Modeling Usher 1B pathogenesis:
Which readouts are pertinent for therapeutic beneficial outcomes?

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Presentation outline:

1- Myosin VIIa/USH1B in the inner ear

2- Myosin VIIa/USH1B in the eye

3- Unifying themes for Usher protein in audition, balance and vision

USH1B workshop, Sept 13, 2021

The Usher syndrome (USH)

First cause of deafness-blindness in humans

Three clinical subtypes : USH1, USH2 and USH3

	Hearing impairment		Vestibular dysfunction		Retinitis Pigmentosa
USH1 (5-6 genes)	**Profound and congenital**		**Severe**		**Prepubertal onset**
USH2 (3 genes)	**Mild to severe and congenital**		**absent**		**Postpubertal onset**
USH3 (1 gene)	**Postlingual, mild and progressive**		**variable**		**variable**

How do the eye and ear work ?

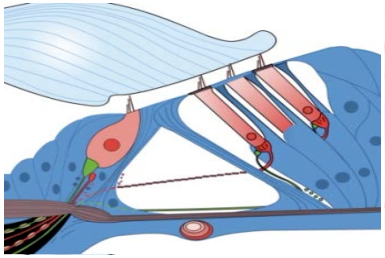
Sound & head/ body motion stimuli

The ear

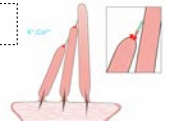
The inner ear

Vestibule:
Balance organ

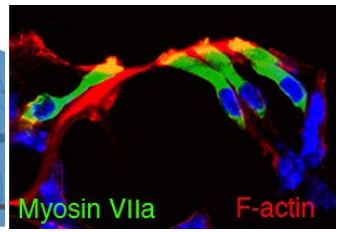
Cochlea:
hearing organ

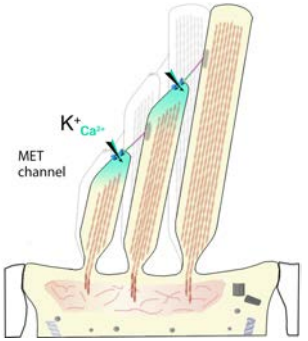


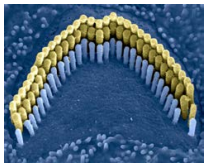
The hair bundle



inner (IHC) and outer (OHCs) hair cells







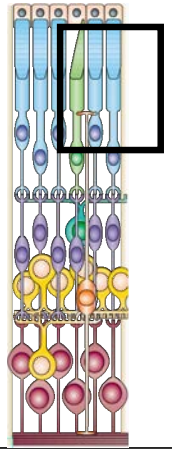
The hair bundle:
50 to 200 F-actin filled stereocilia

site of mechano-electrical transduction

Light stimuli

The eye

The retina

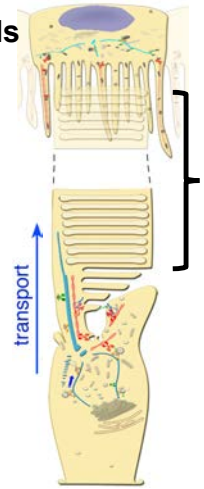


rods: night vision cones: day vision

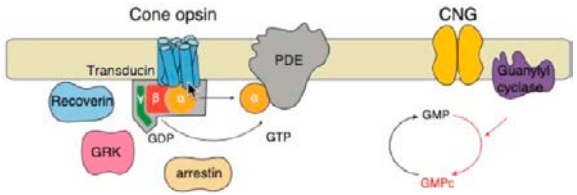
photoreceptor cells

RPE cells

~2000 opsin molecules per minute transit through the connecting cilium



site of light signal phototransduction

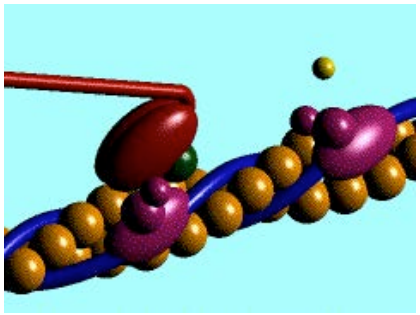


The outer segment:
~ 100 to 1000 phototransducing disks

Understanding Myosin VIIa properties & functions ?

The Usher syndrome type IB (USH1B), 70 to 80% of USH1 cases:

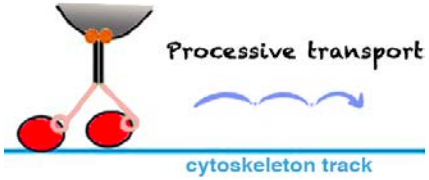
Myosin VIIa, an actin based, multi-domain containing motor protein



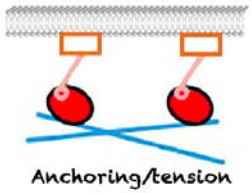
- **Motor head domain**
  - **Neck region:** 5 IQ (Isoleucine/glutamine) motifs
    - **C terminal region:**
      Dimerization domain
      Tandem MyTH4 /FERM domains
      SH3 (Src homology domain)

Potential role(s)?

✓ Protein and organelle transport ?



✓ Actin organization ? Anchoring & tension force at specific membrane microdomains

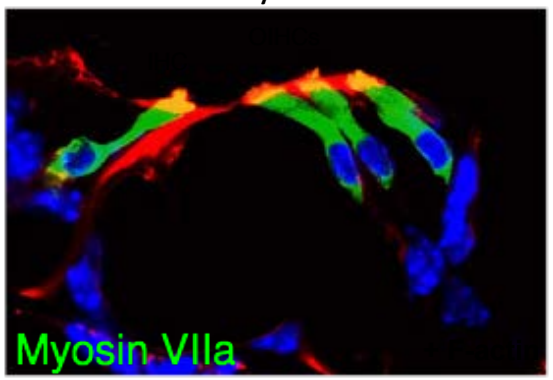


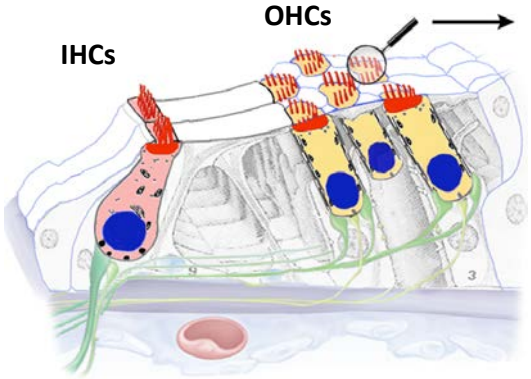
Cellular and subcellular targets of USH1B protein ?

The ear

❖ Inner ear: the sensory hair cells & the mechano-sensitive hair bundles

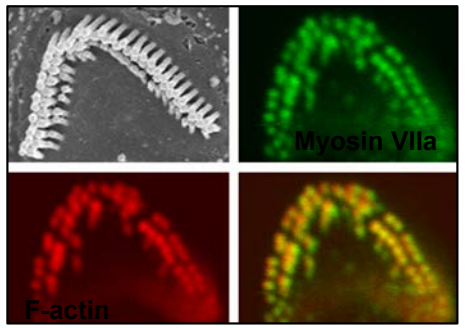
Auditory hair cells





Auditory sensory organ: organ of Corti

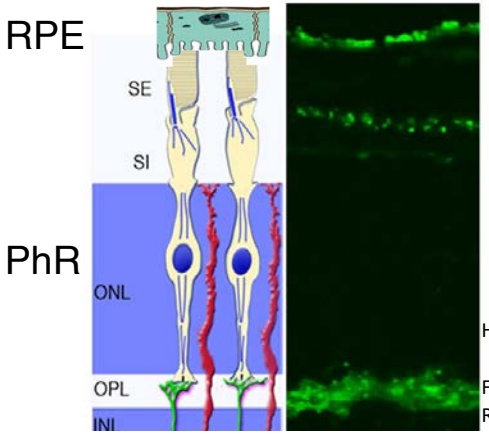
Sound receptive-hair bundle



El-Amraoui A, et al., HMG 1996

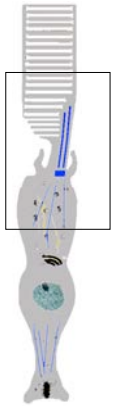
The eye

❖ Retina: Photoreceptors (PhR) & retinal pigment epithelial cells (RPE)



Human retina

From Reiners et al. 2003



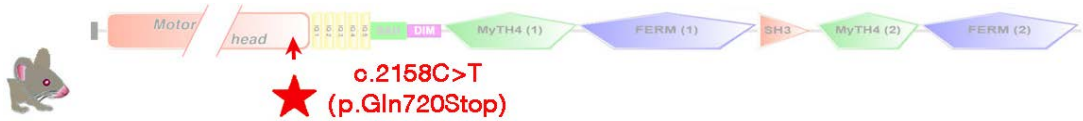
Myosin VIIa

Liu X et al.-> Williams D 1997

Source: U. Wolfrum 2000

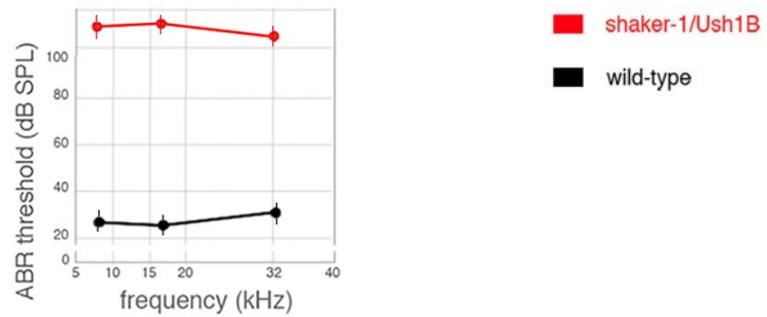
Myosin VIIa defective shaker-1 mutants

Shaker-1 Myo7a $^{-/-}$ mice



In absence of a functional myosin VIIa, the mutant mice display:

- **Congenital, profound hearing loss**



profound hearing loss
(threshold over 100 dB)

- **Bilateral vestibular dysfunction**

Open field exploratory behavior (@P30)

Vestibular balance deficits
(hyperactivity and circling behavior)

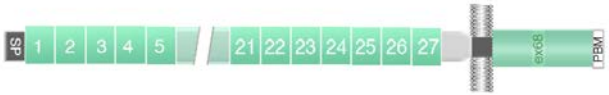
Genes responsible for Usher syndrome (USH)

USH1

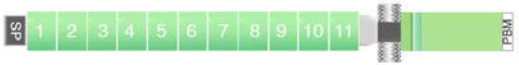
USH1B (*MYO7A*, 11q13.5 - OMIM 276903) : **myosin VIIa**

USH1C (*USH1C*, 11p15.1 - OMIM 605242) : **harmonin**

USH1D (*CDH23*, 10q22.1 - OMIM 605516) : **cadherin-23**



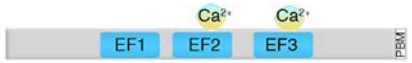
USH1F (*PCDH15*,10q21.1 - OMIM 605514) : **protocadherin-15**



USH1G (*USH1G*, 17q25.1 - OMIM 607696) : **Sans**

Atypical form

DFNB48/USH1J (*CIB2*, 15q25.1 - OMIM 605564) : **calcium integrin binding protein 2**

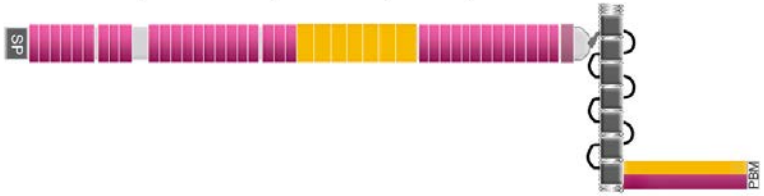


USH2

USH2A (*USH2A*, 1q41 - OMIM 608400) : **usherin**

«transmembrane form»

USH2C (*GPR98*, 5q14.3 - OMIM 602851) : **ADGVR1** (adhesion G-protein coupled receptor V1)

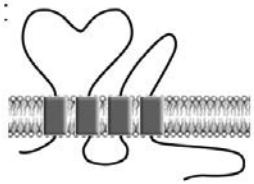


USH2D (WHRN, 9q32 - OMIM 607928) : **whirlin**

Long isoform (L)

USH3

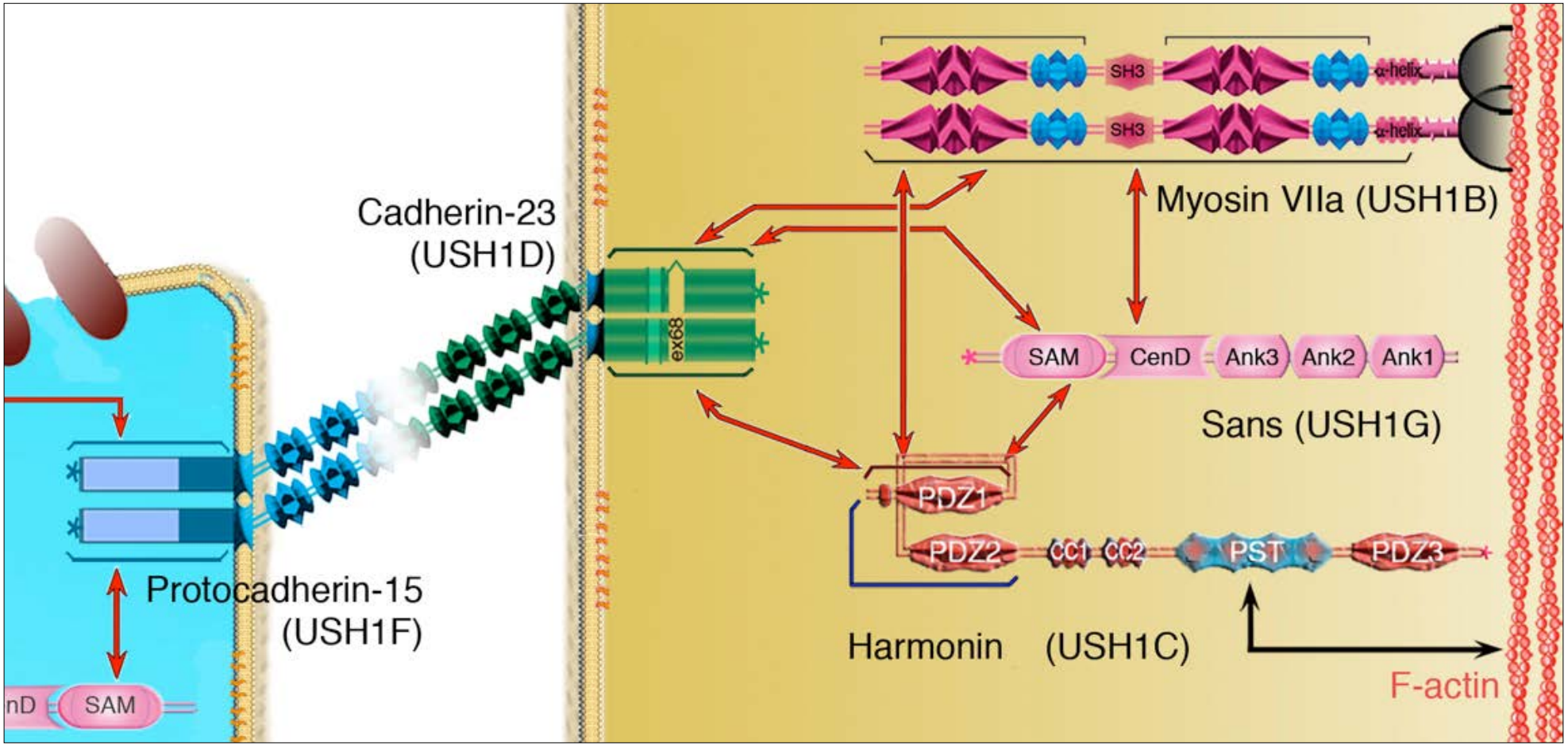
USH3A (*CLRN1*, 3q25.1 - OMIM 606397) : **clarin-1**



Geleoc G & El-Amraoui A, Hear Res. 2020

Genes responsible for Usher syndrome (USH)

The Usher type I « interactome » : each Usher 1 protein interacts with at least one other Usher 1 protein

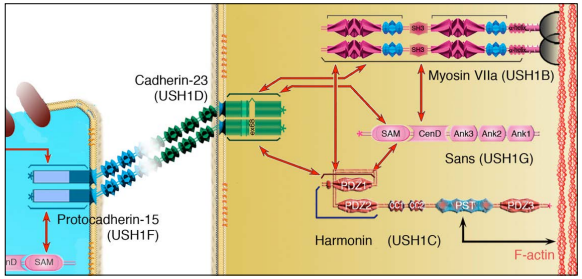


Geleoc G & El-Amraoui A, Hear Res. 2020

Usher syndrome type I (USH) genes & mechanisms

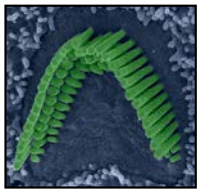
Five Usher 1 proteins:

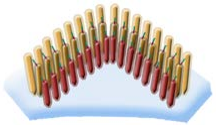
The Usher type I « interactome



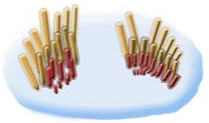
There exist mouse mutants for each of the Usher genes:
they all display profound deafness, balance deficits (for USH1), similar to human patients

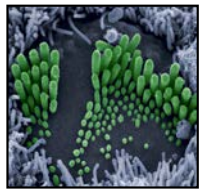
Origins of the hearing loss in the Usher syndrome ?





without
Usher 1 proteins

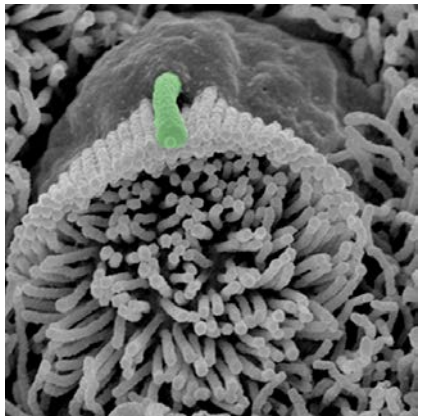




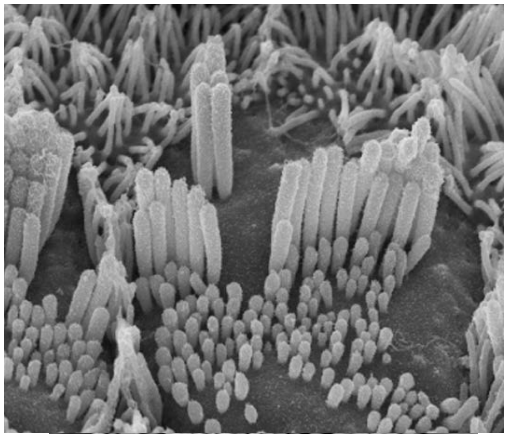
The lack of any USH1 protein, leads to the same type of abnormality:

fragmentation of the hair bundle, the sound receptive structure

control



Ush1b **-/-**

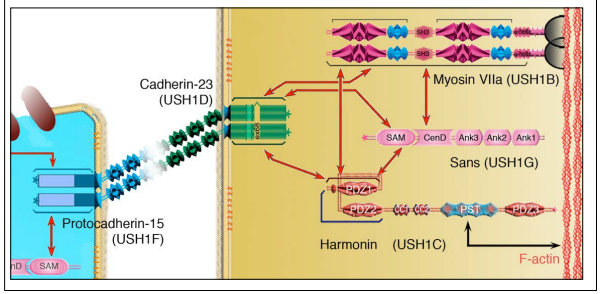


Kussel *et al. EMBO J* (2000); Boëda *et al. EMBO J.* (2002); Weil *et al. Hum. Mol. Genet. (2003);* Adato *et al. HMG* .(2005); Michel *et al.* (2005); Lefèvre *et al. Development* (2008; Bahloul *et al.. HMG.* (2010)

Usher syndrome type I (USH) genes & mechanisms

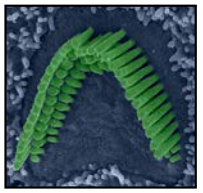
Five Usher 1 proteins:

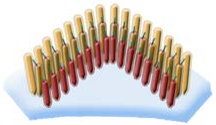
The Usher type I « interactome »



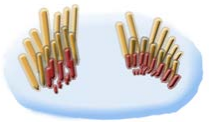
There exist mouse mutants for each of the Usher genes: they all display profound deafness, balance deficits (for USH1), similar to human patients

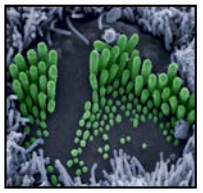
Origins of the hearing loss in the Usher syndrome ?





without
Usher 1 proteins

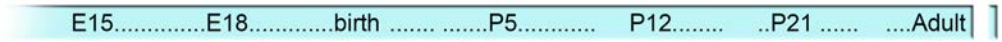




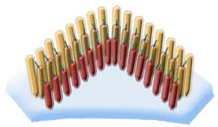
The lack of any USH1 protein, leads to the same type of abnormality:

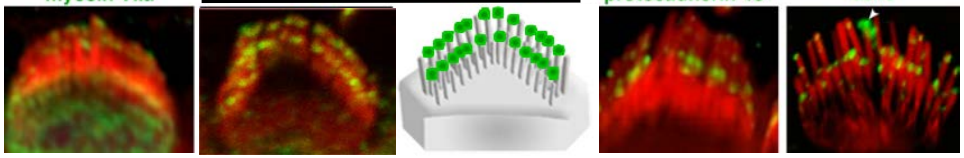
fragmentation of the hair bundle, the sound receptive structure

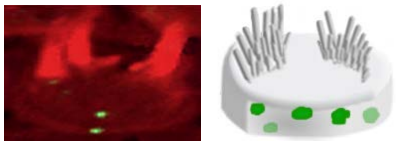
All five Usher 1 proteins colocalize at the apical region of the stereocilia, where sound transduction occurs:



Ush1 proteins expression





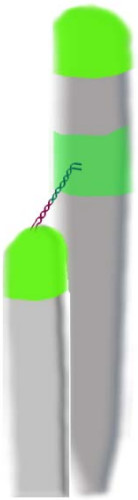


Without Myo7a (shaker-1 mouse)

(No USH1C in the stereocilia ?)

Usher 1 proteins
@stereocilia tips

(site of MET transduction)



Myosin VIIa cooperates with other USH1 proteins to shape properly the hair bundle

Usher 1 proteins form the apical inter-stereocilia links

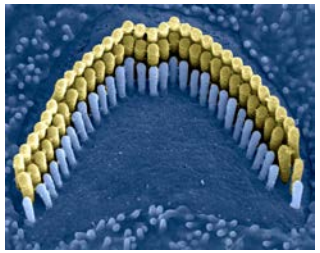
Myosin VIIa (USH1B)

Harmonin (USH1C)

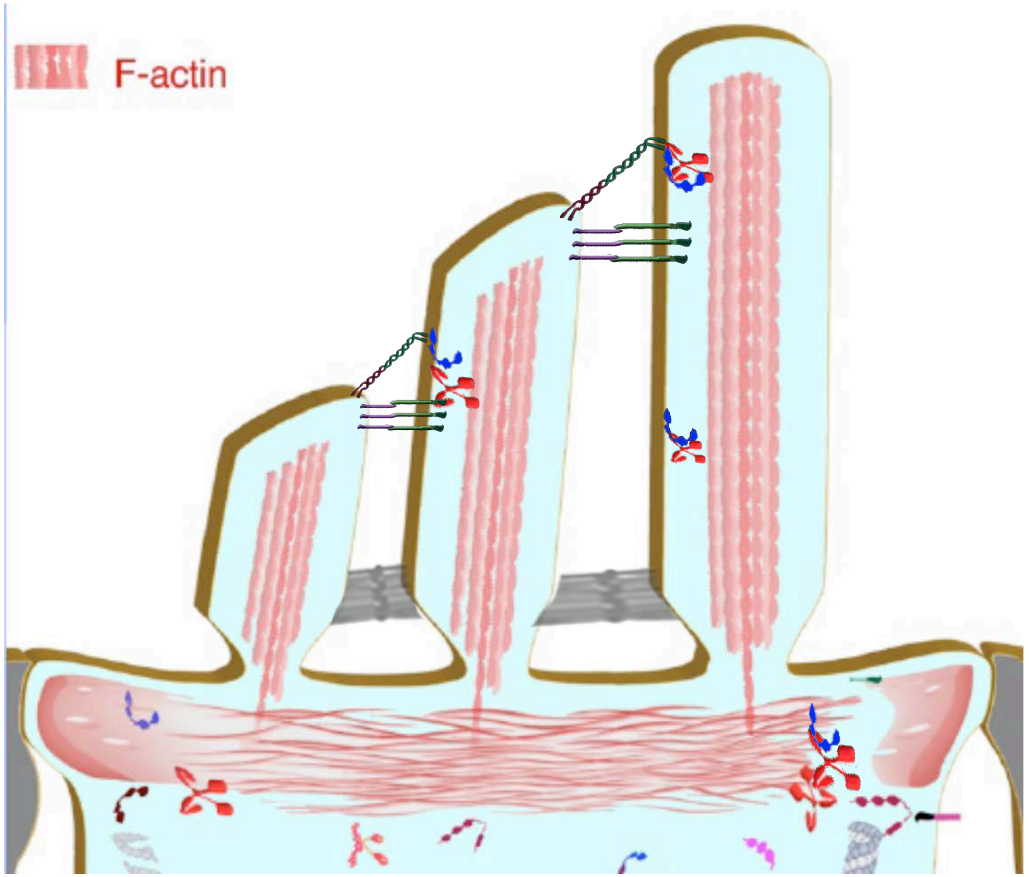
Protocadherin-15 & cadherin-23

USH1F/USH1D heterodimers

SANS (USH1G)



Normal Auditory Hair Bundle



- ✓ Myosin VIIa (USH1B) is required for the transfer of some USH1 and USH2 proteins into the stereocilia
- ✓ Myosin VIIa (USH1B) and Harmonin (USH1C) anchor the inter-stereocilia fibrous links to actin filaments
- ✓ Myosin VIIa (USH1B) is necessay for normal mechano-electrical transduction in mature hair bundles

Weil D *et al. PNAS* (1996); Verpy E *et al. Nat. Genet.* (2000); Kussel P *et al. EMBO J* (2000); Boëda B *et al. EMBO J.* (2002); Weil D *et al. Hum. Mol. Genet. (2003);* Adato A *et al. Hum. Mol. Genet.*(2005); Michel V *et al. Dev Biol.* (2005); Lefèvre G *et al. Development* (2008), Bahloul A et al. Hum Mol Genet. (2010); Bonnet C & EL-Amraoui A, Curr Opin Neurobiol.. 2012; El-Amraoui A & Petit C, CR Biol, 2014; Geleoc G & EL-Amraoui A, hear. Res. 2020

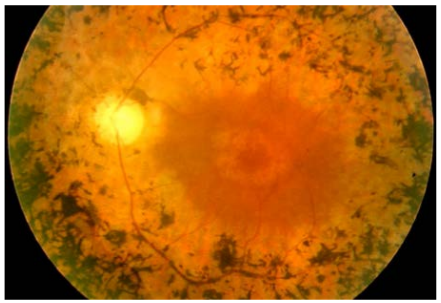
Usher type I syndrome: the origin of vision loss ?

	Hearing impairment	Vestibular dysfunction	Retinitis Pigmentosa
USH1	Profound and congenital	Severe	Prepubertal onset

Phenotype discrepancy between USH1 patients and related mouse models ?

Human

- Congenital deafness
- Circling behavior



- Retinitis pigmentosa

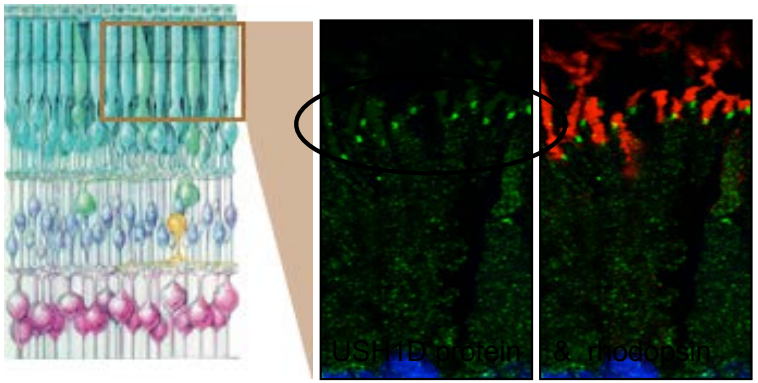
Mouse

- Congenital deafness
- Circling behavior

- No retinitis pigmentosa

Molecular and structural differences between mouse and primate photoreceptors

Primate (Human, Monkey**), Pig, Frog**

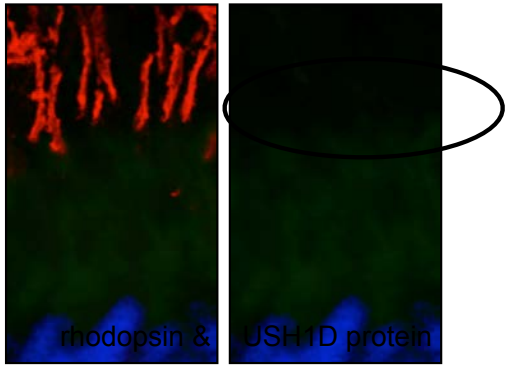


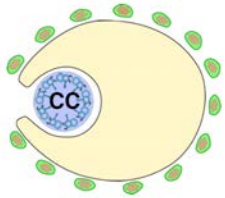
Mouse

The outer segment (OS):
the light sensitive
structure

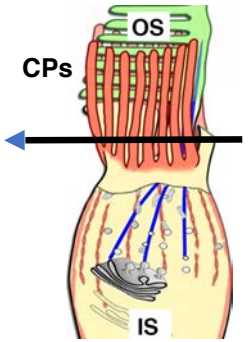
Connecting cilium
(CC)

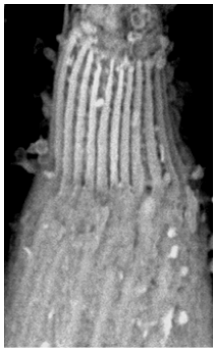
The inner segment (IS):
the metabolic
compartment





ring of
F-actin-labelled
calyceal processes



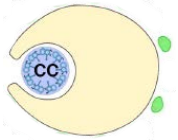


Sahly I. et al.
J. Cell Biol. (2012)

The outer segment
(OS)

Connecting cilium
(CC)

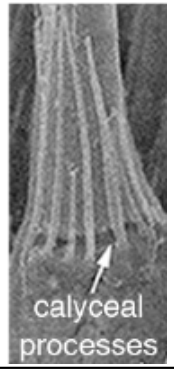
The inner segment
(IS)



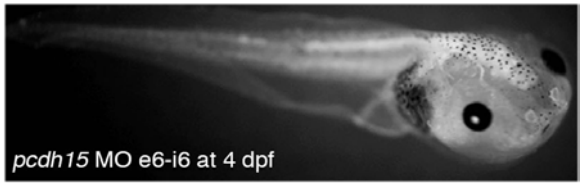
No ring of **calyceal processes (CPs)** in mouse
photoreceptor cells

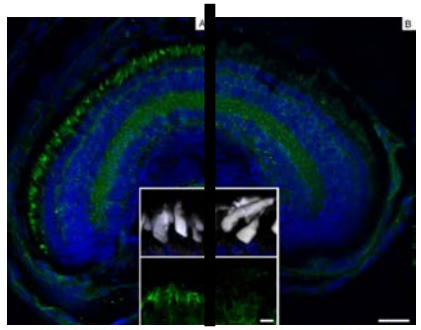
Production and characterization of pertinent animal models for USH1 retinopathy

THE AMPHIBIAN MODEL *(XENOPUS TROPICALIS)*

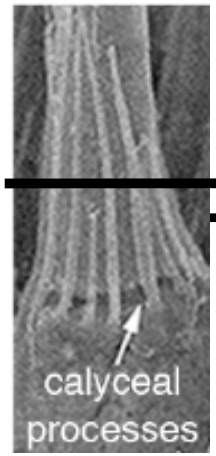


Morpholine (MO)-based approach to knock-down USH1 genes



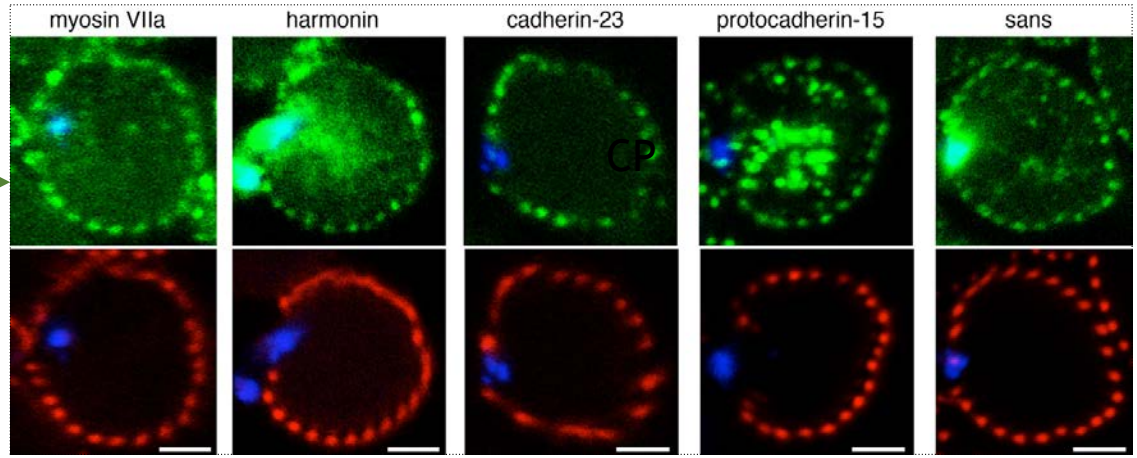


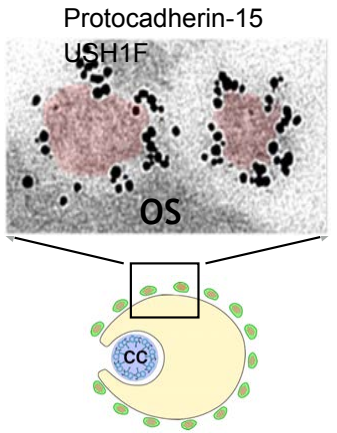
Collabs.
M. Perron (Paris Orsay
JA Sahel (IDV, Paris))



Usher 1 proteins

Cross-section of a photoreceptor cell



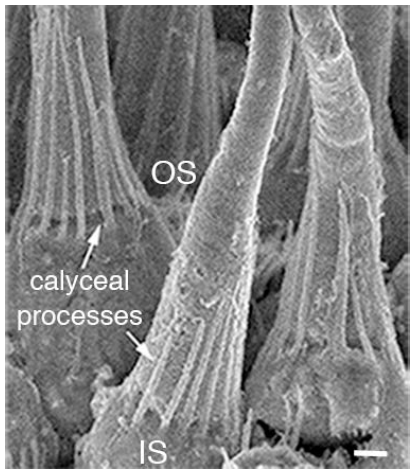


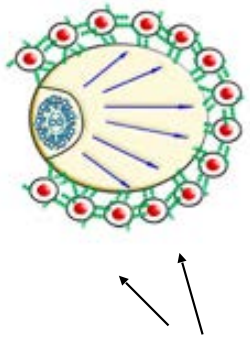
All USH1 proteins — myosin VIIa, harmonin, cadherin-23, protocadherin-15, sans — are detected in the calyceal processes of frog photoreceptors

Schietroma C *et al. J. Cell Biol.* (2017)

Production and characterization of pertinent animal models for USH1 retinopathy

control

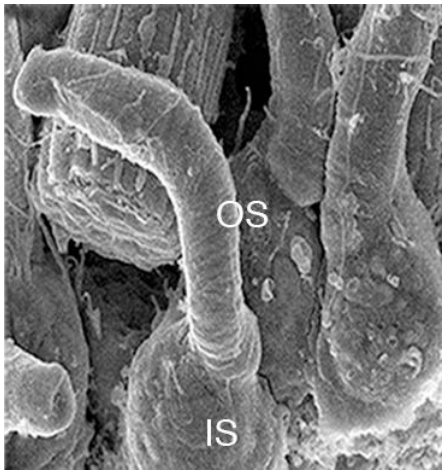


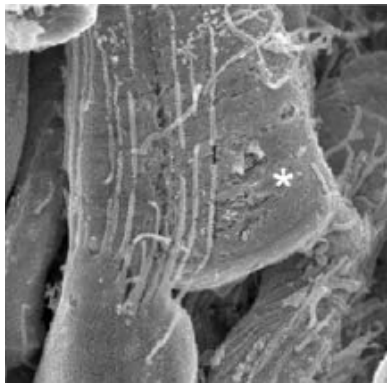


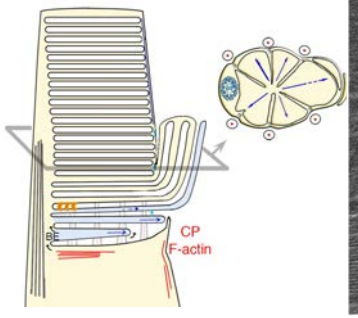
USH1 deficiency leads to alterations in the calyceal processes, along with impaired shape and function of photoreceptor outer segments

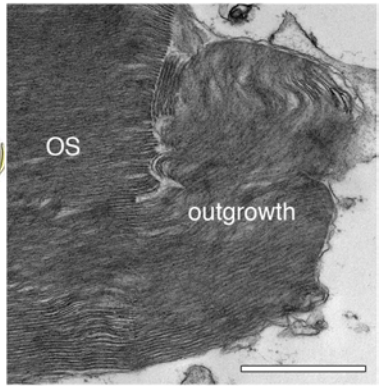
Pcdh15/USH1F morphant

no calyceal
processes





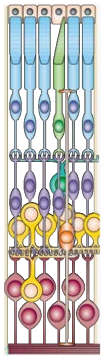




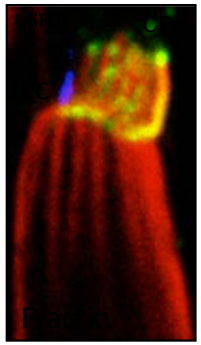
Molecular, structural, and phenotype differences between mouse and primate photoreceptors

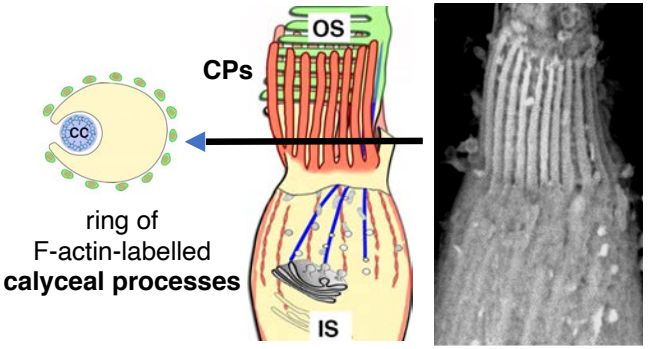
Primate (Human, Monkey**), Pig, Frog**

retina



transport





ring of
F-actin-labelled
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Sahly I. et al. *J. Cell Biol.* (2012)

Mouse

The outer segment
(OS)

Connecting cilium
(CC)

The inner segment
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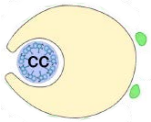
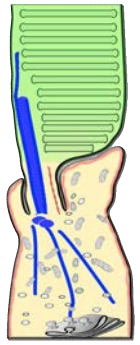
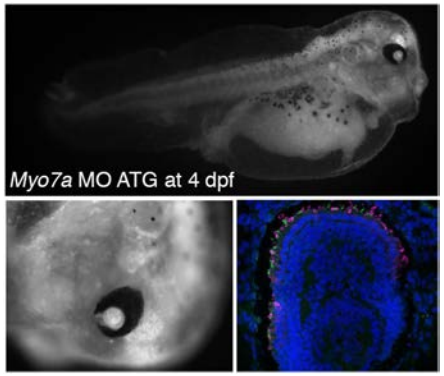




No ring of **calyceal processes (CPs)** in mouse photoreceptor cells

❖ Loss of USH1 function leads to defective calyceal processes & impaired outer segment disks morphogenesis

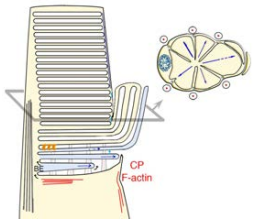
Morpholino-Based approach in Xenopus to study USH1 role in the retina

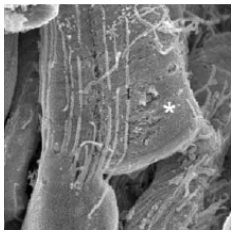


Schietroma C *et al.*
J. Cell Biol. (2017)

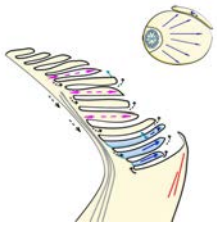
***X. tropicalis pcdh15/USH1F* morphant**

rod photoreceptor





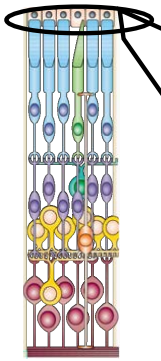
cone photoreceptor



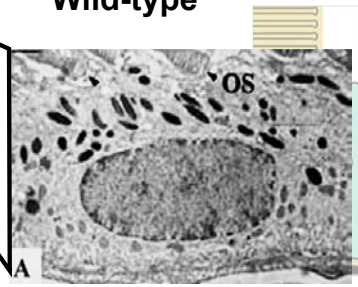
Retinal abnormalities in shaker-1 mice (defective myosin VIIa)

- **Decreased outer segment phagocytosis in RPE cells** *Gibbs D et al, …> Williams DS, PNAS 2003*
- **Melanosome mislocalization in RPE cells** *Liu X et al, …> Williams DS, 1998*

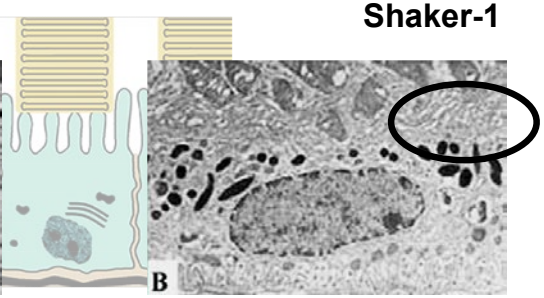
RPE

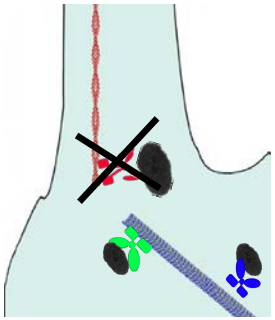


Wild-type



Shaker-1

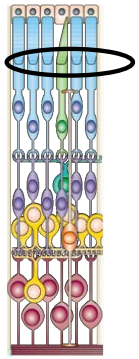


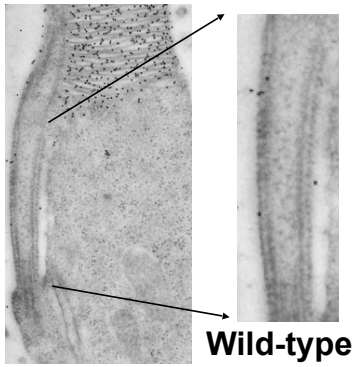


No transfer in absence of myosin VIIa

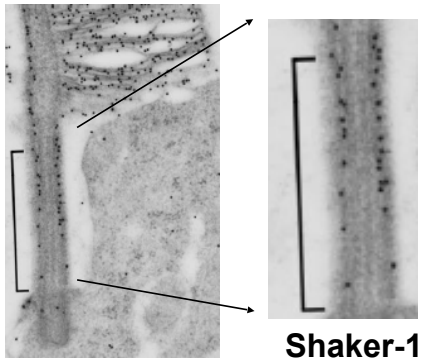
- **Opsin transport delay in photoreceptor cells** *Liu X et al, …> Williams DS, 1999, Wolfrum U & Schmitt A, 2000*

Photoreceptor cells





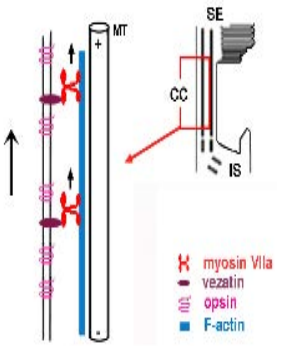
Wild-type



Shaker-1

Connecting cilium

Slower transport in absence of myosin VIIa

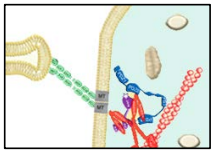


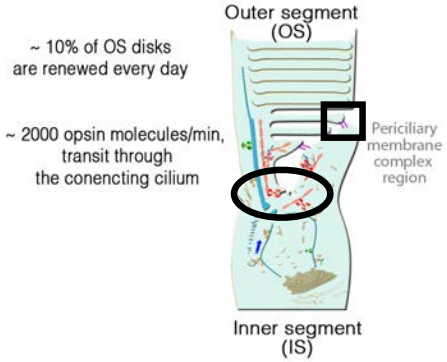
Conclusion (1)

Two key functions for myosin VIIa in Usher target cells?

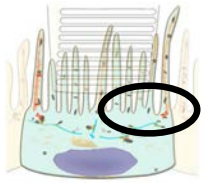
RPE and Photoreceptor retinal cells

The light-sensitive outer segment of photoreceptor cells



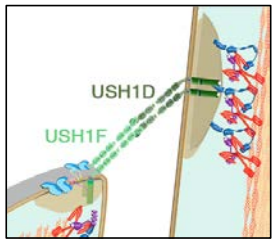


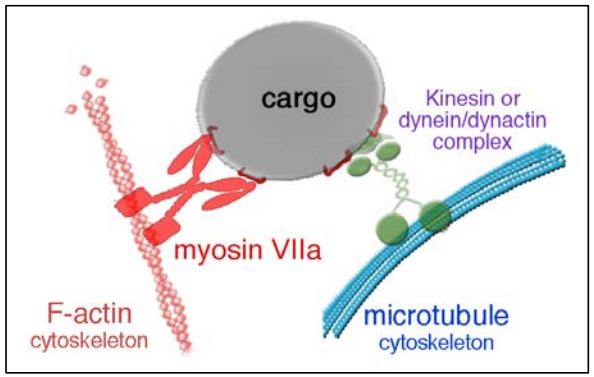
The microbvilli of retinal pigment epithelium (RPE) cells

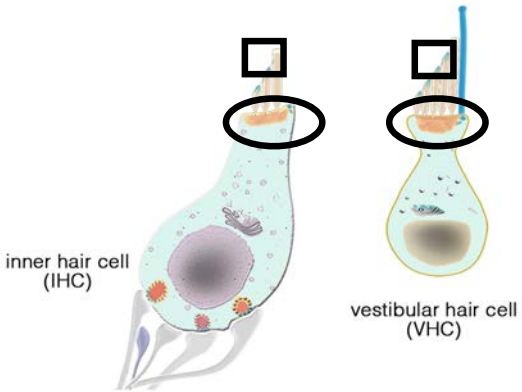


Inner ear Hair cells

The sound-receptive hair bundle of hair cells

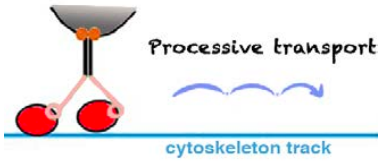






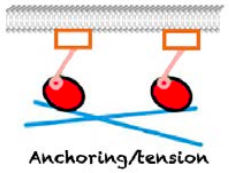
TRANSPORT FUNCTION

✓ Protein and organelle transport ?



TENSER FUNCTION

✓ Anchoring & tension force at specific membrane microdomains



The inner ear, as a window to look into the eye

Multi-scale phenotypic characterization of Myo7a defective mice?

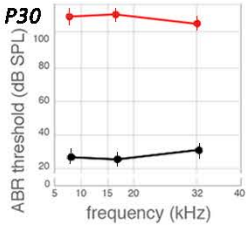
A 1. Hearing phenotyping:

e.g. **ABRs**, DPOAEs, MET activity, ...

shaker-1/Ush1B

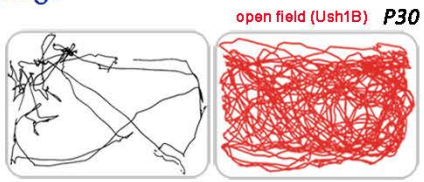
wild-type

profound hearing loss (threshold over 100 dB)



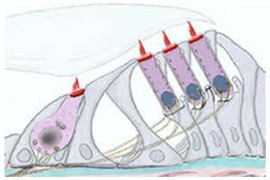
A 2. Balance phenotyping:

e.g. **Exploratory behavior**, swiming tests, righting reflex, rotarod tests, VORs, ...

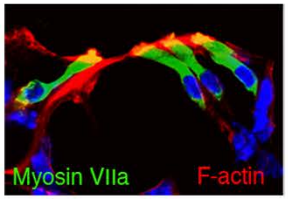


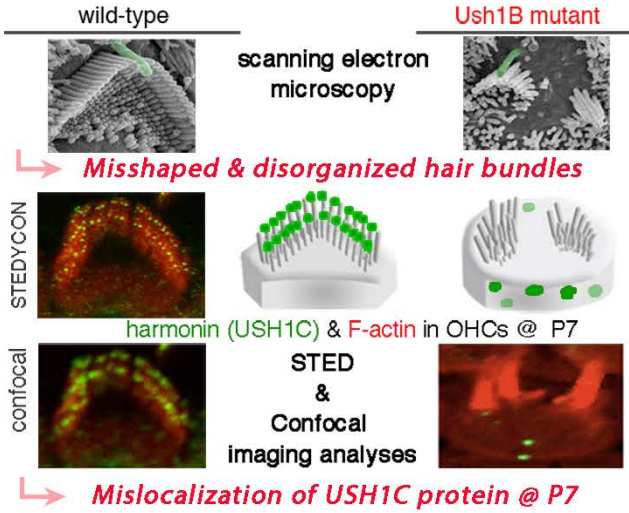
Vestibular balance deficits (hyperactivity and circling behavior)

A 3. Hearing organ architecture and function



Mature auditory hearing organ & P21





The inner ear

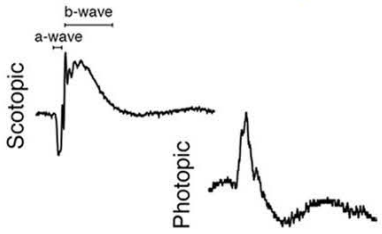
Balance organ

hearing organ

Non-invasive tests

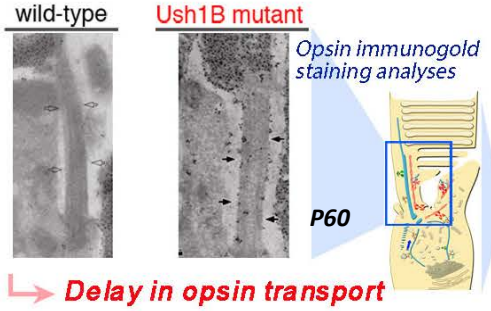
Morpho-molecular analyses

B1. Functional ERG responses



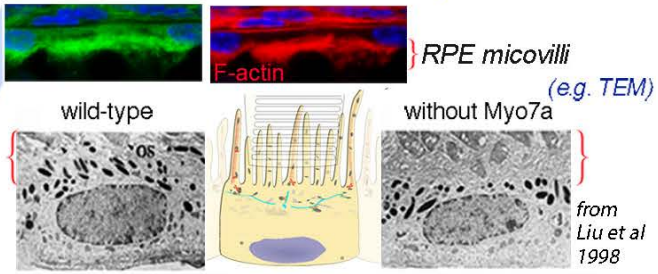
25% decrease of ERGs @ M6

B2. Photoreceptors architecture and activity



Delay in opsin transport

B3. RPE architecture and activity



Misposition of melanosomes in RPE cells

vision

The eye

INSTITUT DE LA VISION

Collab: Deniz Dalkara Serge Picaud,

Progressive Sensory Disorders

FROM MOLECULES TO PATHWAYS | **FROM PATHOPHYSIOLOGY TO THERAPY**

Sedigheh DELMAGHANI
Ass. Prof.

Sylvie NOUAILLE
Tech

Sandrine VITRY
Ass. Prof.

Audrey MAUDOUX
PHC / AP-HP

Pranav PATNI
Postdoc

Emilia WYSOCKA
Postdoc

Samantha PAPAL,
Postdoc

Maureen WENTLING
PhD

Maria POTTER
M2

Nawal YAHIAOUI
M1/ENS

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Serge Picaud, José-Alain Sahel
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Université Bordeaux-II, FR

François Simon & **Mathieu Beraneck**
Paris Descartes, St Pères, Paris, FR

V Michel, A. Bahloul, & **Christine Petit**
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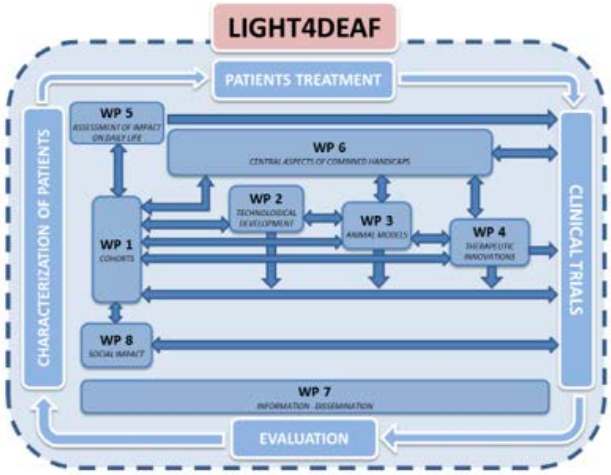
TOUT SAVOIR SUR LE SYNDROME DE USHER

Marie-José **Duran**, Ph

Work Packages with expected impacts on diagnosis, preclinical elopment of therapeutic strategies and initiation of clinical trials for the deafblind



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