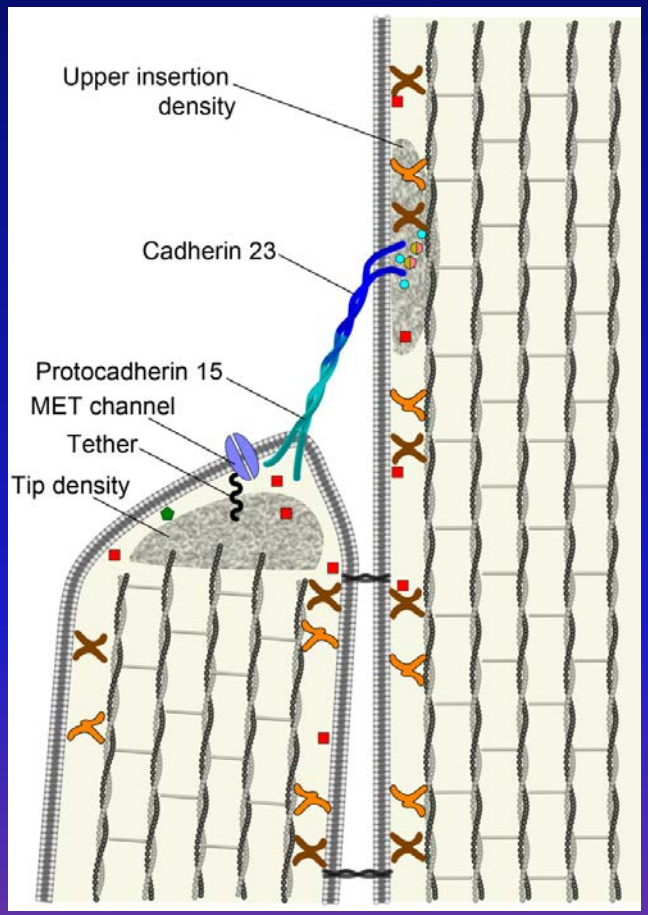
I- What is Usher Syndrome?



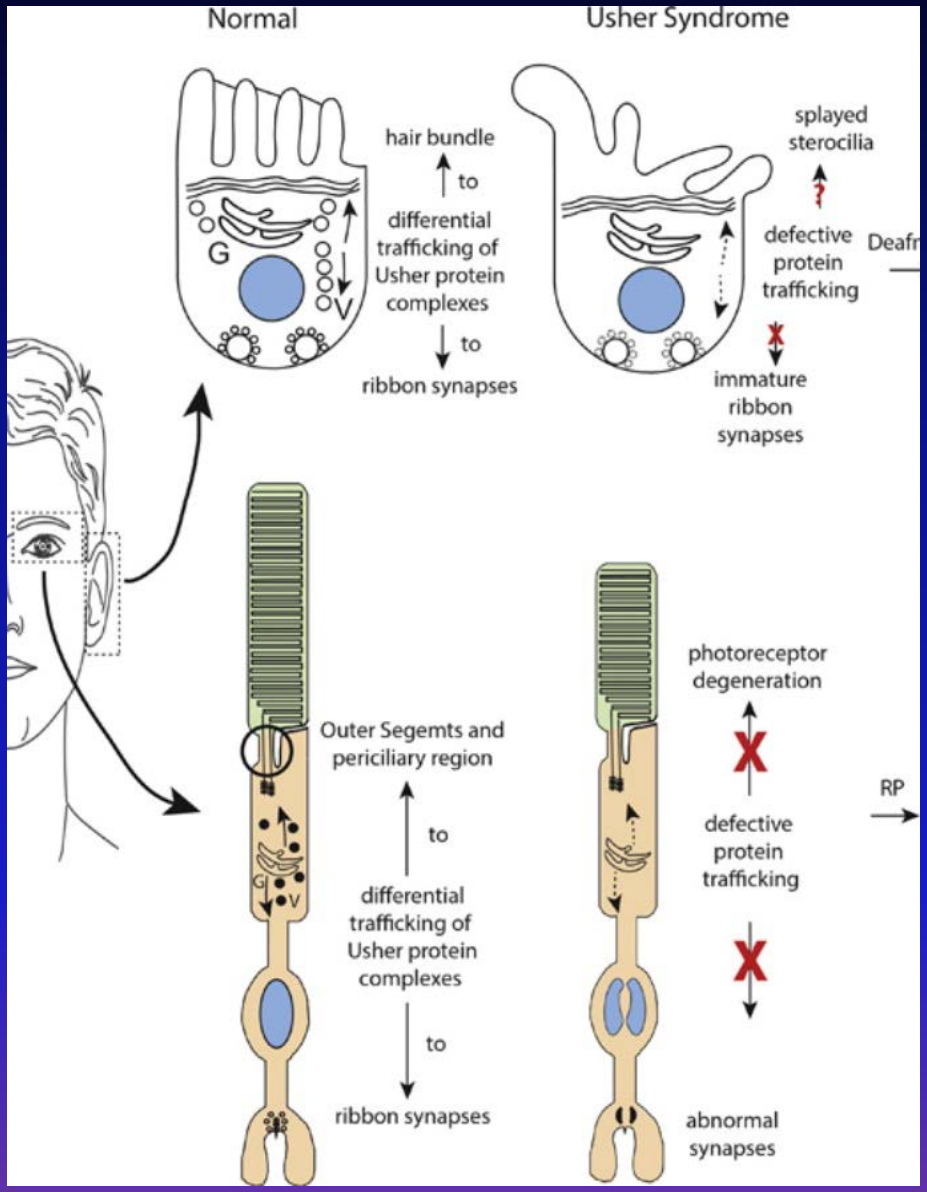
Cosgrove and Zallocchi, 2014, IJBCB

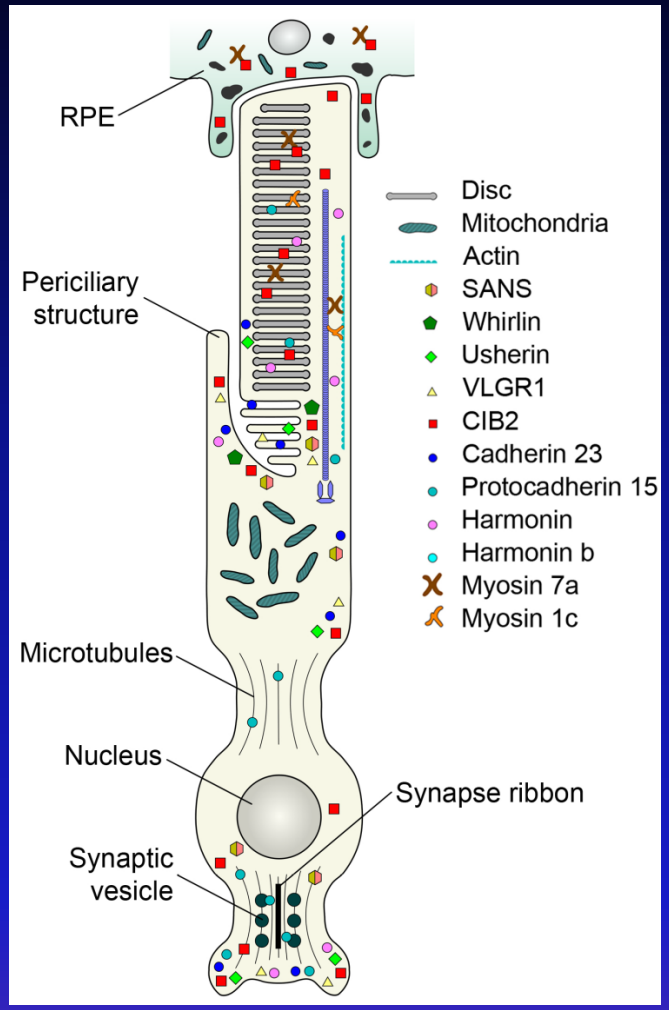
IE: Usher proteins play structural and developmental roles in stereocilia and ribbon synapses.





I- What is Usher Syndrome?



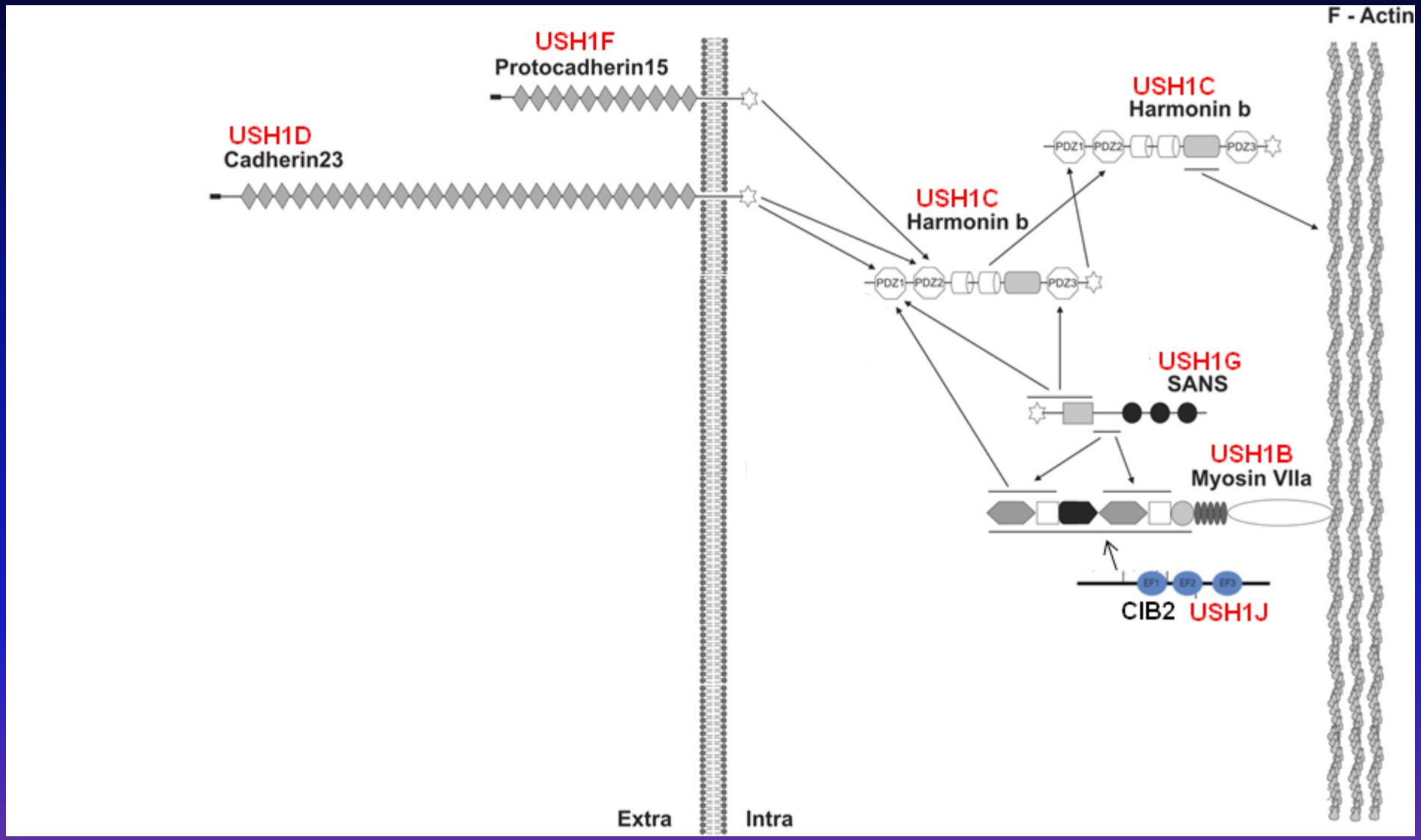


RE: Usher proteins are associated with the connecting cilium, outer limiting membrane and ribbon synapse of photoreceptors.

Cosgrove and Zallocchi, 2014, IJBCB

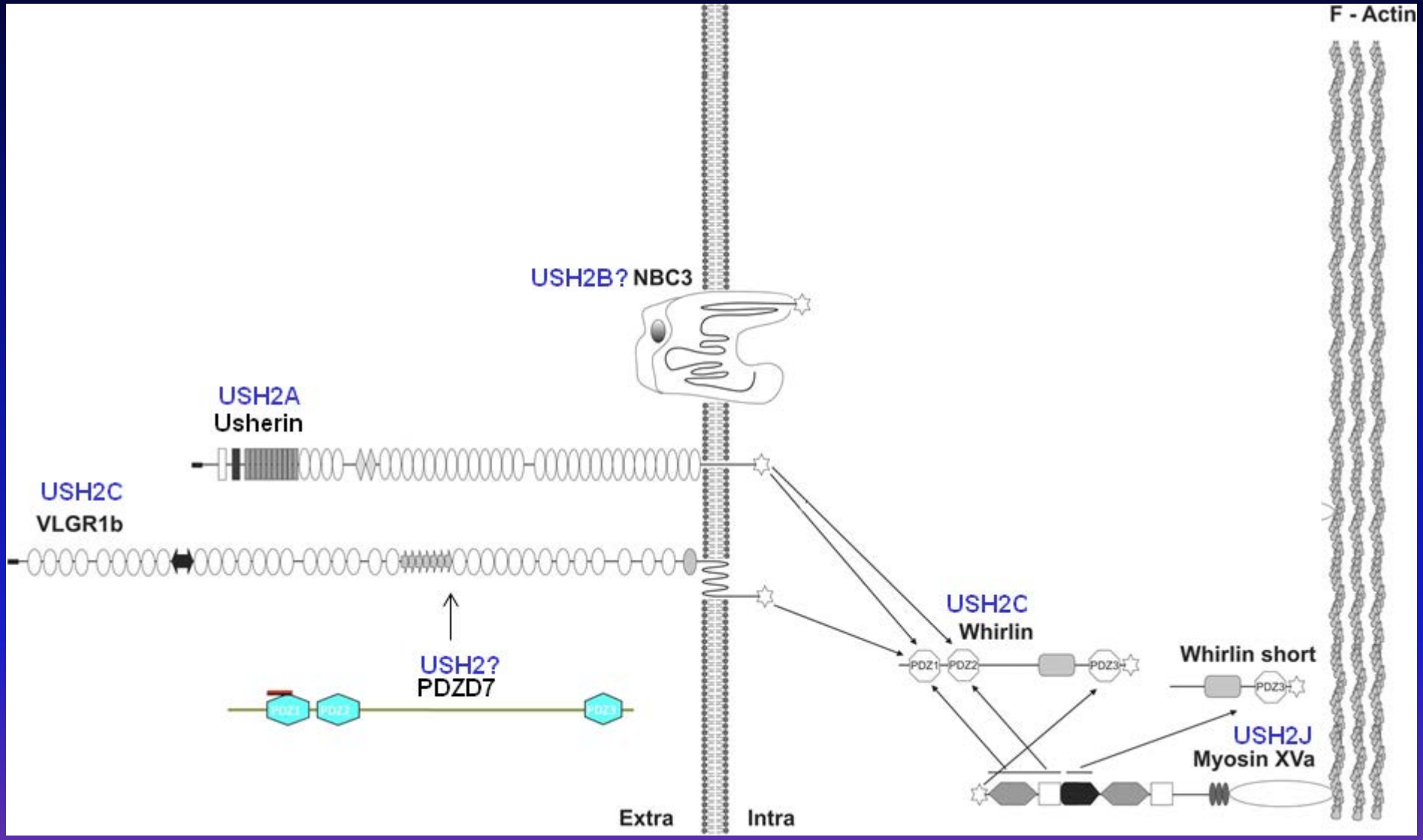
The Usher Interactome

USH1



The Usher Interactome

USH2



II- Advances in understanding the pathophysiology of Usher Syndrome

Christine Petit

"Gathering basic knowledge towards the development of therapeutic approaches."

Uwe Wolfrum

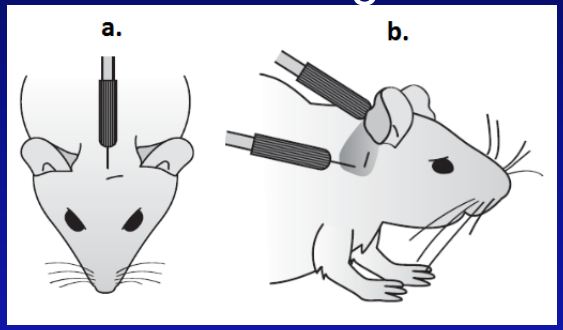
"Decoding of Usher Syndrome protein networks reveals insights in the molecular basis of the disease."

Monte Westerfield

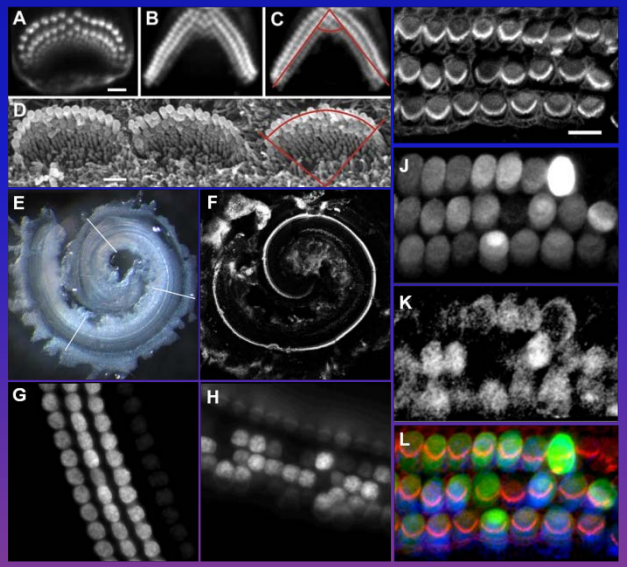
"Defective protein complex assembly produces ER stress that causes cell death in Usher Syndrome"

II- Advances in understanding the patho-physiology of Usher Syndrome

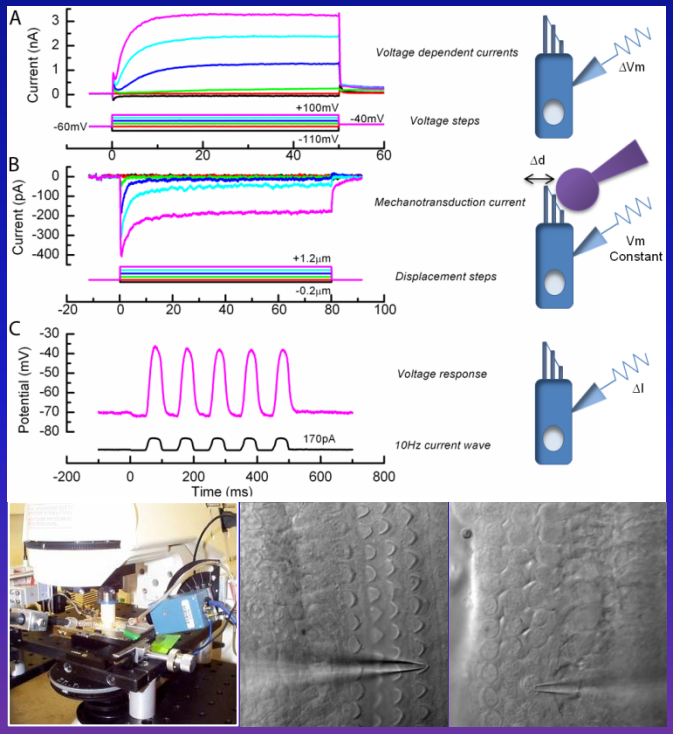
Auditory and Balance Testing



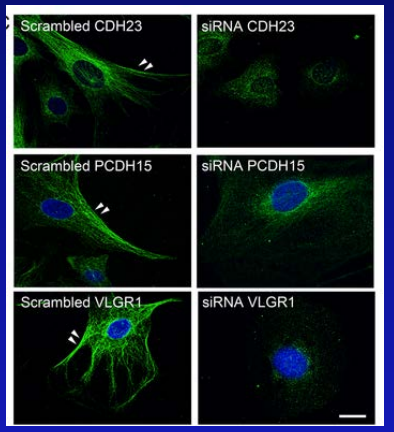
Imaging

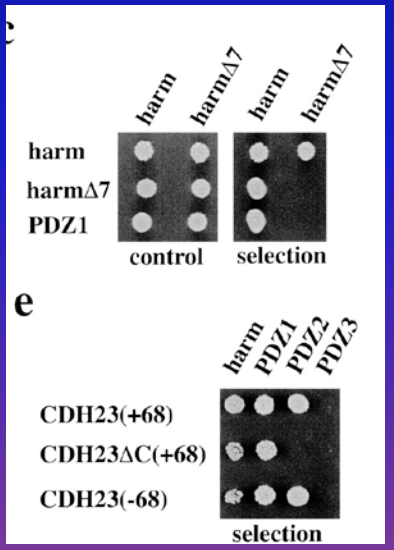


Physiology



Cellular/Molecular work

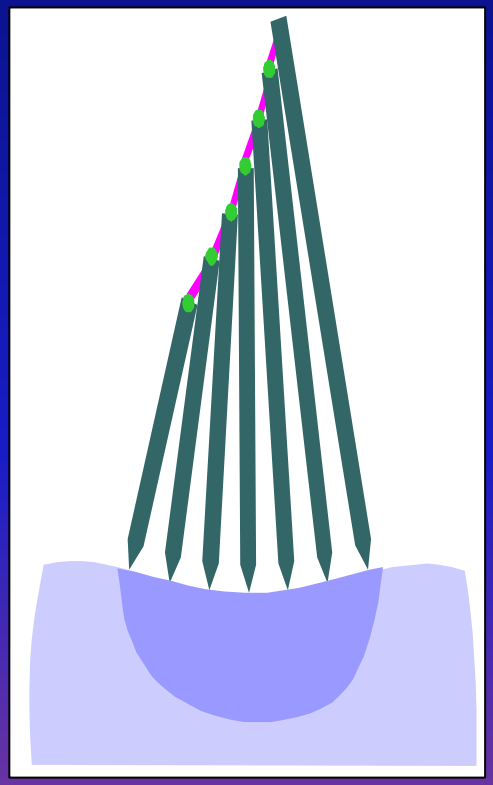


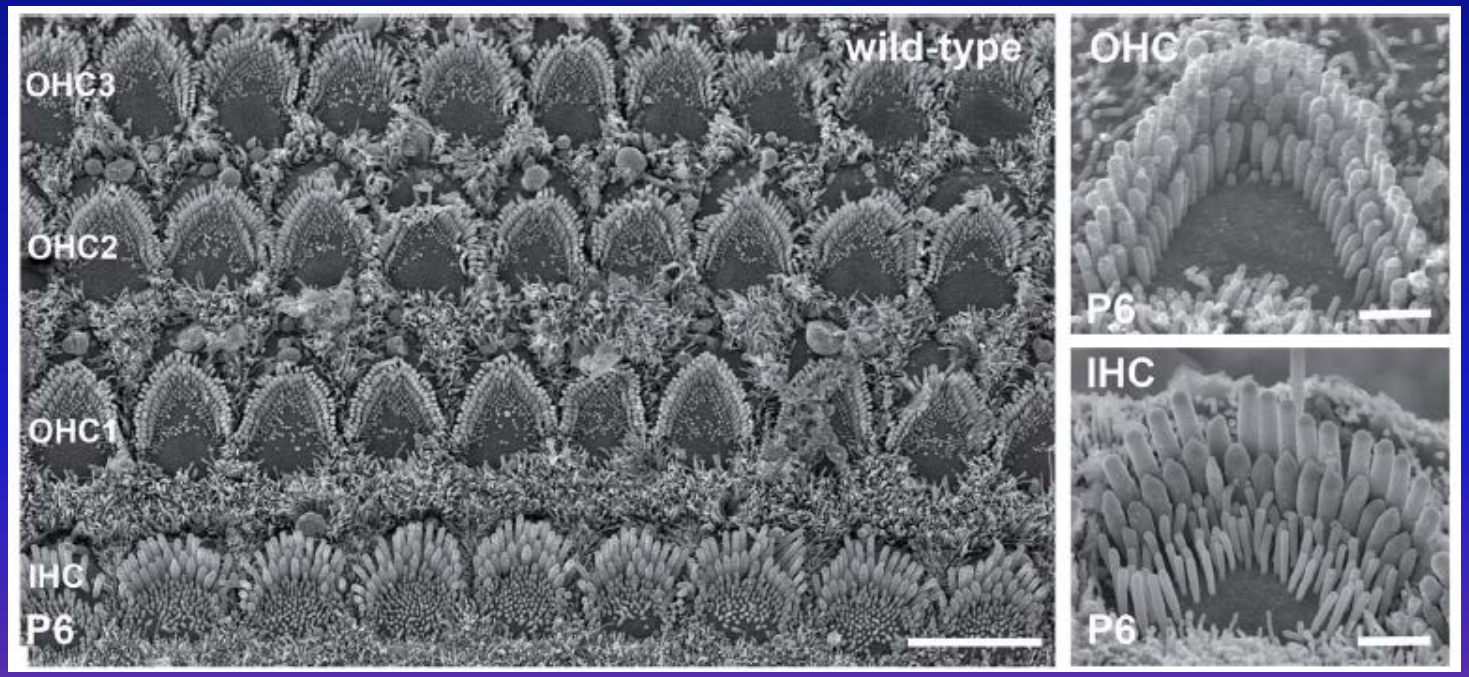


II- Advances in understanding the patho-physiology of Usher Syndrome

Christine Petit

"Gathering basic knowledge towards the development of therapeutic approaches."





Pflugers Arch - Eur J Physiol
DOI 10.1007/s00424-014-1552-9

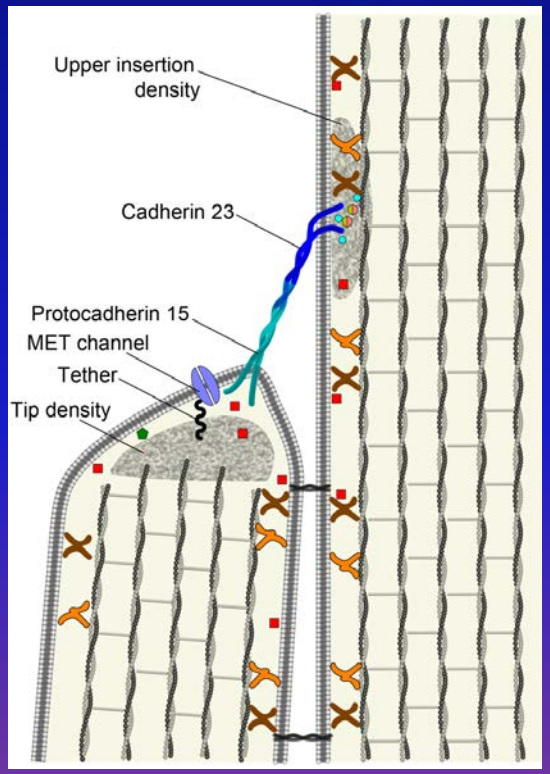
INVITED REVIEW

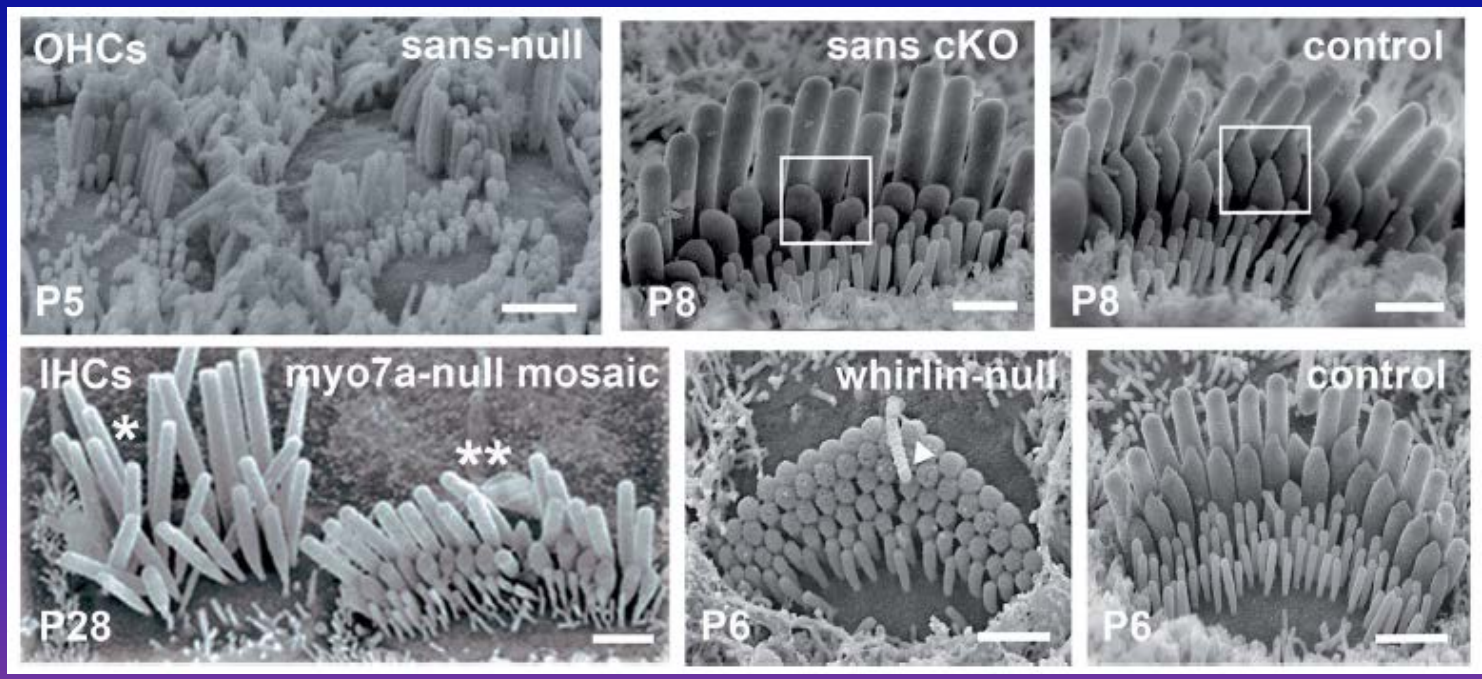
Genetics of auditory mechano-electrical transduction

II- Advances in understanding the patho-physiology of Usher Syndrome

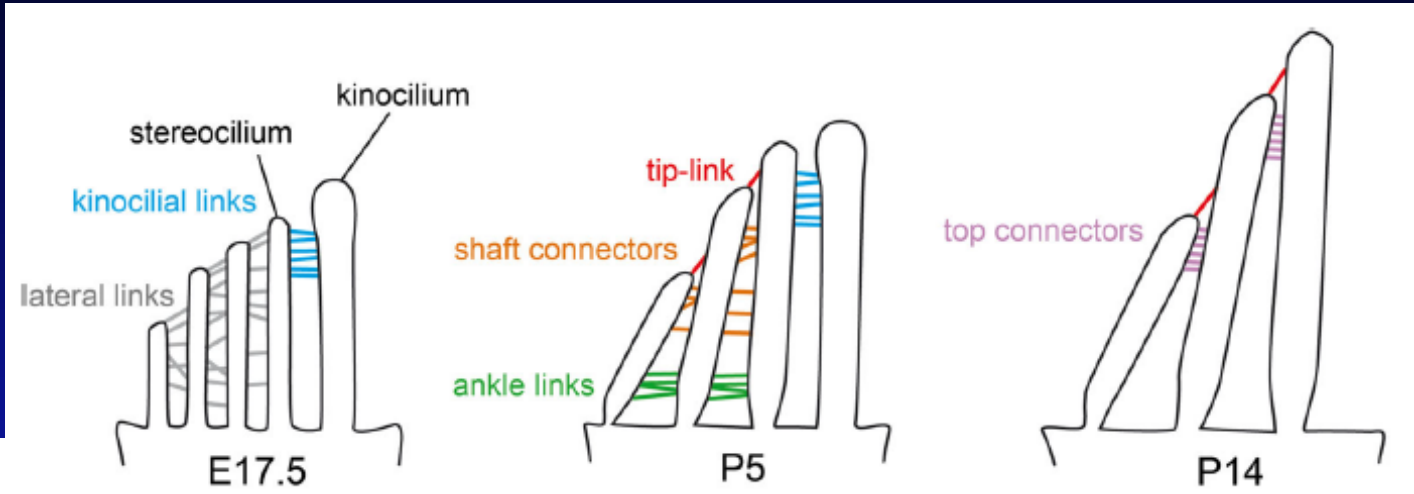
Christine Petit

"Gathering basic knowledge towards the development of therapeutic approaches."





II- Advances in understanding the patho-physiology of Usher Syndrome



lateral link complex	
link	protocadherin-15, cadherin-23
submembranous	harmonin
actin-binding	myosin-VIIa, sans
others	unknown

tip-link complex (MET machinery)	
link	protocadherin-15, cadherin-23
submembranous*	harmonin b, sans, harmonin a (?)
actin-binding	harmonin b, myosin-VIIa, myosin-Ic (?)
others	TMHS, TMC1/2 (MET channel ?)

ankle link complex	
link	VLGR1, usherin **
submembranous	whirlin, PDZD7
actin-binding	myosin-VIIa
others	vezatin (transmembrane protein)

top connector complex	
link	unknown
submembranous	unknown
actin-binding	unknown
others	stereocilin (extracellular protein?)

kinocilial link complex	
link	protocadherin-15, cadherin-23
submembranous	unknown
actin-binding	unknown
others	unknown

shaft connector complex	
link	PTPRQ **
submembranous	unknown
actin-binding	unknown
others	unknown

II- Advances in understanding the patho-physiology of Usher Syndrome

Christine Petit

"Gathering basic knowledge towards the development of therapeutic approaches."

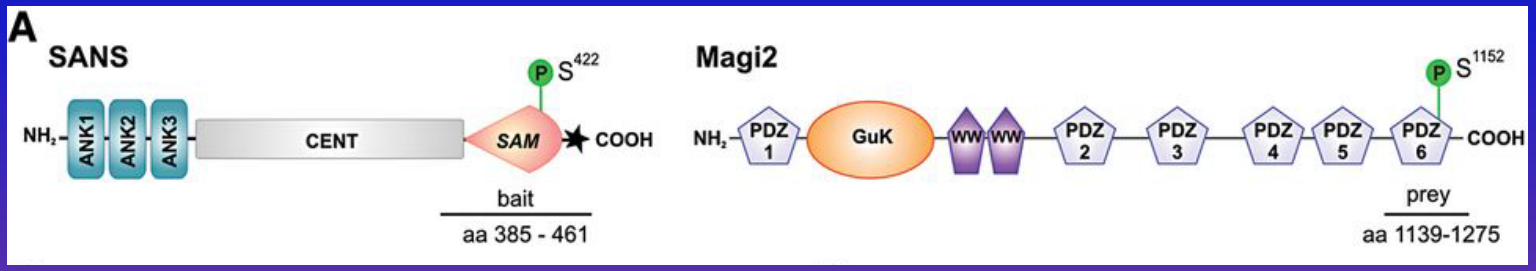
Bicycle, 1980s; Raleigh; Component count: 893. Thames & Hudson.

II- Advances in understanding the patho-physiology of Usher Syndrome

Uwe Wolfrum

"Decoding of Usher Syndrome protein networks reveals insights in the molecular basis of the disease."

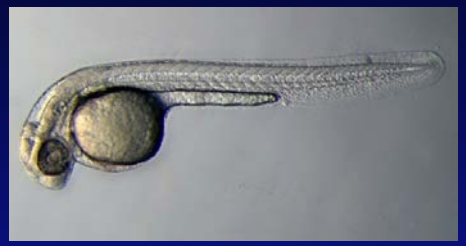
- Large contribution to our understanding of USH1/USH2 interactome in the eye and the ear (ex: Interfering with Sans/Ush2A interaction leads to UHS1G)
-Recently demonstrated that USH1G (Sans) contributes to periciliary protein networks in the retina
- Now his lab has identified a new binding partner that is important for endocytosis (*process by which cells absorb molecules by engulfing them*)



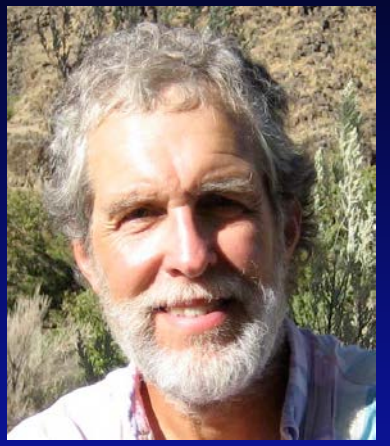
MAGi2/Sans are present at the base of the primary cilia and both play a role in ciliogenesis

Human Molecular Genetics

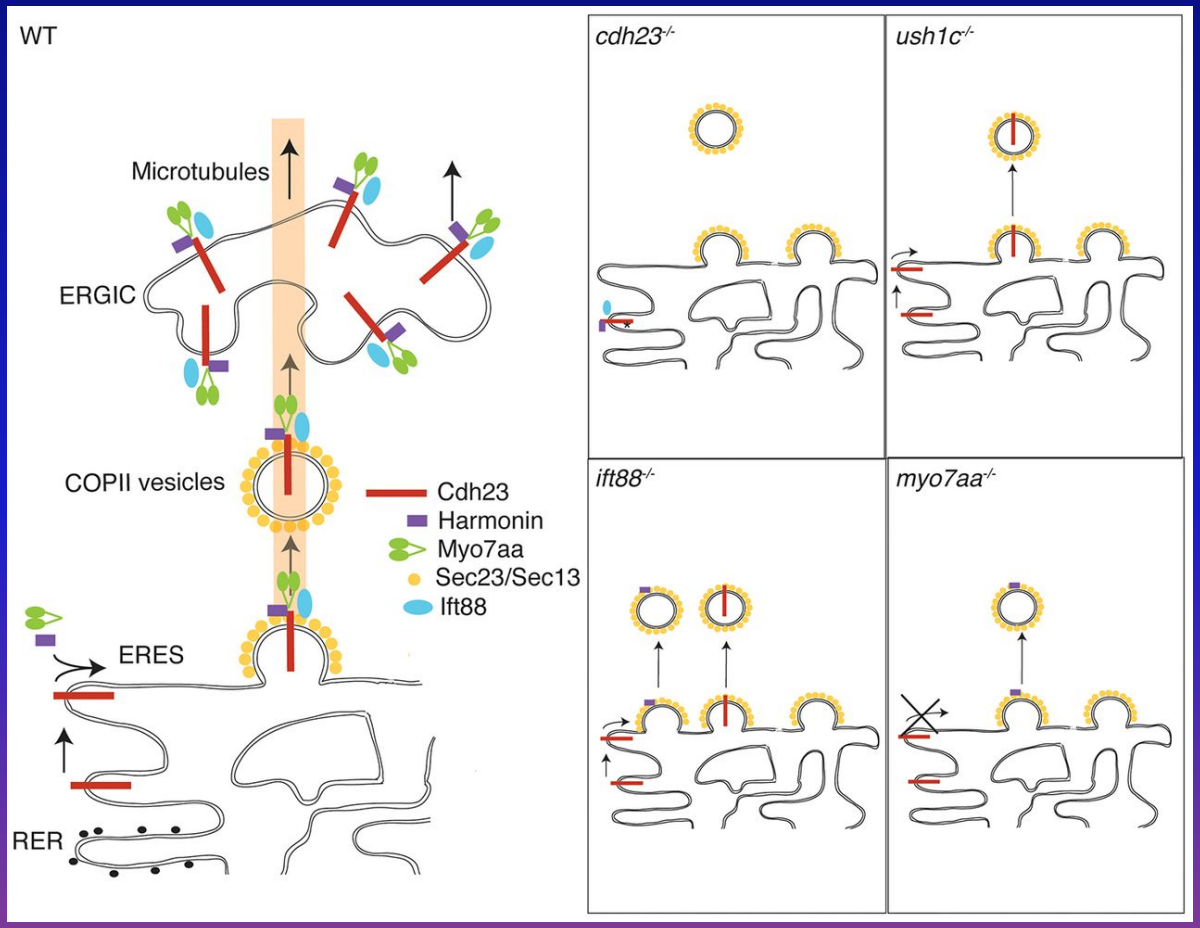
II- Advances in understanding the patho-physiology of Usher Syndrome



Monte Westerfield



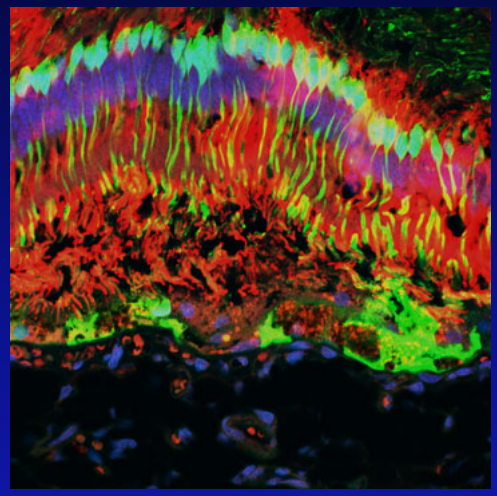
"Defective protein complex assembly produces ER stress that causes cell death in Usher Syndrome"



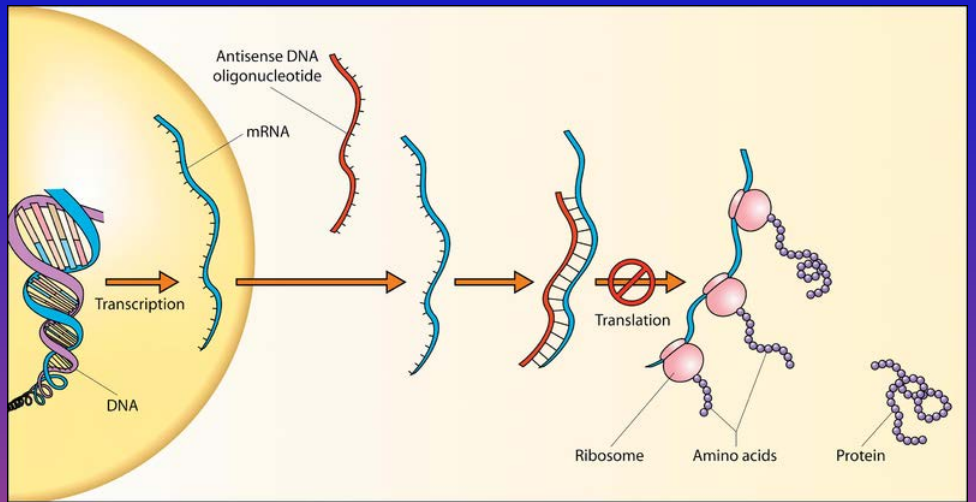
Ush1 proteins are shown to pre-assemble in the endoplasmic reticulum. Disruption in any one of the Ush1 protein partially disrupts trafficking of the complex as well as Ush2 proteins. This defect leads to ER stress and apoptosis (cell death).

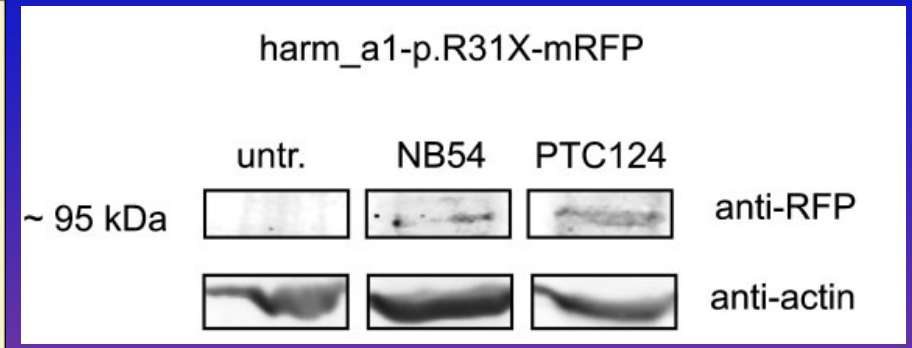
III- Treatment: Strategies for restoring function

- Reinsertion of the missing link
  - a- Engineering AAV vectors: *Luk Vandenberghe*
  - b- Gene Therapy for Usher Syndrome Interaction partners. *Jeff Holt*



- Correction of translation
  - a- Antisense Oligonucleotides *Jennifer Lentz*
  - b- Translation read-through: *Kirsten Nagel Wolfrum*





III- Treatment: Strategies for restoring function

Reinsertion of the missing link

Engineering AAV vectors: *Luk Vandenberghe*

REVIEW

Novel adeno-associated viral vectors for retinal gene therapy

Gene Therapy (2012) 19, 162–168

AAV : non pathogenic, highly stable viral vector (carrier)

At least 30 virus of different serotypes have been identified

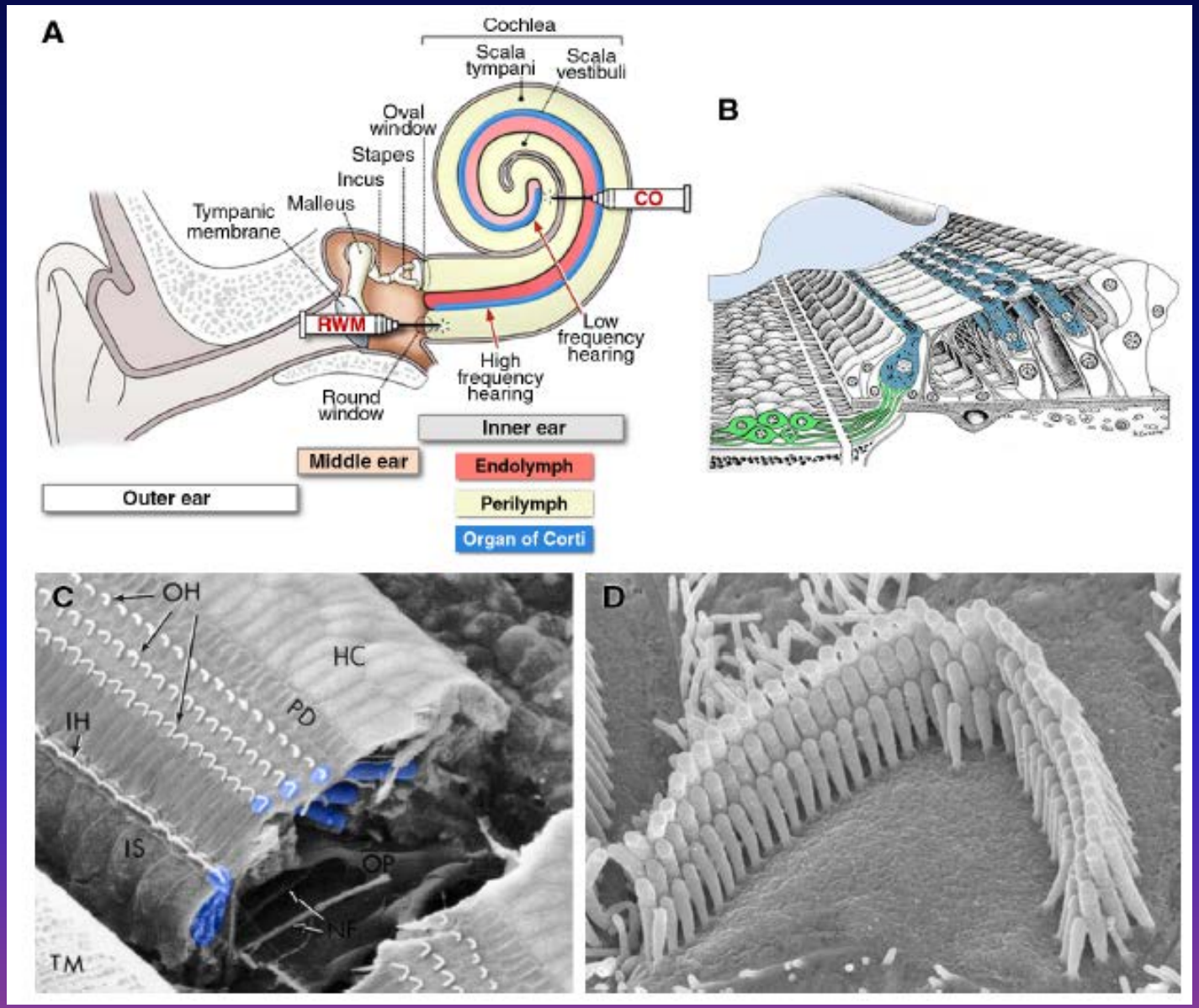
Important goal: Develop vectors for which the **host immune response is limited**.

Subretinal injection of adeno-associated viral vector (AAV) with different types of AAV, each of which have distinct specificity for the various retinal cell types. Peter Mallen

III- Treatment: Strategies for restoring function

Reinsertion of the missing link

- *Gene Therapy for Usher Syndrome Interaction partners. Jeff Holt*



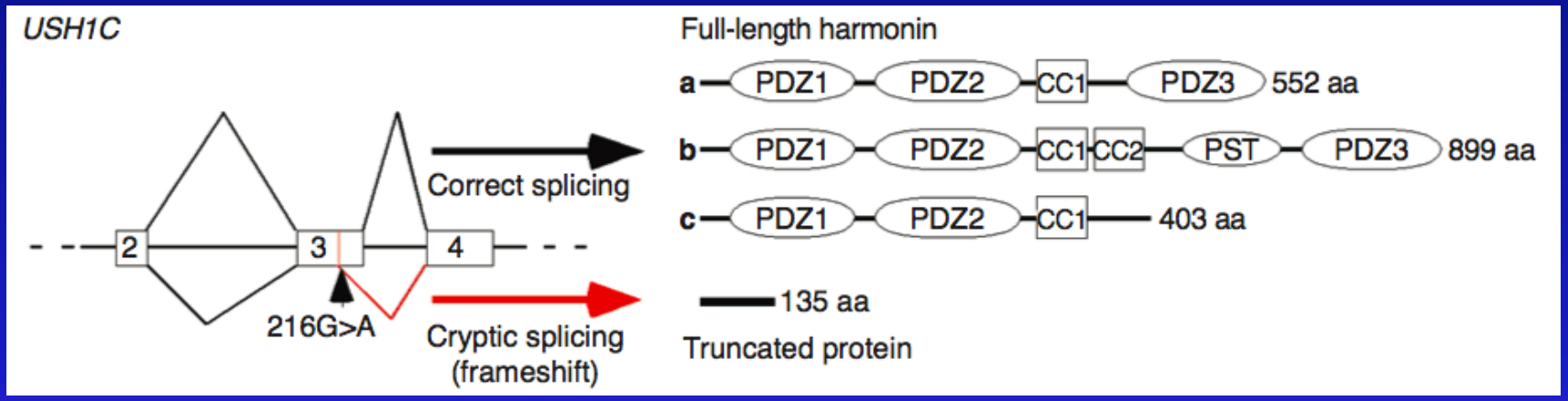
III- Treatment: Strategies for restoring function

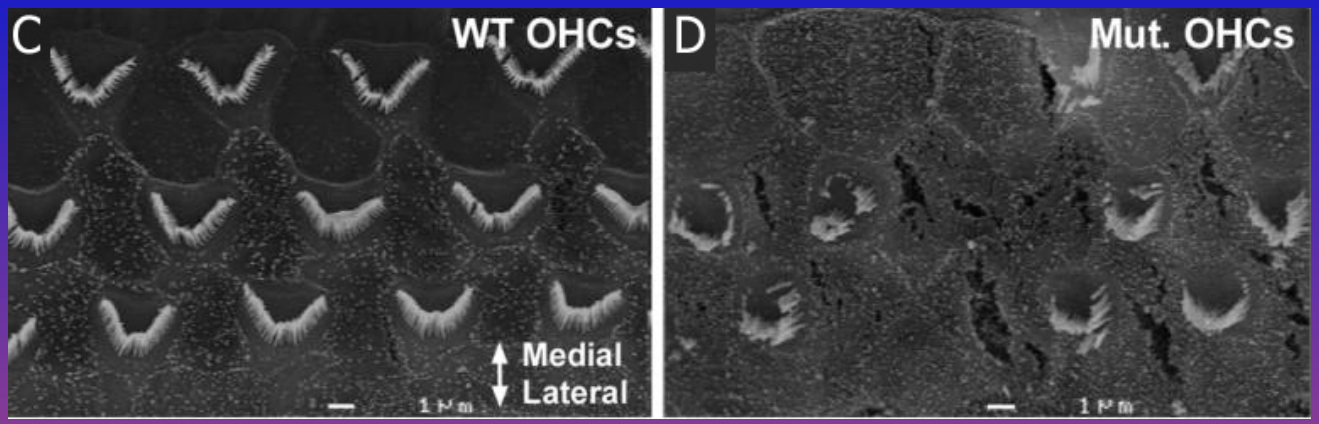
Correction of translation

Jennifer lentz (USH1C)

"Antisense oligonucleotides effectively treat Usher Syndrome in mice."

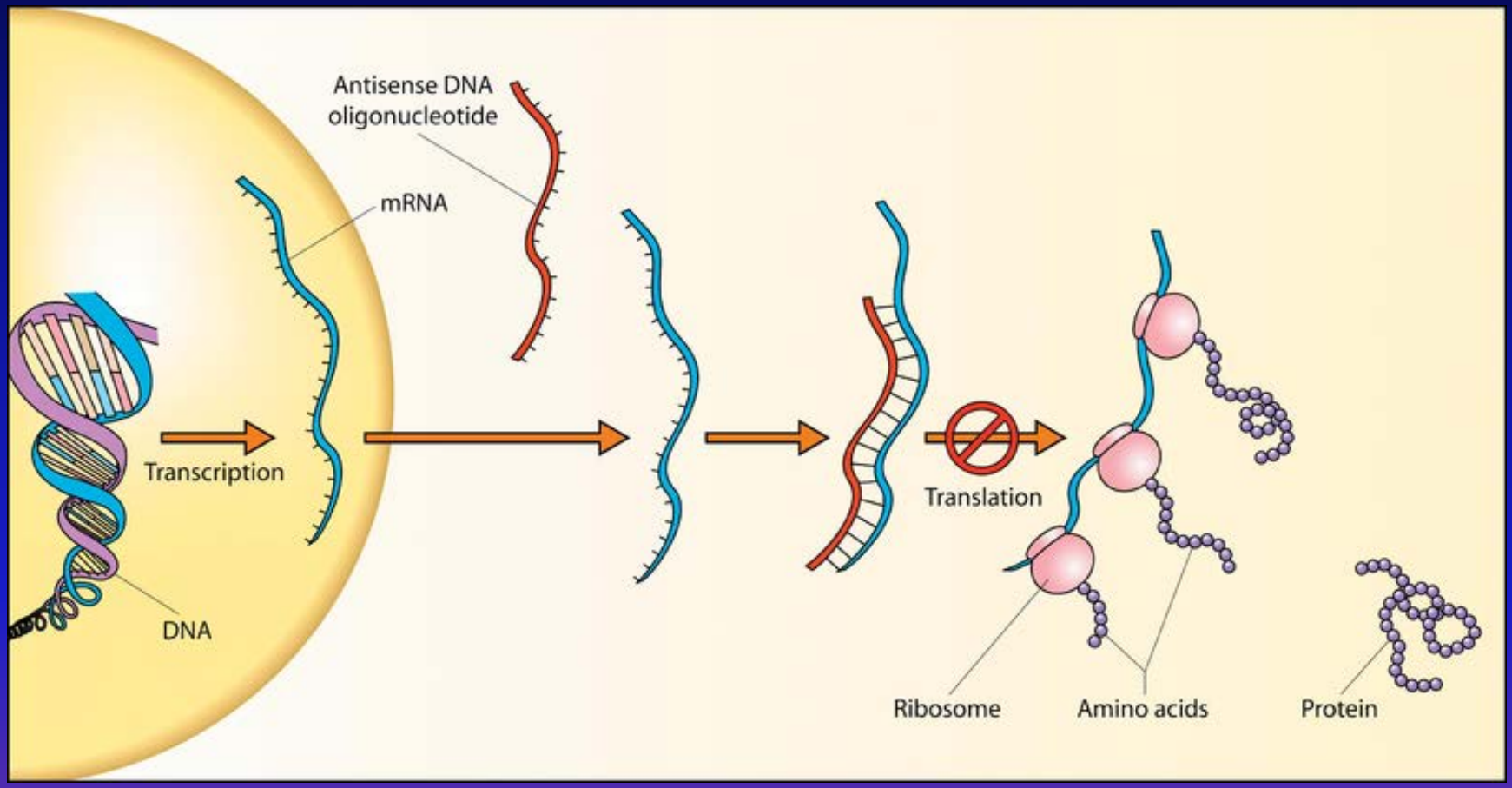
Frame shift mutation found in French-Acadian USH1C patients
Results in a severely truncated protein and affects all harmonin isoforms





III- Treatment: Strategies for restoring function

Correction of translation



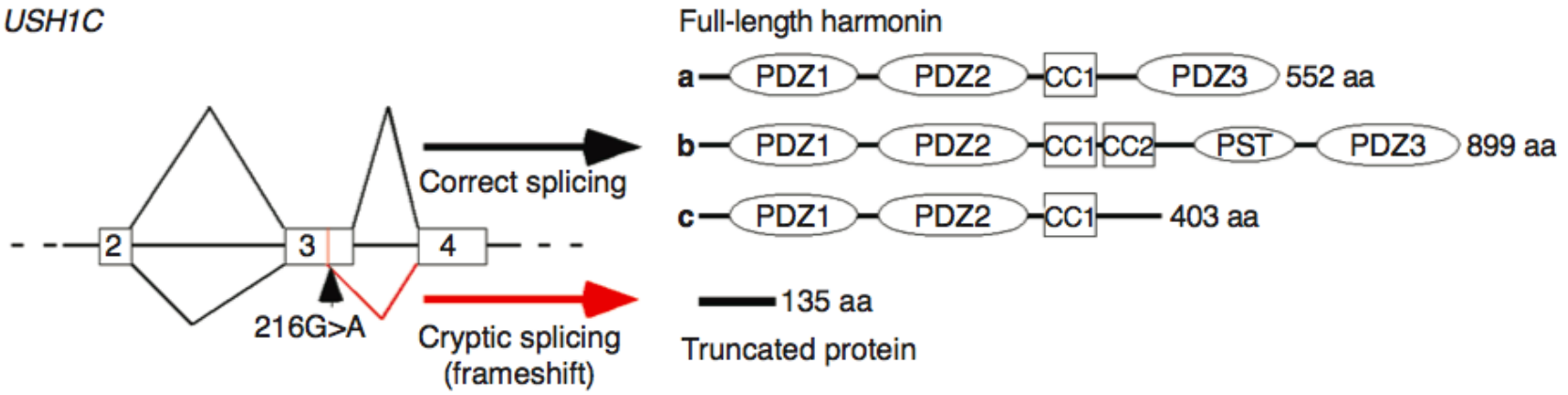
III- Treatment: Strategies for restoring function

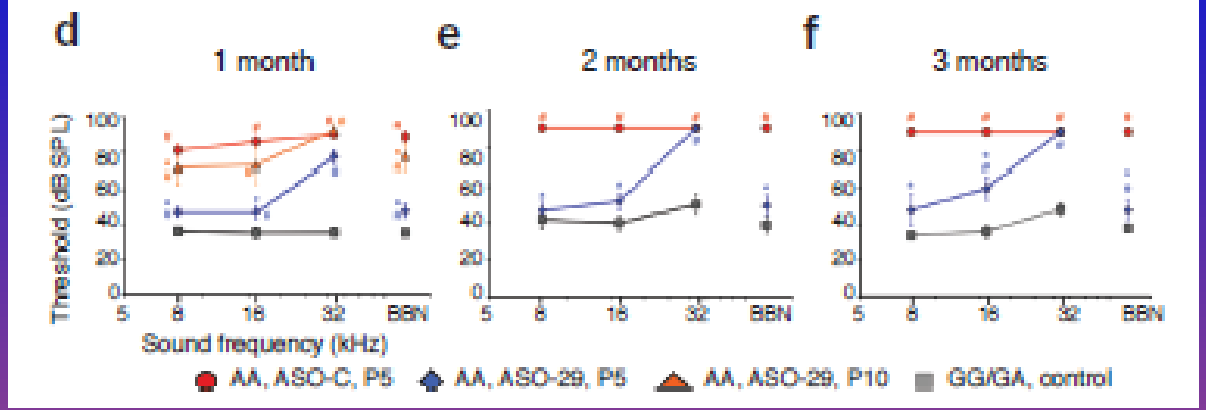
Correction of translation

nature medicine

Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness

Jennifer J Lentz[1,6], Francine M Jodelka[2,6], Anthony J Hinrich[2,6], Kate E McCaffrey[2], Hamilton E Farris[1], Matthew J Spalitta[1], Nicolas G Bazan[3], Dominik M Duelli[4], Frank Rigo[5] & Michelle L Hastings[2]





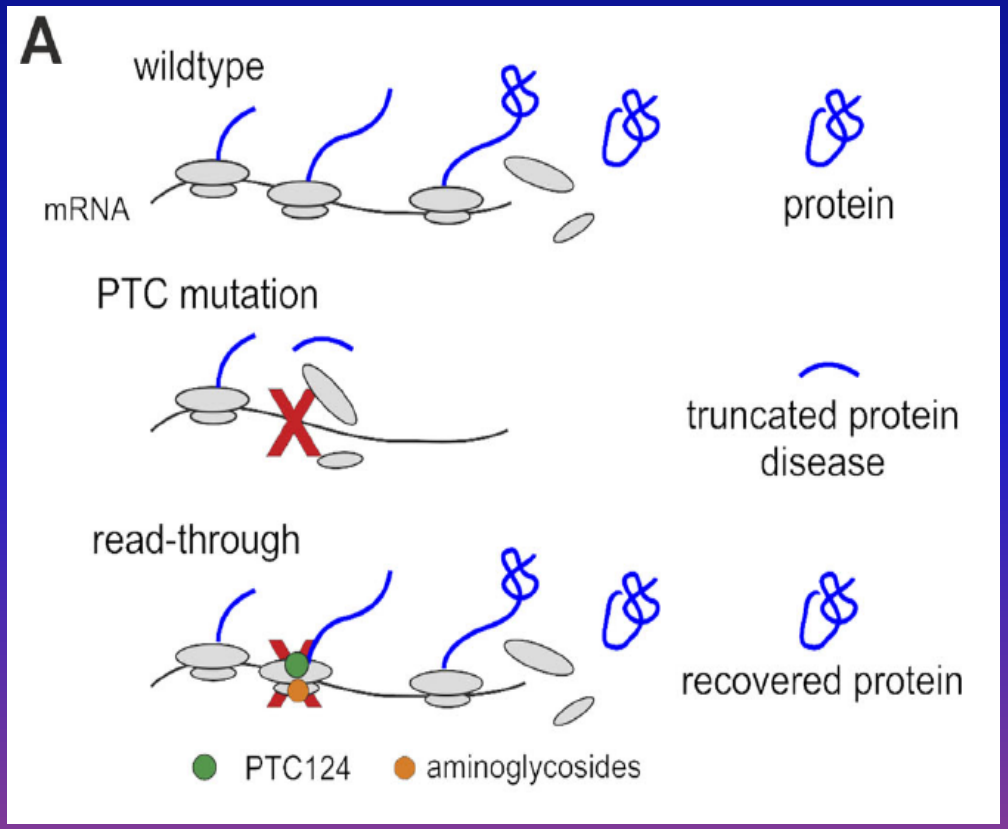
III- Treatment: Strategies for restoring function

Correction of translation

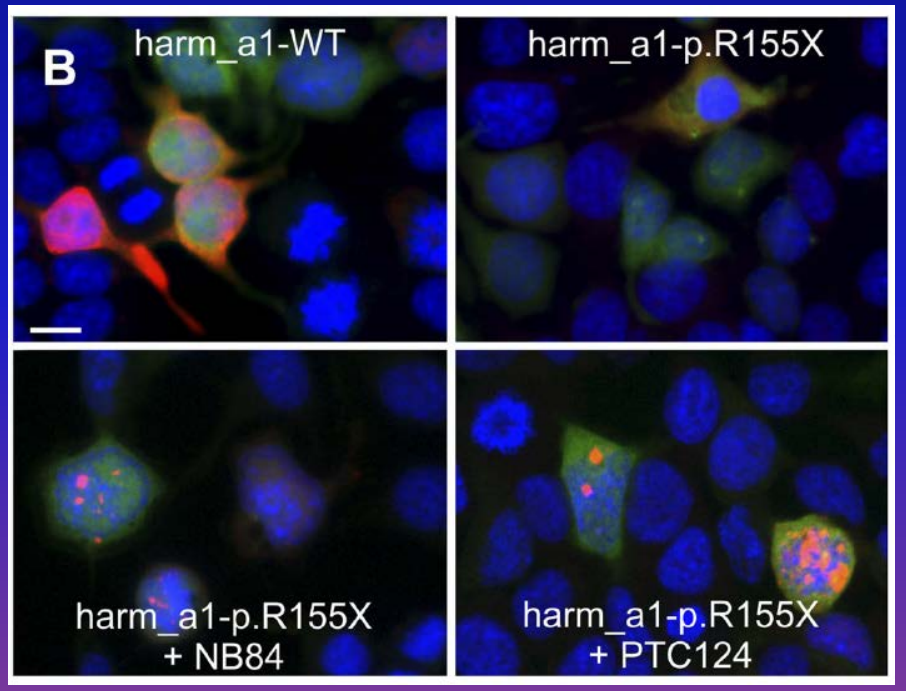
Kirsten Nagel Wolfrum

"Translation read-through to treat hereditary retinopathy"

TRIDS: Drugs that target in-frame non sense mutations (premature stop)- Most studied: Amynoglycosides

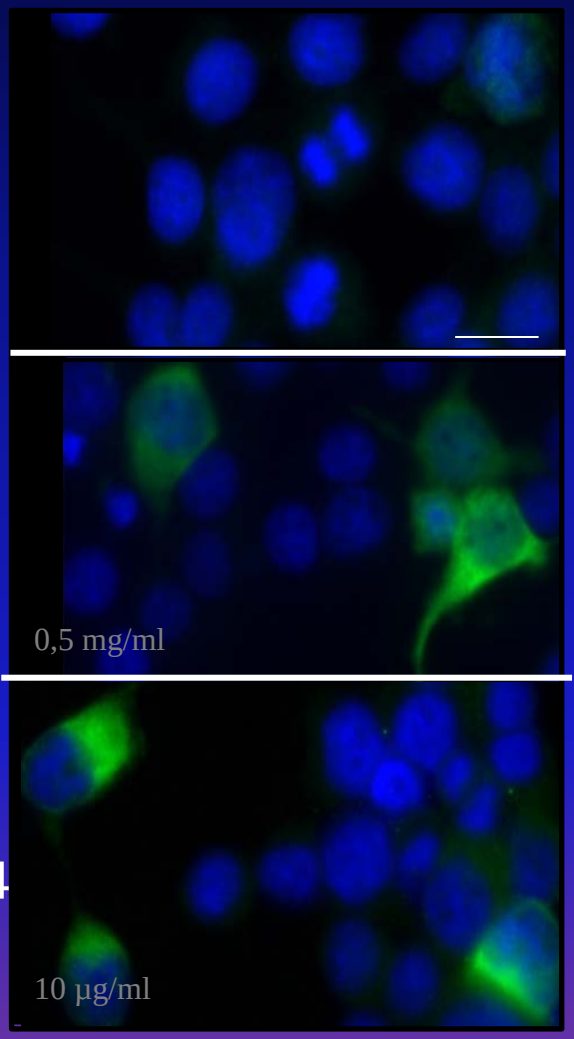


pR155X USH1C in-frame non sense mutation (harmomin: red)

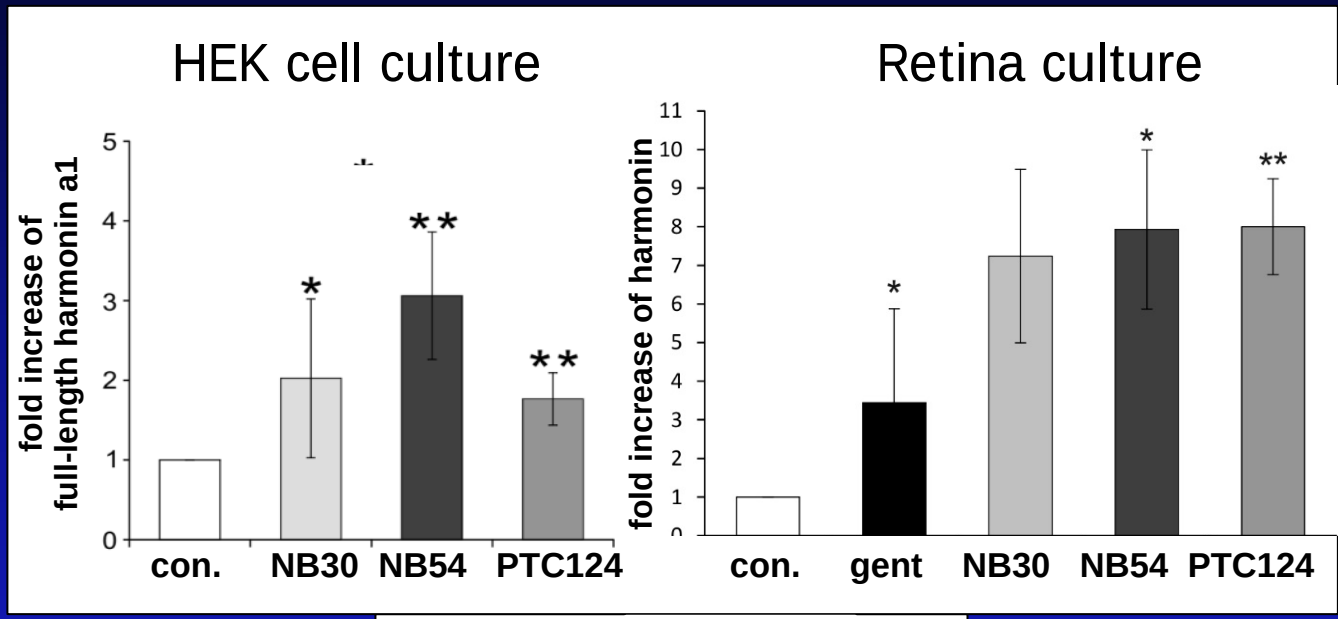


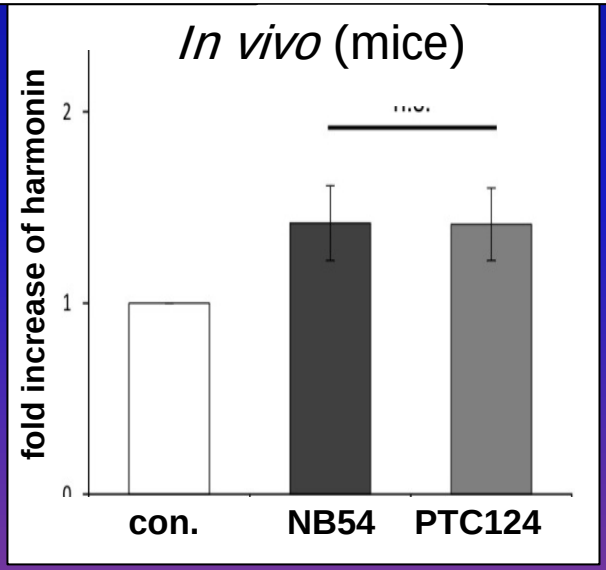
III- Treatment: Strategies for restoring function

Recovered harmonin protein expression after translational read-through



Harmonin (green)





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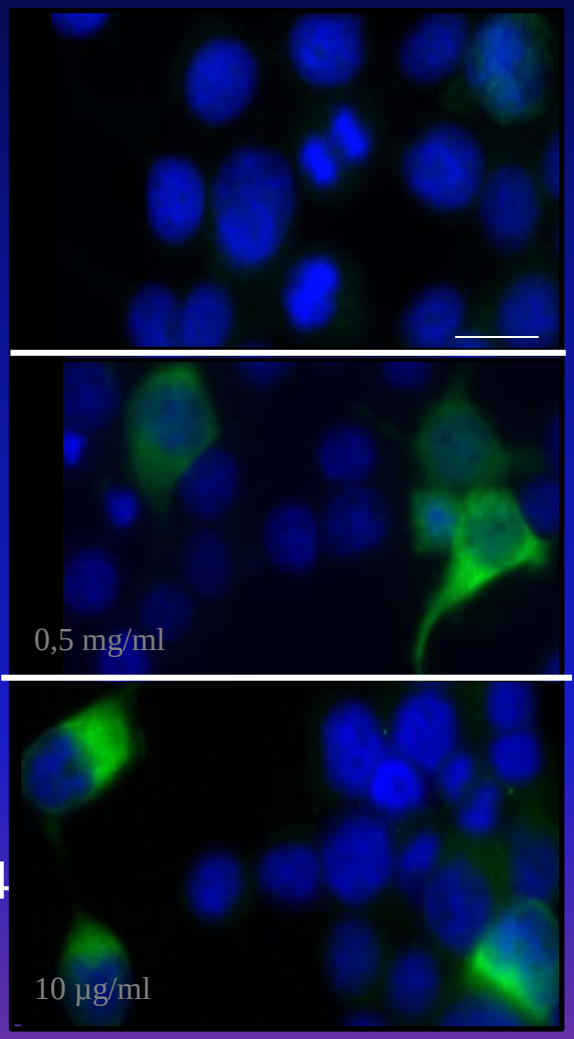
Goldmann et al. (2012) EMBO Mol Med 4:1186-99

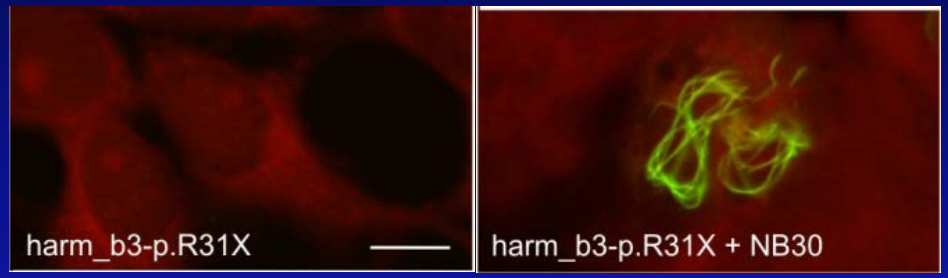
III- Treatment: Strategies for restoring function

Recovered harmonin protein expression after translational read-through
Recovered functional harmonin



Harmonin (green)

Restored harmonin_b3 actin bundling capacity:



Also now study with other Usher causing non-sense mutations:

- USH1F (PCDH15)
- USH2A (Usherin)
- USH2C (GPR98)
- USH3A (Clarin 1)

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III- Treatment: Strategies for restoring function

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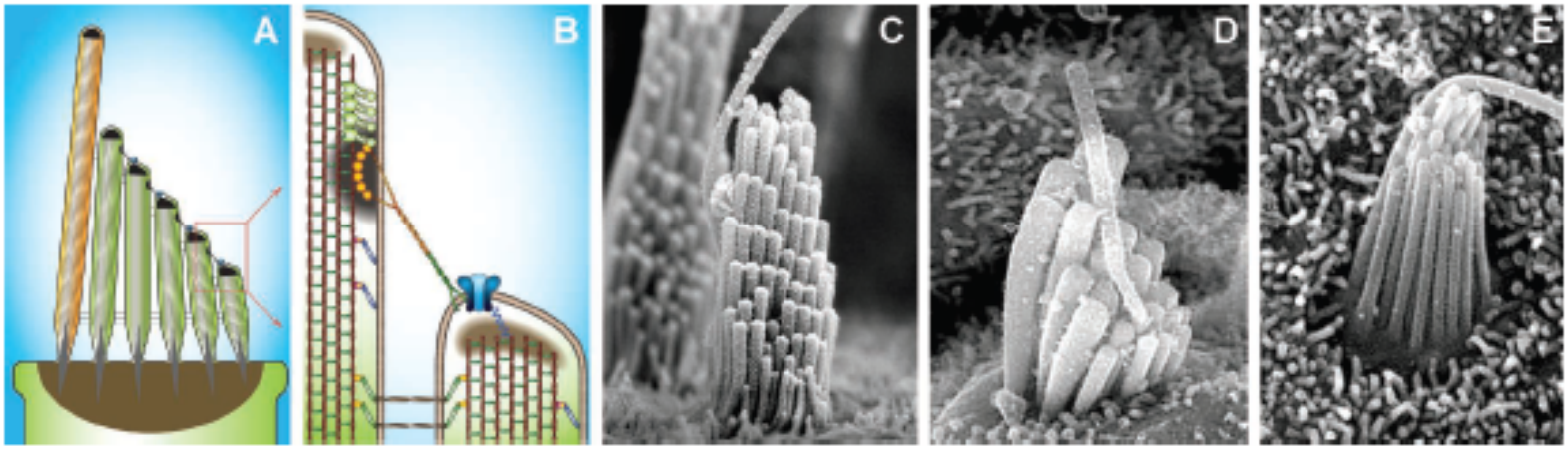
Sound Strategies for Hearing Restoration

Gwenaëlle S. G. Géléoc and Jeffrey R. Holt*

Background: Sensorineural hearing loss is the most common sensory deficit in the world, with nearly 300 million affected individuals. The problem is multifactorial and can arise from damage or death of the primary sensory cells or the inner-ear neurons that relay auditory information to the brain. The inner-ear sensory cells and neurons can be damaged by environmental insult (such as exposure to infectious agents, drugs such as aminoglycoside antibiotics or chemotherapeutics, or overexposure to loud sounds) or by a host of genetic factors. Because mature inner ears lack the capacity for self-repair, the cellular damage is permanent. In addition to acquired hearing loss, more than 300 genetic loci have been linked to hereditary hearing loss, with about 70 of the causative genes identified. Eighty percent of genetic hearing loss is recessive, with the rest inherited as a dominant trait. More subtle genetic defects that cause a predisposition toward age-related hearing loss are poorly understood. Unfortunately, there is no cure for acquired, inherited, or age-related hearing loss.

Advances: Efforts to restore and repair damaged inner-ear cells have intensified over the past 10 years. Thus far, a major thrust has been to adapt three biological strategies for use in the inner ear: gene therapy, stem-cell therapy, and molecular therapy. Using these approaches, researchers have restored sensory function at the cellular level in animal models of human hearing loss. A few reports suggest functional recovery at the systems and behavioral levels, although caveats remain.

Outlook: As the population continues to age and expand, so will the number of patients who suffer from clinically serious hearing loss. As such, the need for a deeper and comprehensive understanding of hearing-loss therapies is more pressing than ever. Although the pace of progress is accelerating and clinical trials are on the horizon, it is clear that there are still a number of hurdles to overcome. Overall, there are reasons to be both cautious and optimistic as we attempt to repair and regenerate one of nature's most exquisite mechanosensory devices: the human inner ear.



The many faces of Usher Syndrome Research