Antisense Technology for the Treatment of Usher Syndrome

Michelle L. Hastings, PhD

Rosalind Franklin University of Medicine and Science
Chicago Medical School

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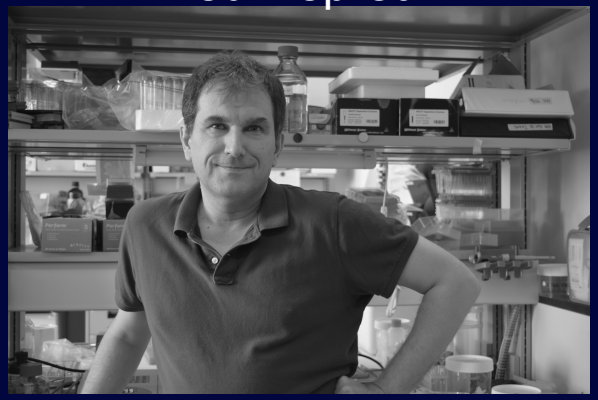
Fran Jodelka

Anthony Hinrich

Fred Depreux



Louisiana State University Health Sciences

Jennifer J. Lentz
Abhilash Ponnath
Russell Amato
Christopher M. Tran
Robert Rosencrans

Ionis Pharmaceuticals

Frank Rigo

Oregon Health & Science University

John V. Brigande
Lingyan Wang
Han Jiang

University of Nebraska-Lincoln

Timothy A. Jones
Sarath Vijayakumar

Northern Illinois University

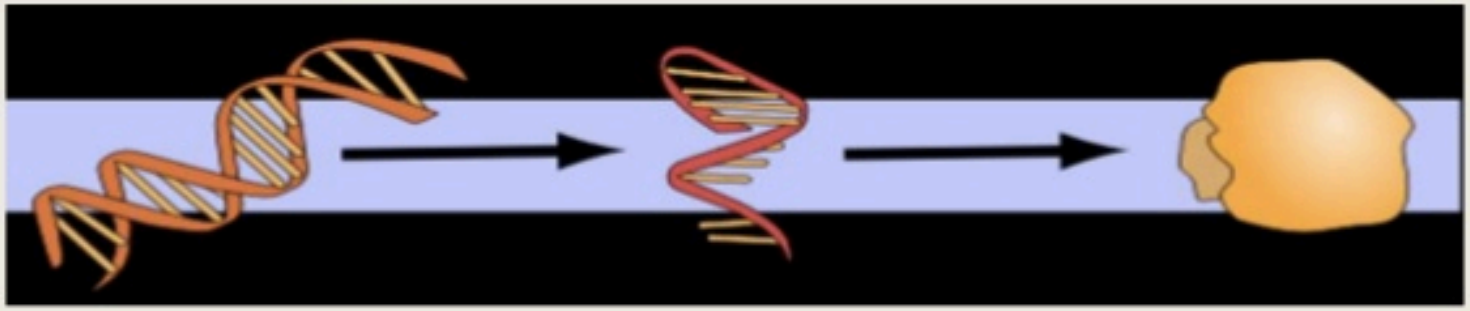
Douglas G. Wallace
Tia N. Donaldson

Harvard Medical School

Gwenaëlle Géléoc

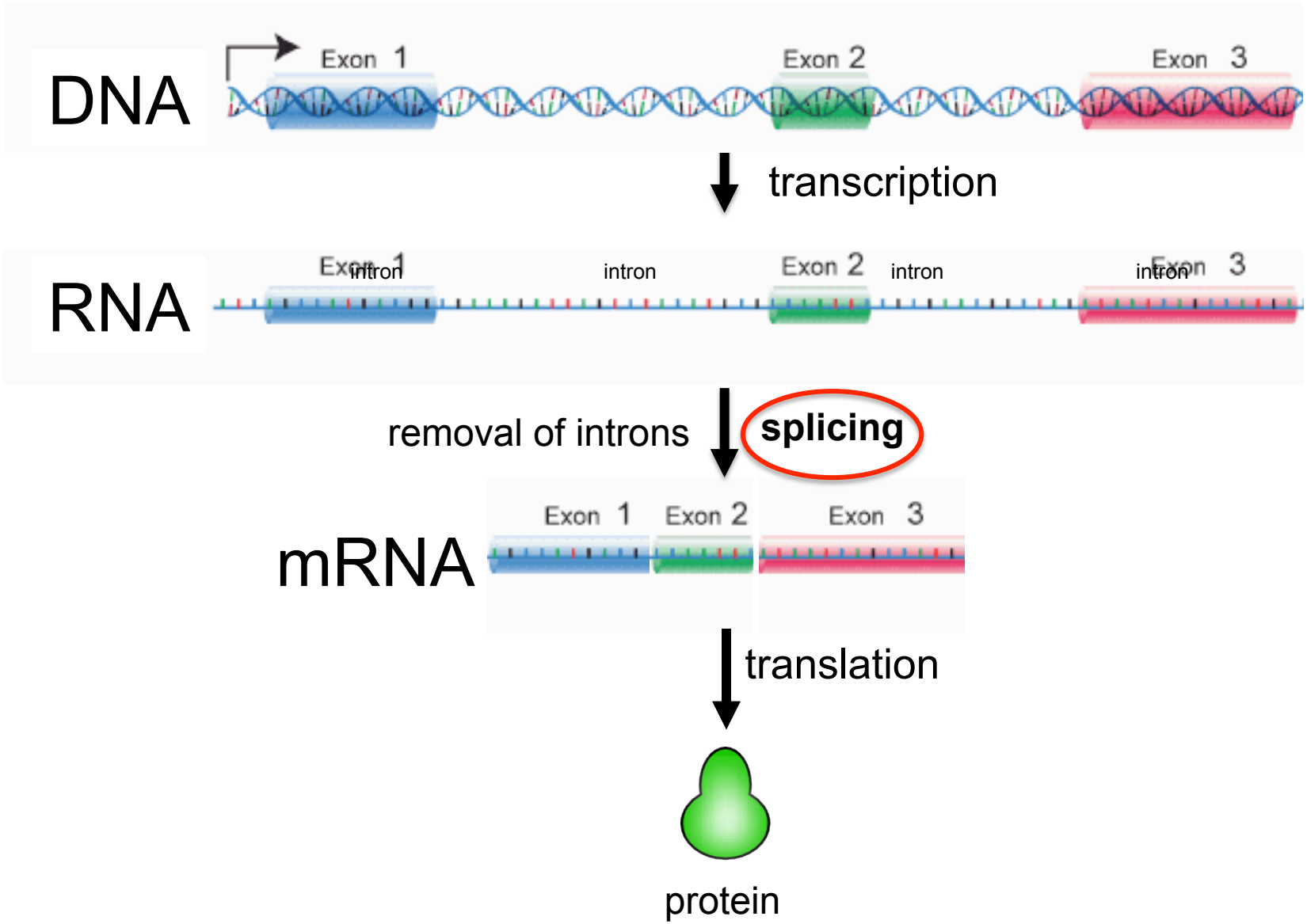
What is Gene Expression?

- A process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins or functional RNA.

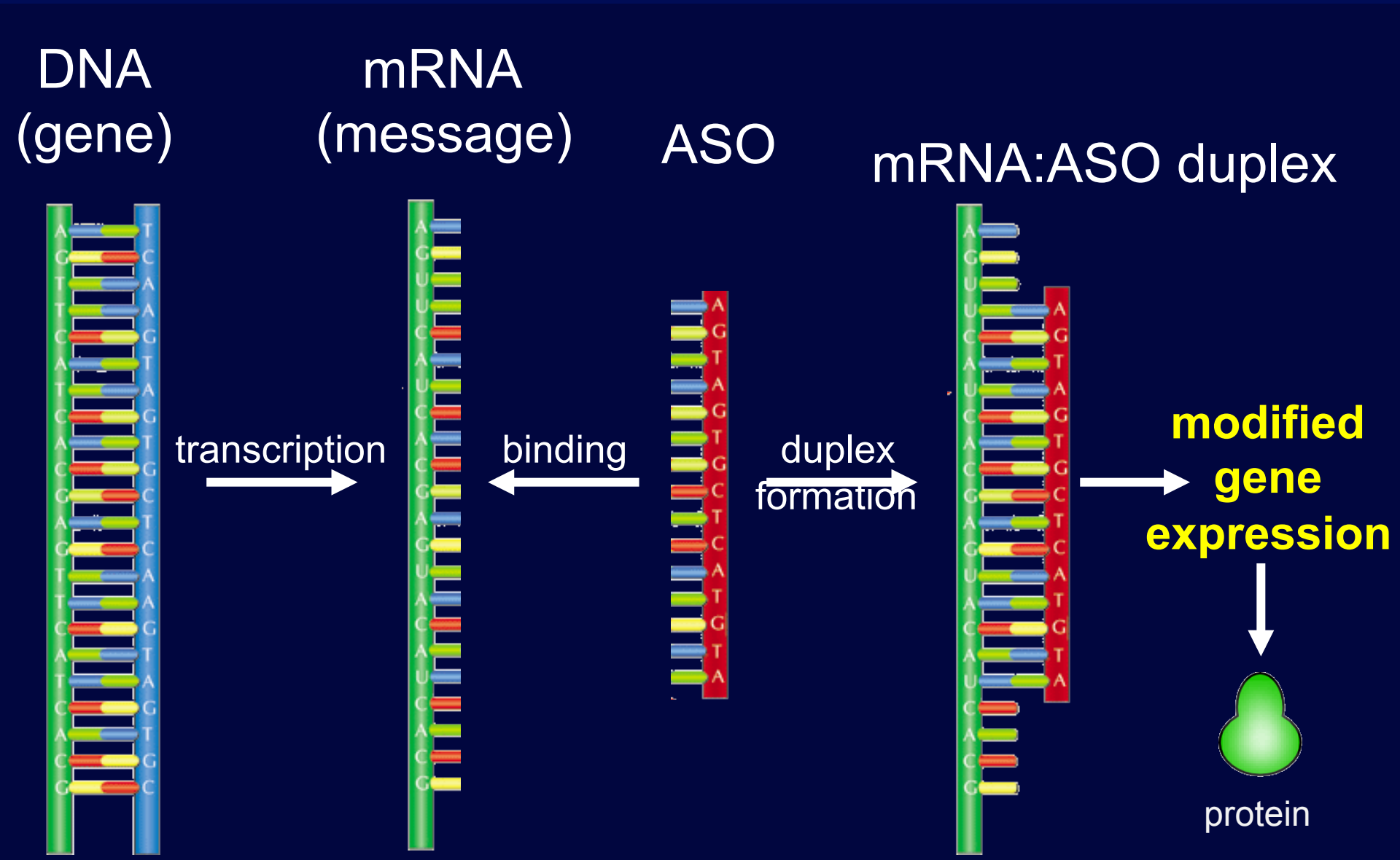


DNA RNA Protein

Gene Expression



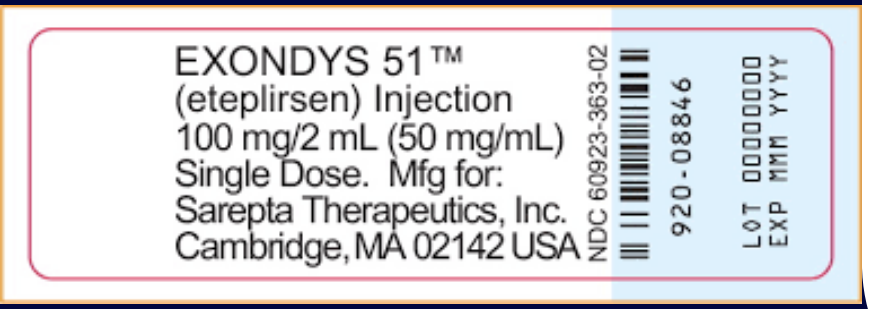
Antisense Oligonucleotides (ASOs)



Antisense Oligonucleotides as Drugs

- Size – small, 15-30 nucleotides
- Specificity – high (base-pairing)
- Stability – high (low clearance; long-lasting)
- Toxicity – low, especially for targeted delivery
- Deliverability – free uptake to many cell types
  - VIRAL DELIVERY NOT REQUIRED OR NECESSARY

FDA Approved Antisense Drugs Targeting Splicing



Spinal Muscular Atrophy

Spinal Muscular Atrophy (SMA) is the #1 genetic killer of children under the age of two. SMA is a devastating and relatively common children's genetic disease.

FDA-approved Antisense Drugs for the Eye

Usher syndromes

Type	Presentation	Locus	Gene	Protein	Function
USH1	Severe-Profound, Congenital Hearing Impairment (HI) Adolescent-onset Retinitis Pigmentosa (RP) Vestibular areflexia	USH1B	*MYO7A*	Myosin VIIa	Actin binding, molecular motor
		USH1C	***USH1C***	**Harmonin**	**Scaffolding**
		USH1D	*CDH23*	Cadherin-23	Cell adhesion
		USH1E	*Unknown*		
		USH1F	*PCDH15*	Protocadherin-15	Cell adhesion, polarity
		USH1G	*USH1G*	Sans	Scaffolding
		USH1H	*Unknown*		
		USH1J	*CIB2*	Calcium and intergin binding protein 2	Calcium binding
		USH1K	*Unknown*		
USH2	Mild-moderate, Congenital HI Adult-onset RP Normal vestibular responses	USH2A	*USH2A*	Usherin	Cell adhesion
		USH2C	*GPR98*	G protein-coupled receptor 98	Signaling
		USH2D	*DFNB31*	Whirlin	Scaffolding
USH3	Post-lingual, progressive HI Late-onset RP Variable Vestibular	USH3A	*CLRN1*	Clarin-1	Scaffolding and adhesion
		USH3C	*HARS*		

USH1C c.216G>A

- *USH1C* codes for the ubiquitously expressed, scaffold protein, **Harmonin**.

- The *USH1C c*.216G>A mutation accounts for nearly all cases of type 1 Usher in the Acadian population in Canada and Louisiana.

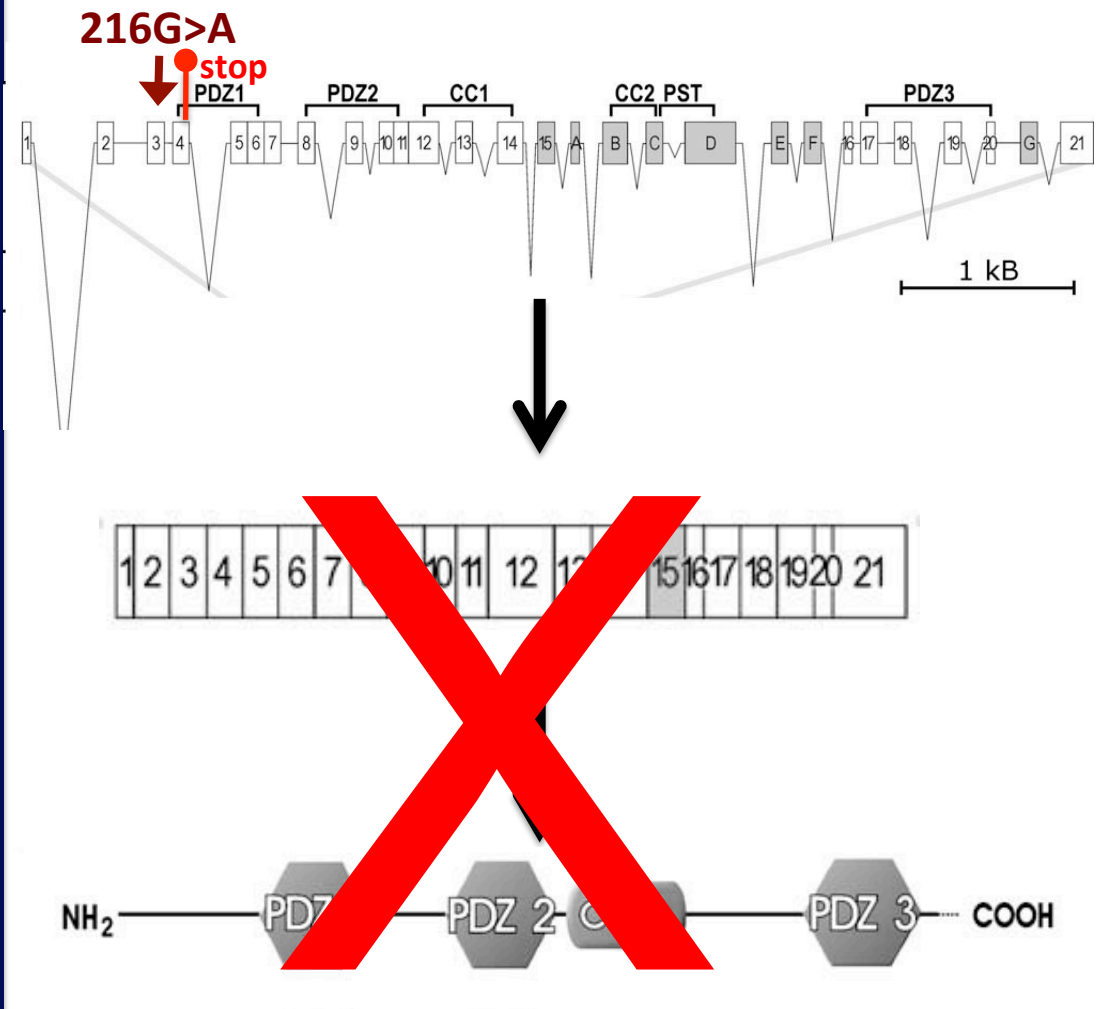
How Does the *USH1C* c.216G>A Mutation Disrupt Gene Expression and Cause Usher Syndrome?

USH1C c.216G>A Mutation Disrupts Gene Expression

***USH1C* gene**

USH1C mRNA

Harmonin protein

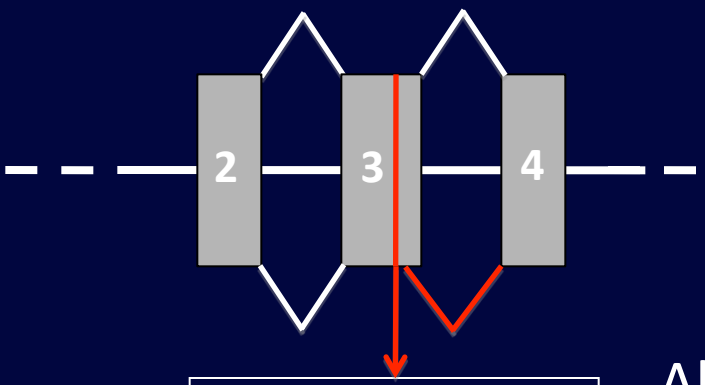


Reiners et al 2006

USH1C/Harmonin 216G>A mutation

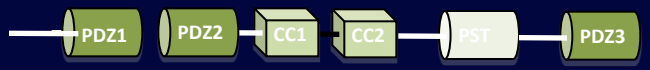
USH1C splicing

Harmonin protein



216G>A new incorrect splicing site

Correct splicing

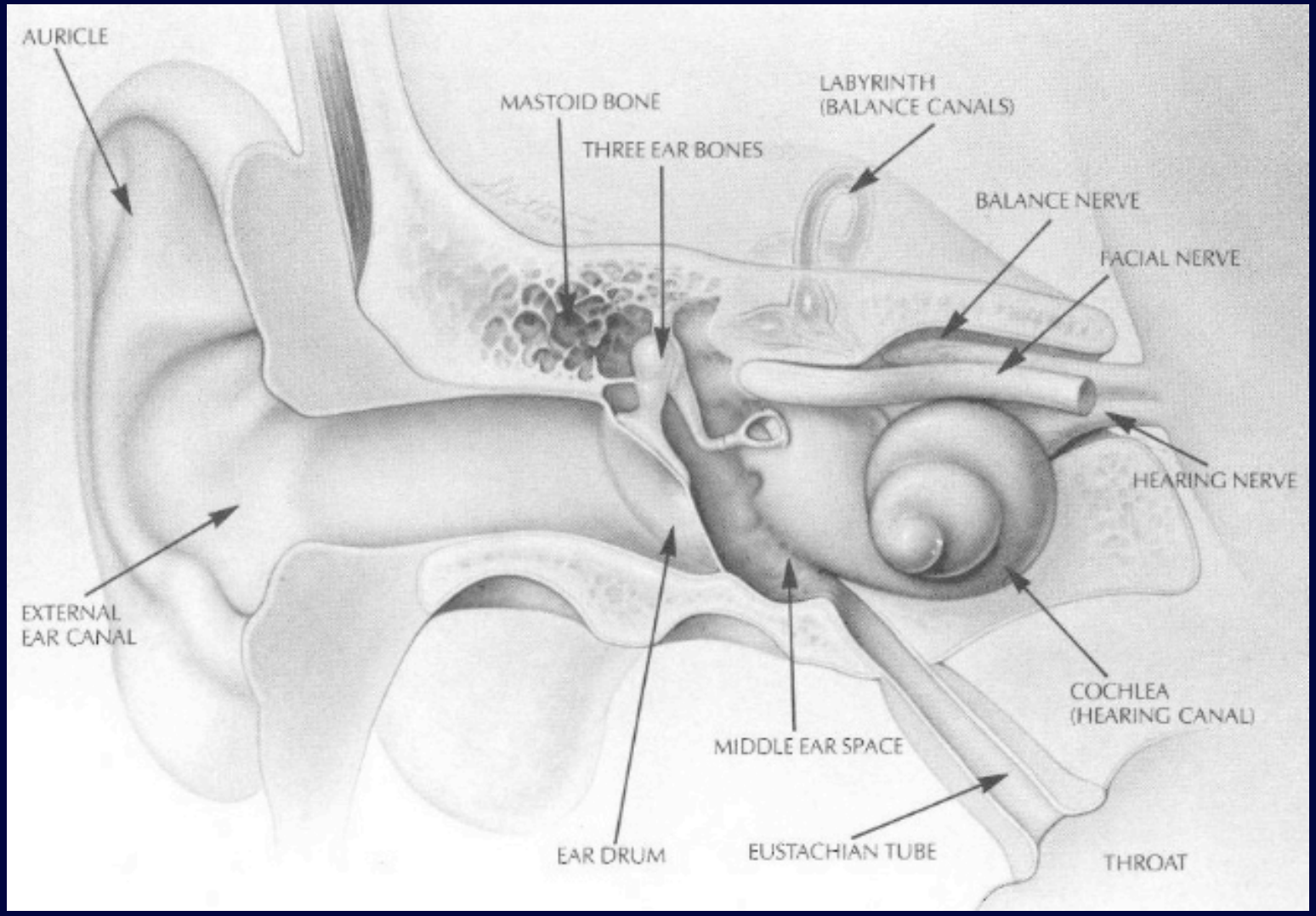


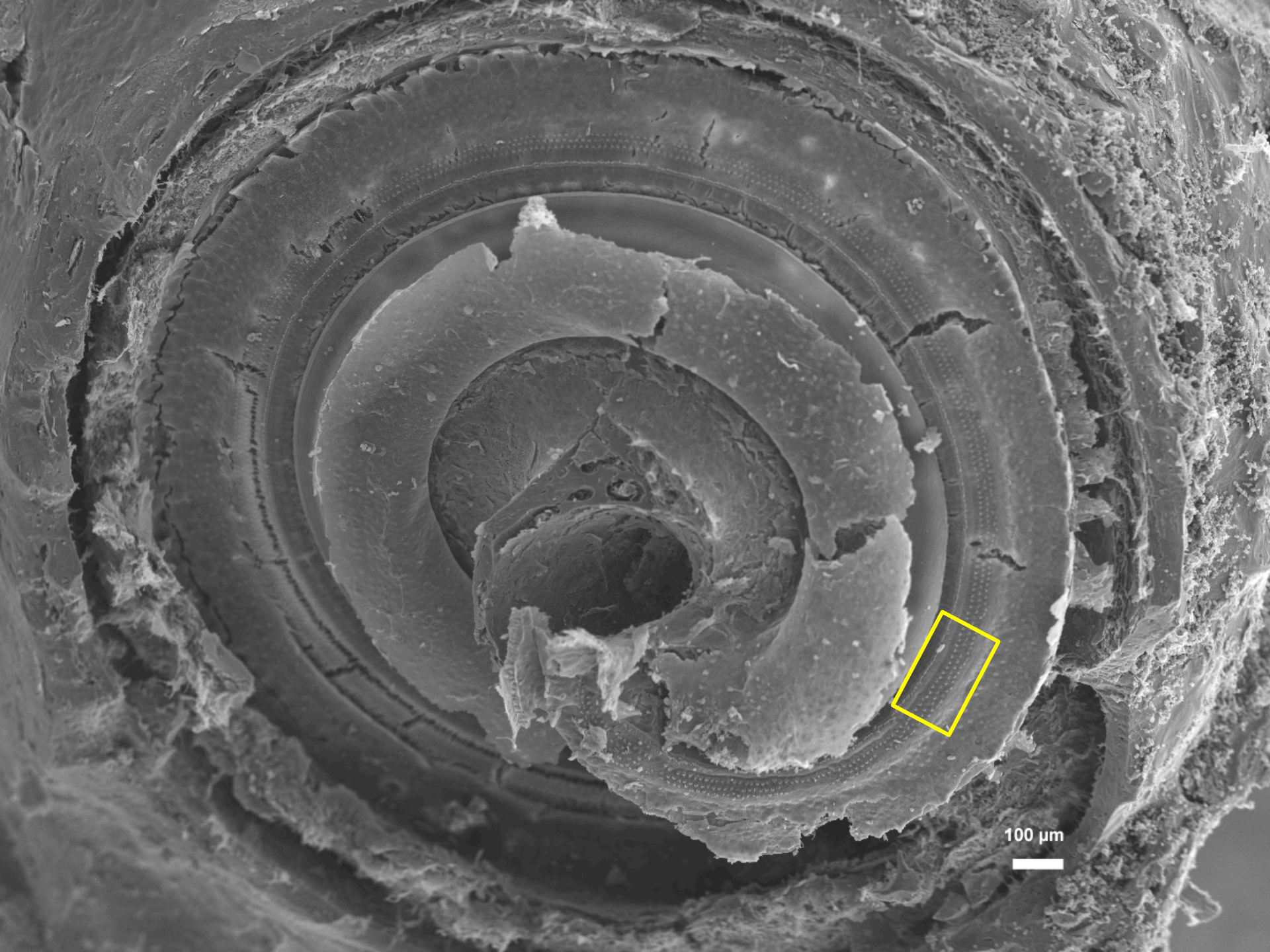
Aberrant splicing (frame-shift)

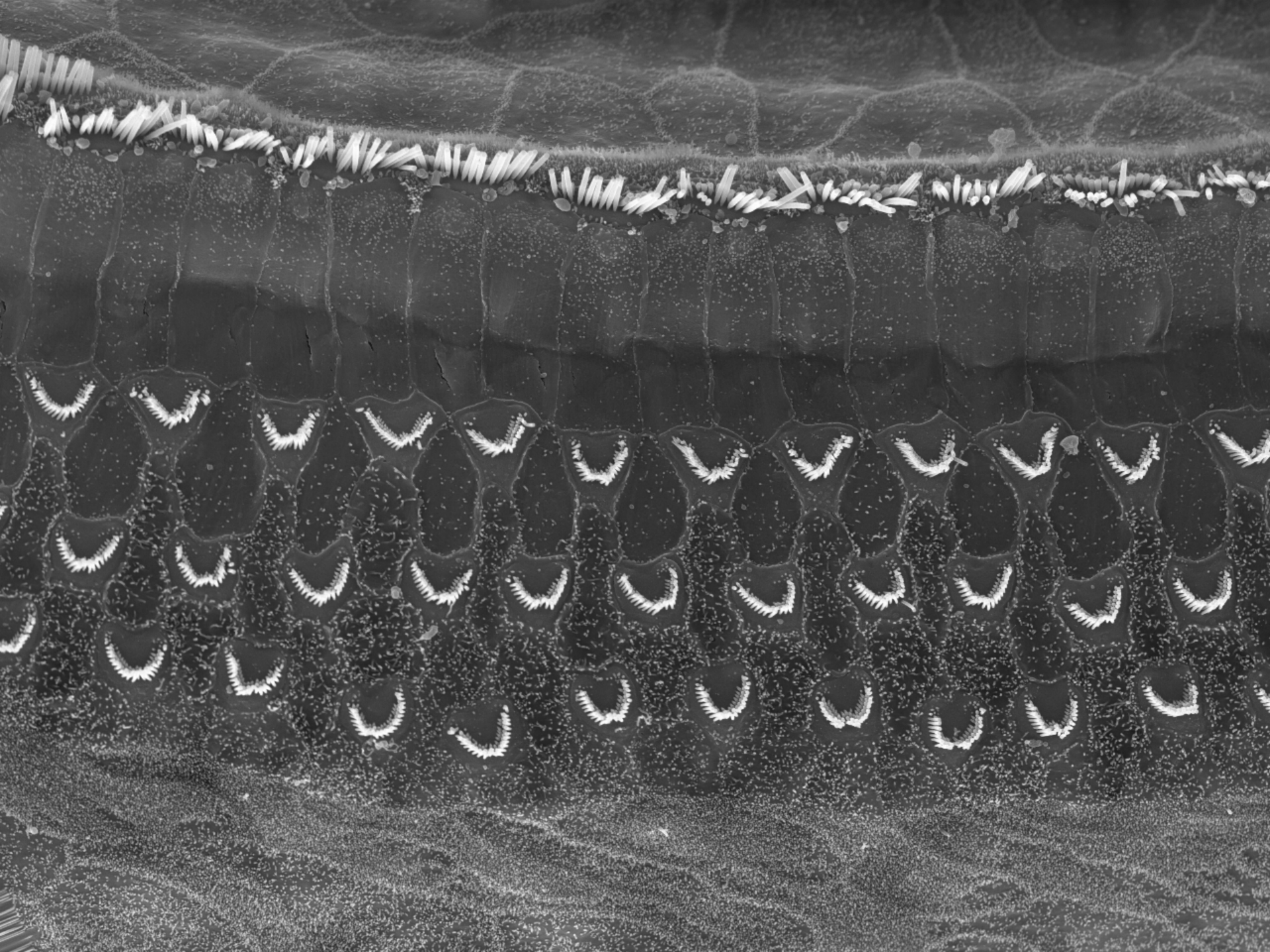
truncated protein

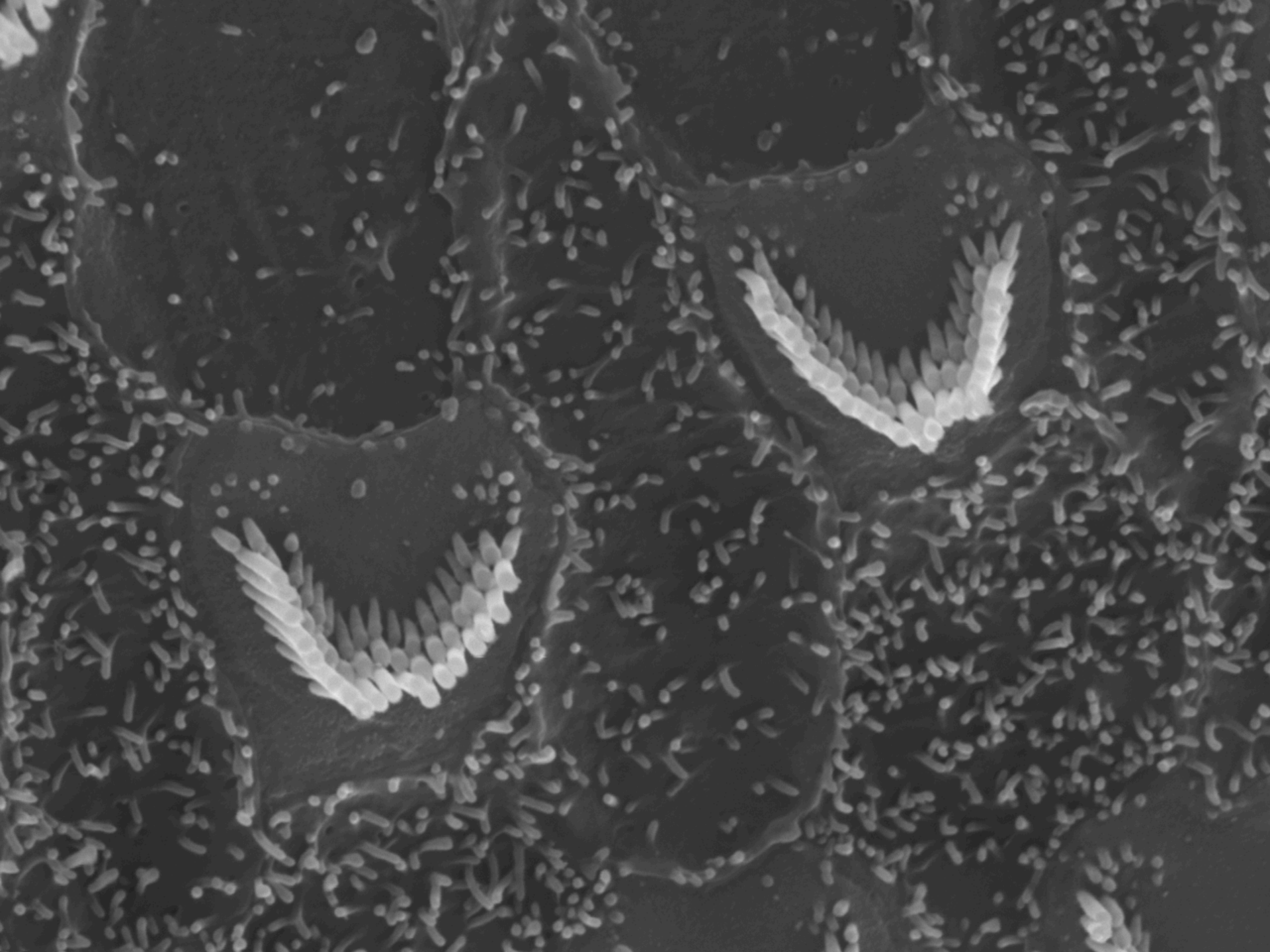
Usher Syndrome

Harmonin and Hearing

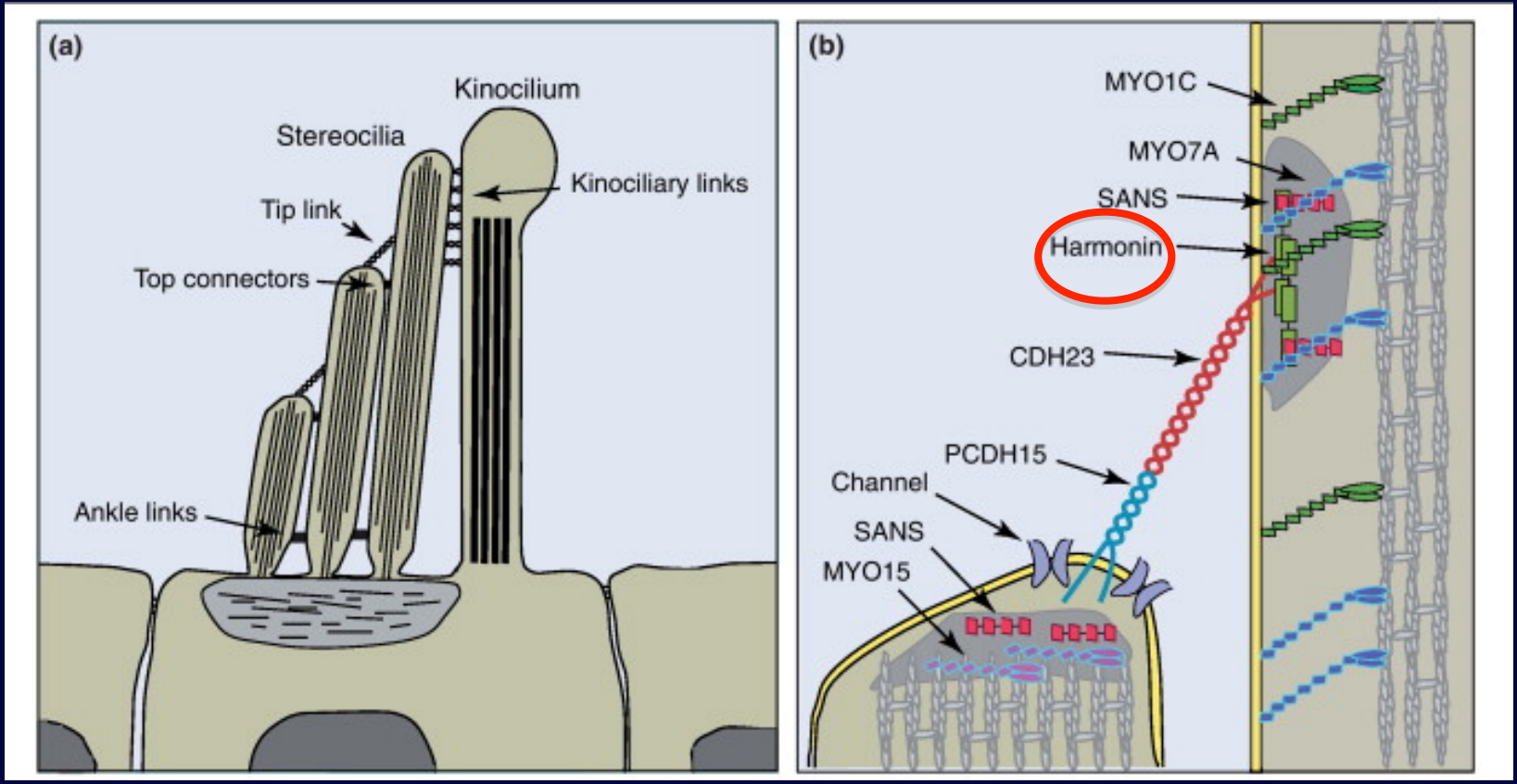



100 µm

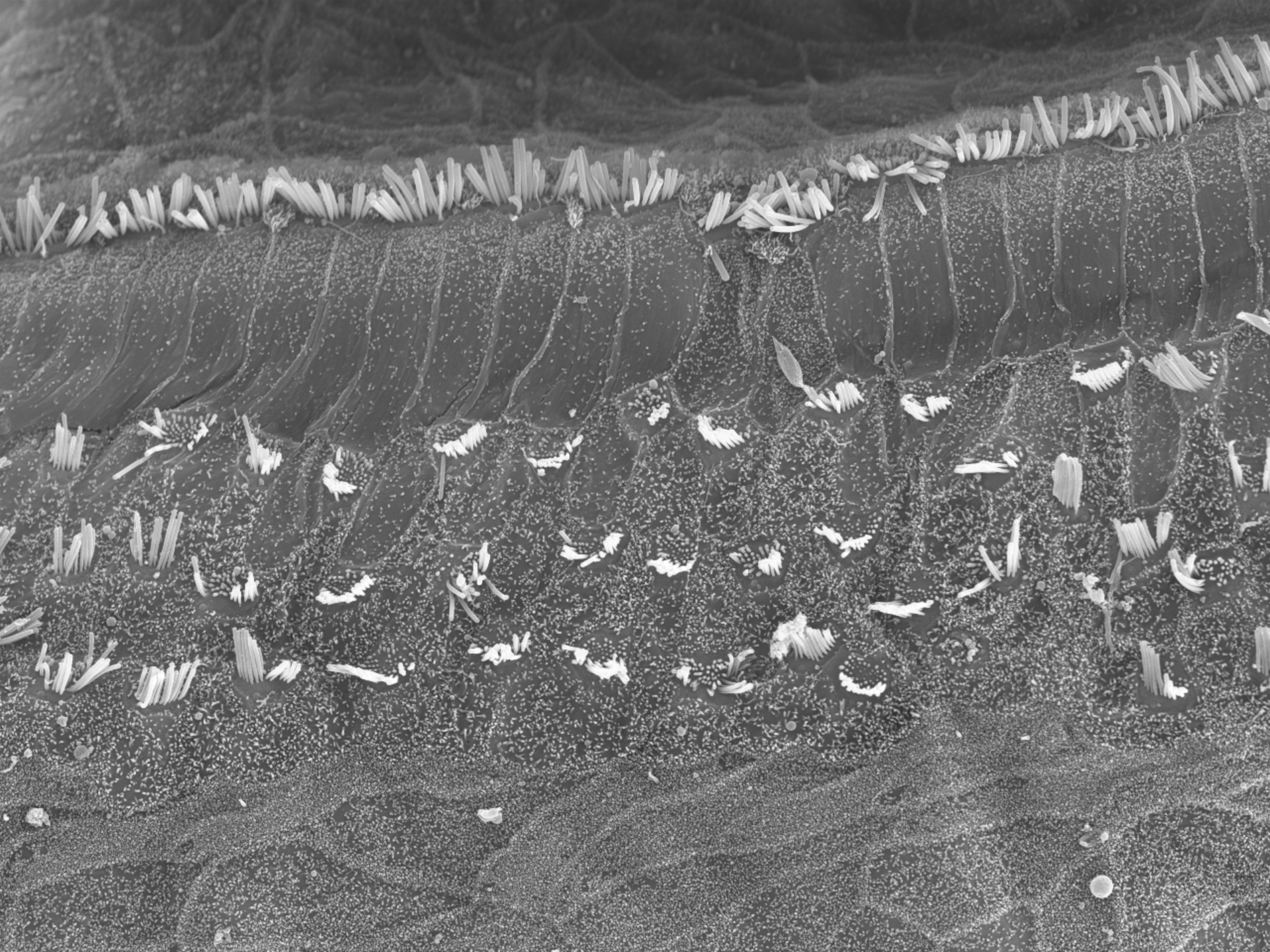




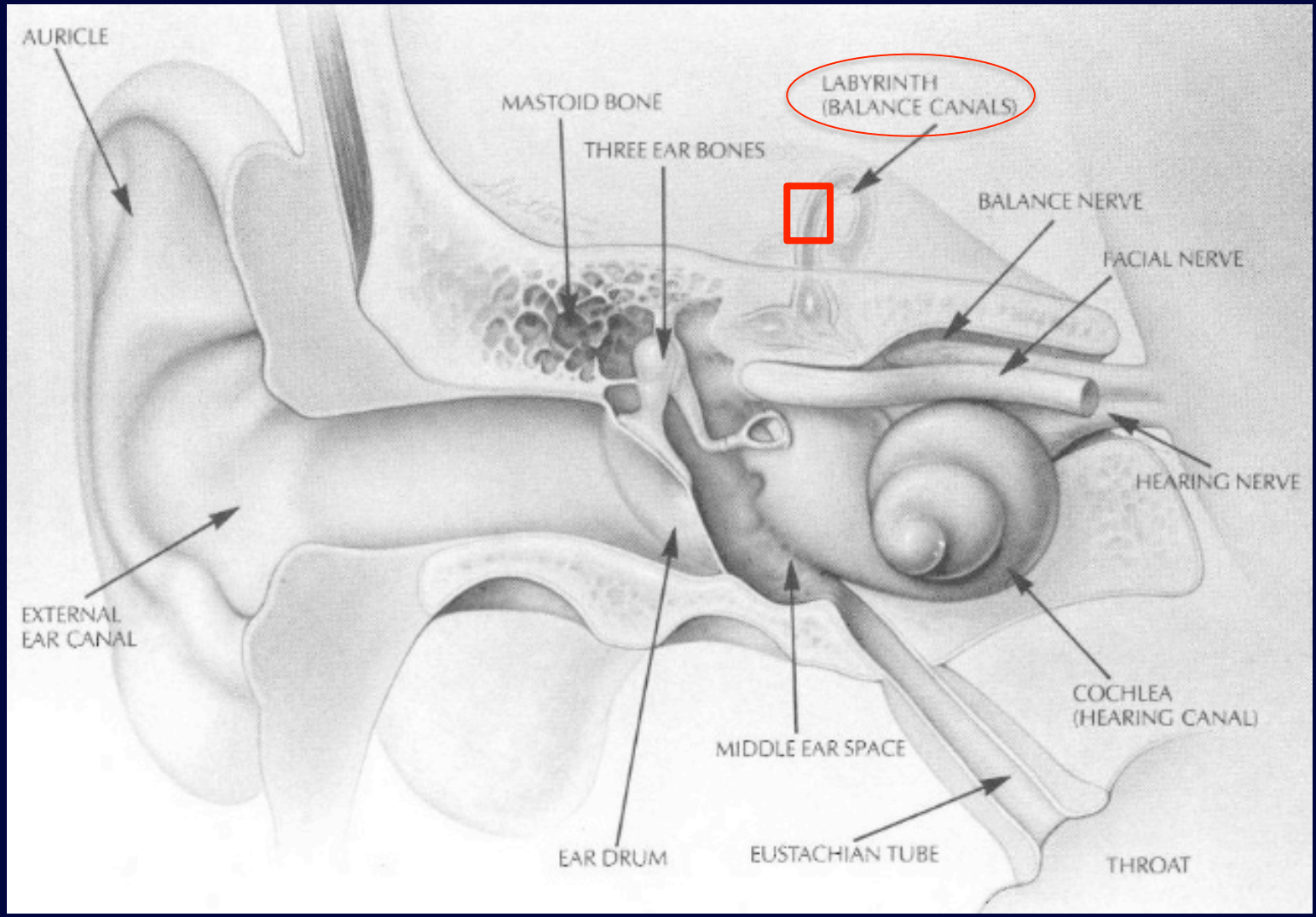
Harmonin / USH1C



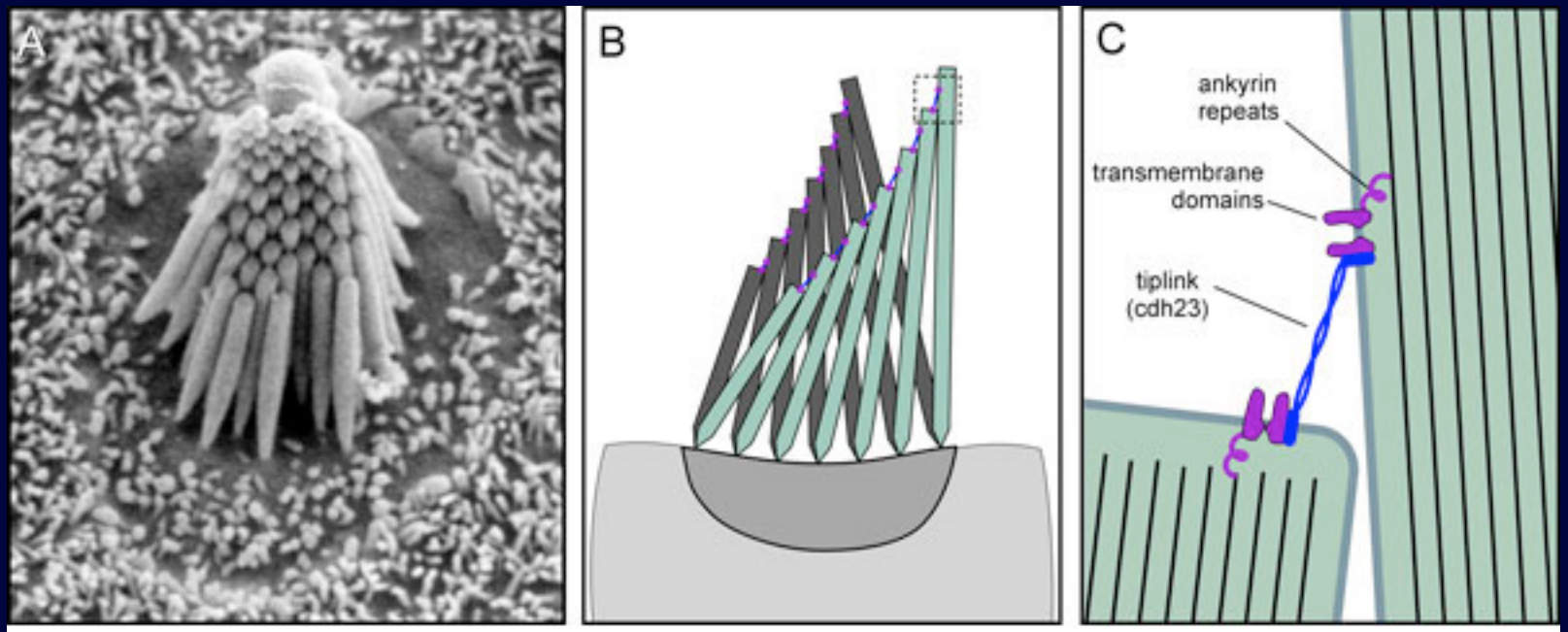
Kazmierczak and Müller (2012) Trends in Neurosciences, 35: 220-229 (2012)



Harmonin and Balance

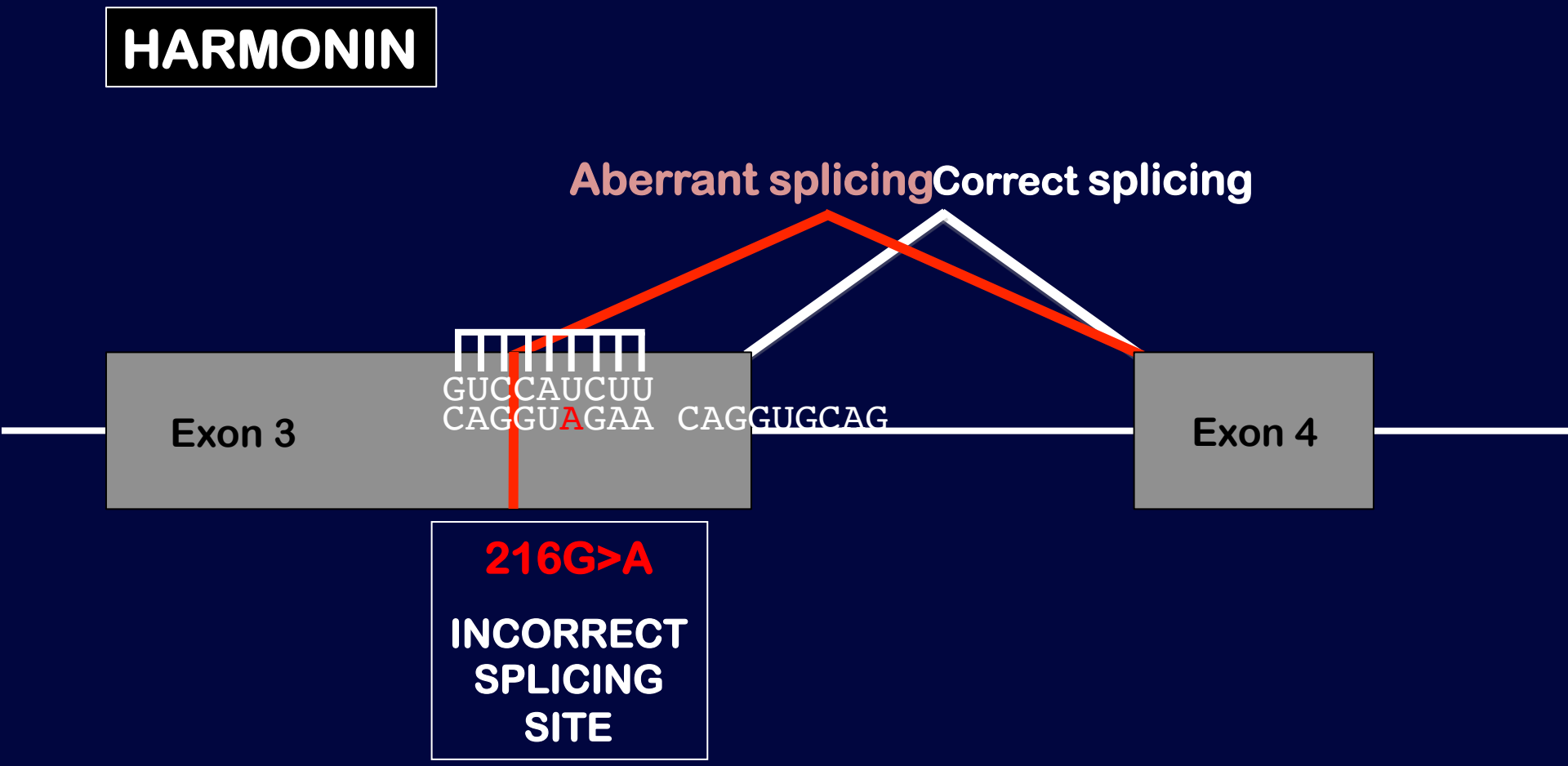


Stereocilia of the Vestibular System



Theoretical and Computational Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign

Correction of *Ush1c G216A* Splicing with Antisense Oligonucleotides



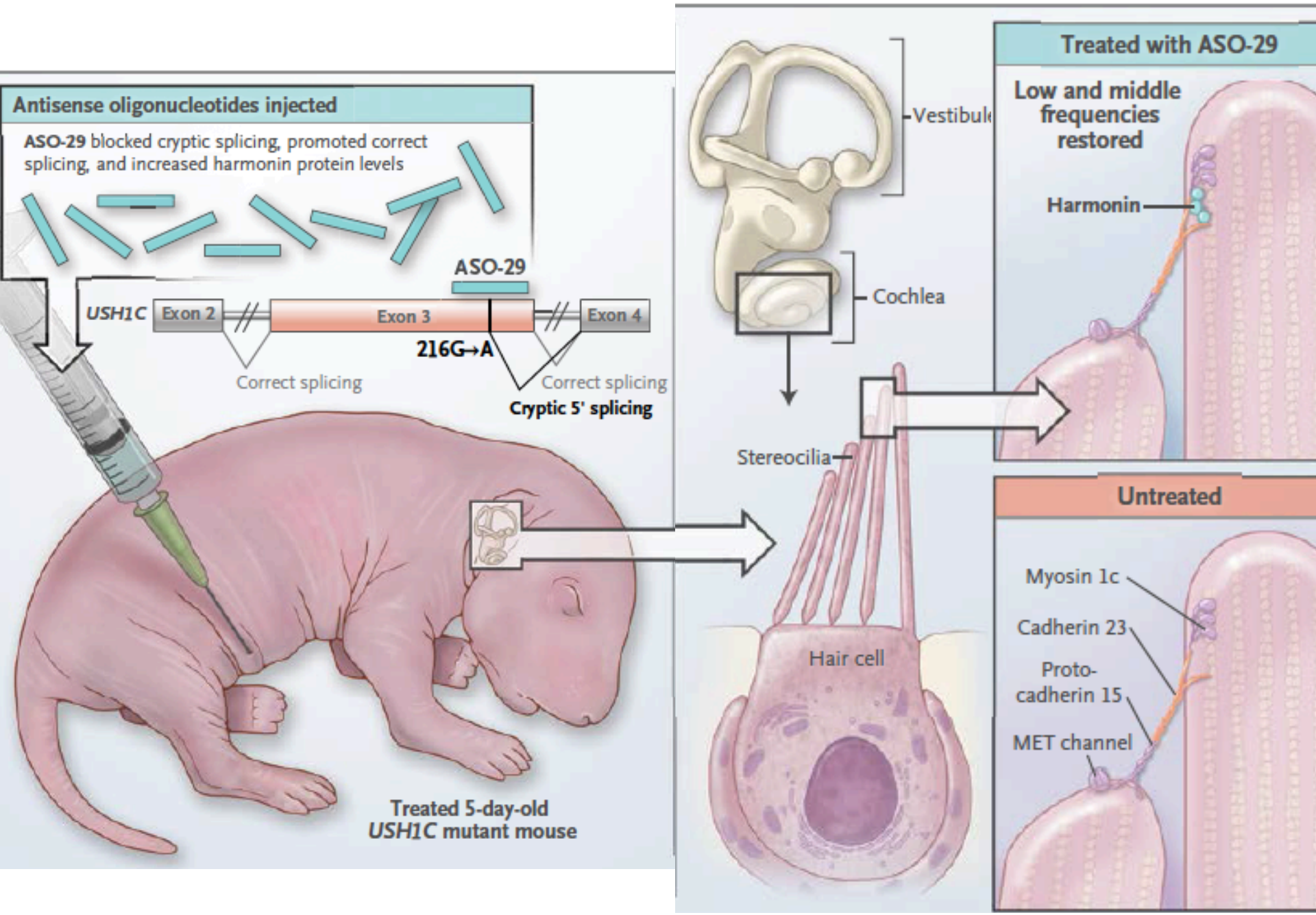
Ush1c^{216AA} Mouse Model: Knock-in of Human Mutation

- Severe Vestibular Dysfunction
- Hearing Impaired
- Visual Deficits

Lentz et al., 2007, 2010

Treatment Strategy

- Treated mice 3-16 days after birth (P3-16)
- Single intraperitoneal injection (IP)



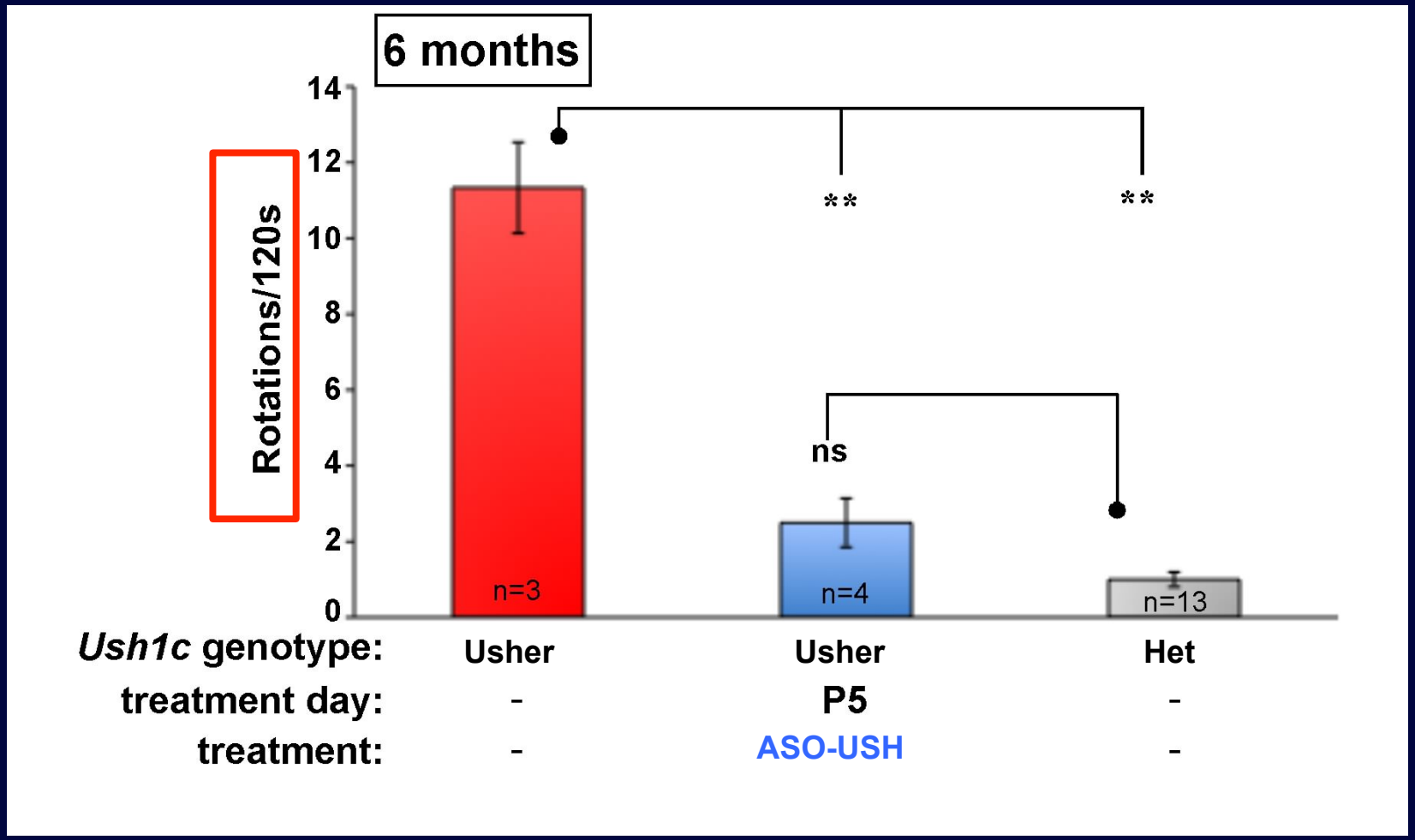
Avraham, N Engl J Med. 2013 O369:1758-60

ASO Treats Usher Symptoms in Mice

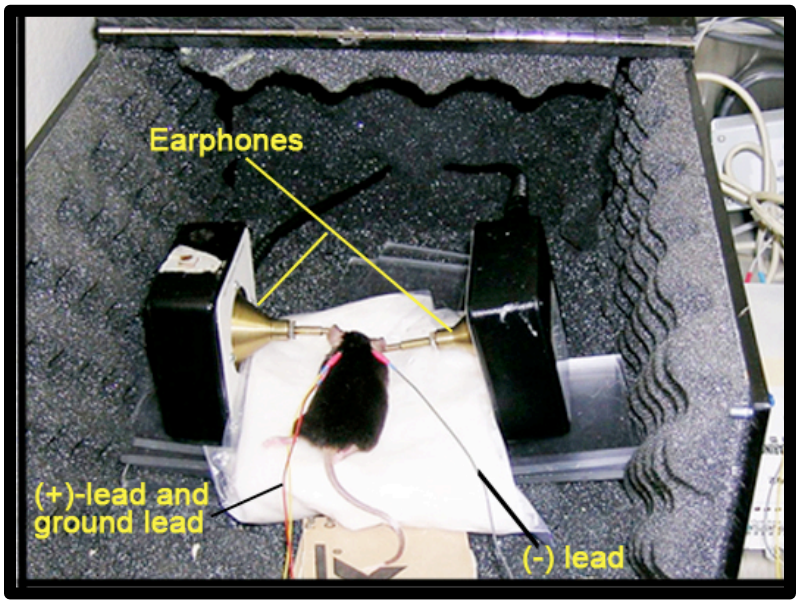
Non-disease mouse
ASO-Control

Usher syndrome mouse
ASO-Control

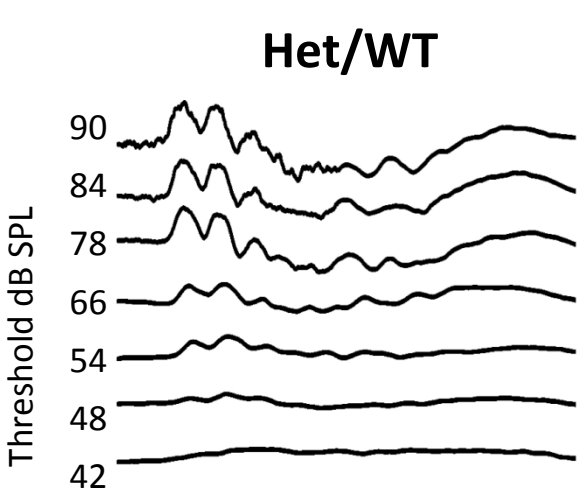
A Single Dose of ASO Cures Balance Deficits

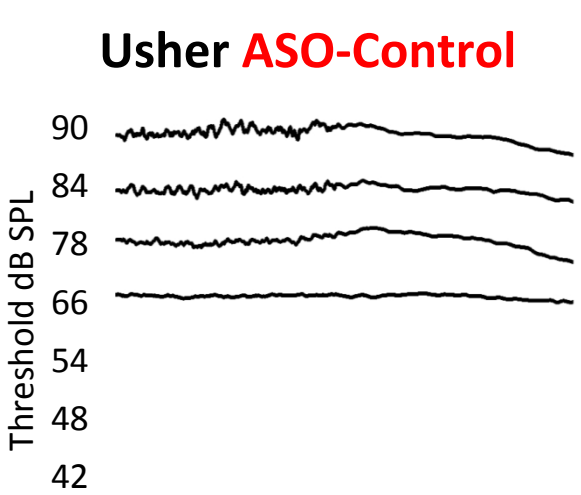


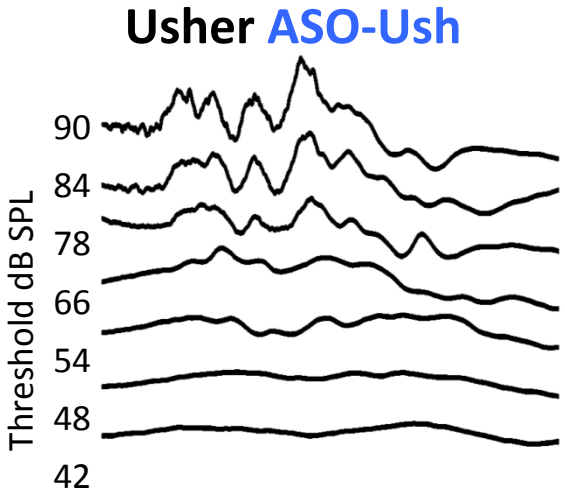
ASO-USH Rescues Hearing (Auditory-Evoked Response)



Jennifer J. Lentz

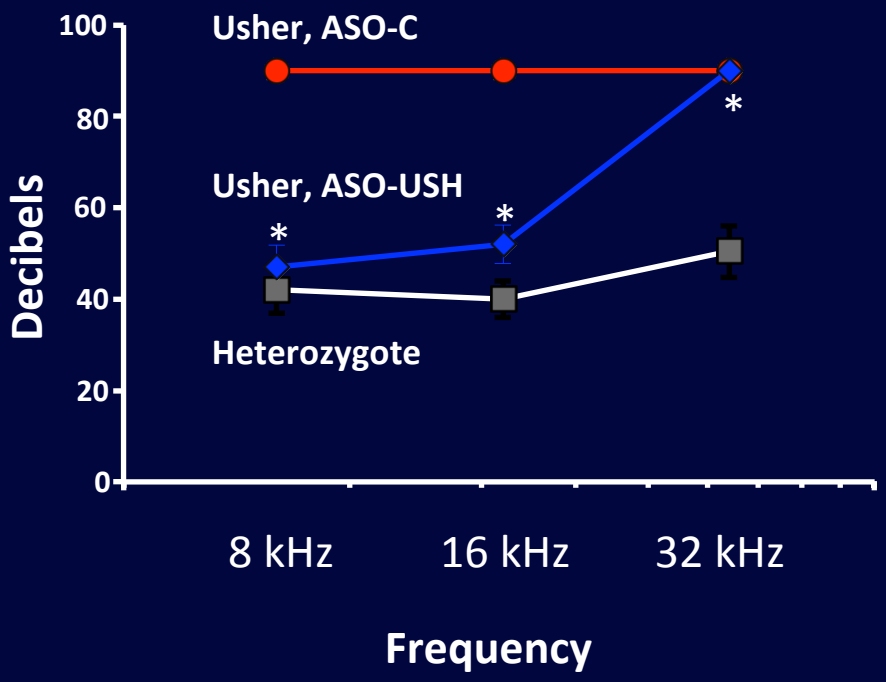




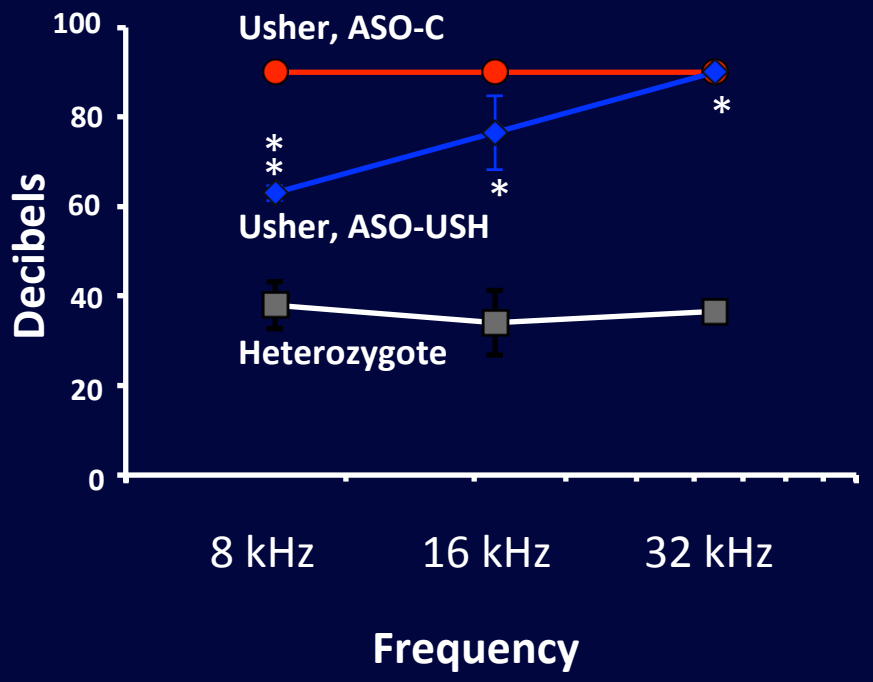


ASO Rescues Hearing for up to 6 months

2 months old



6 months old



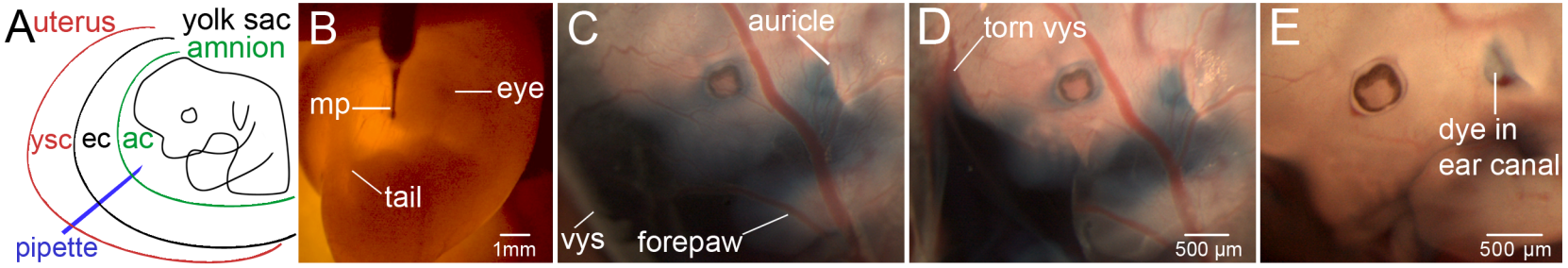
Challenge

Hearing in Humans Develops *in Utero*, Early in Gestation.

In Utero, Intra-amniotic Delivery of Antisense Oligonucleotides

Depreux, F.F, Wang L., Jiang, H., Jodelka, F.M., Rosencrans R.F., Rigo, F., Lentz, J.J., Brigande, J.V., and Hastings, M.L. (2016) *Nucleic Acids Res.* **44**: 9519-9529.

Delivery of ASO-USH to the Amniotic Cavity as Efficient as Direct Injection to Newborn

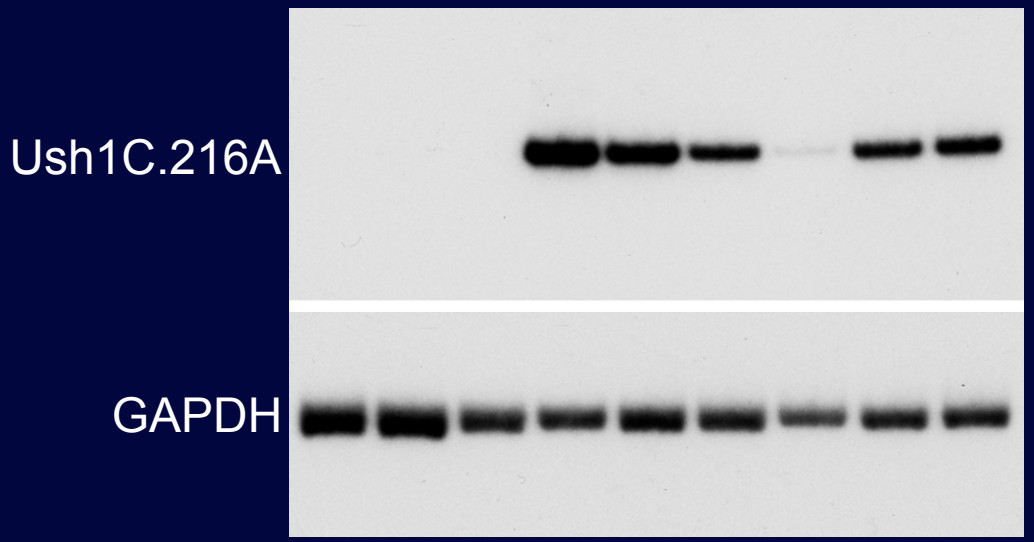


Amniotic, E13.5
80 µg

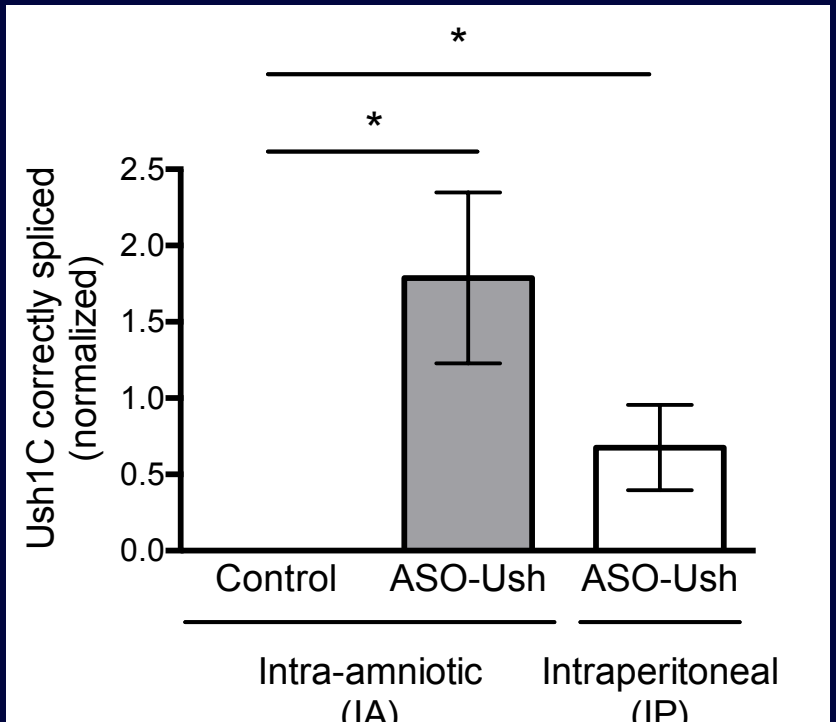
P5, IP
300 µg/g

Control

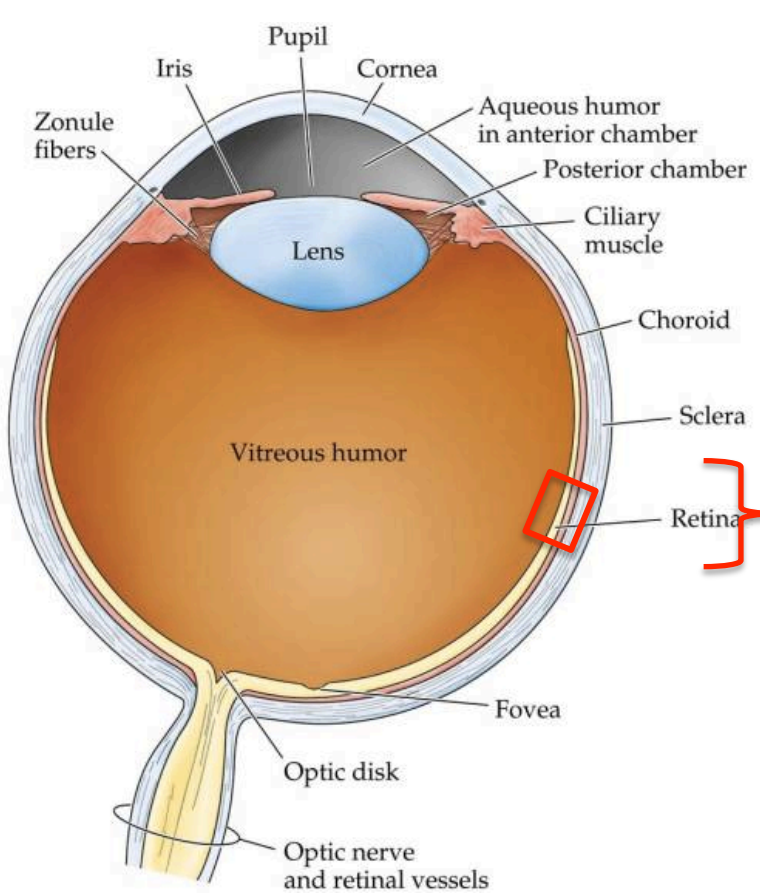
ASO-USH

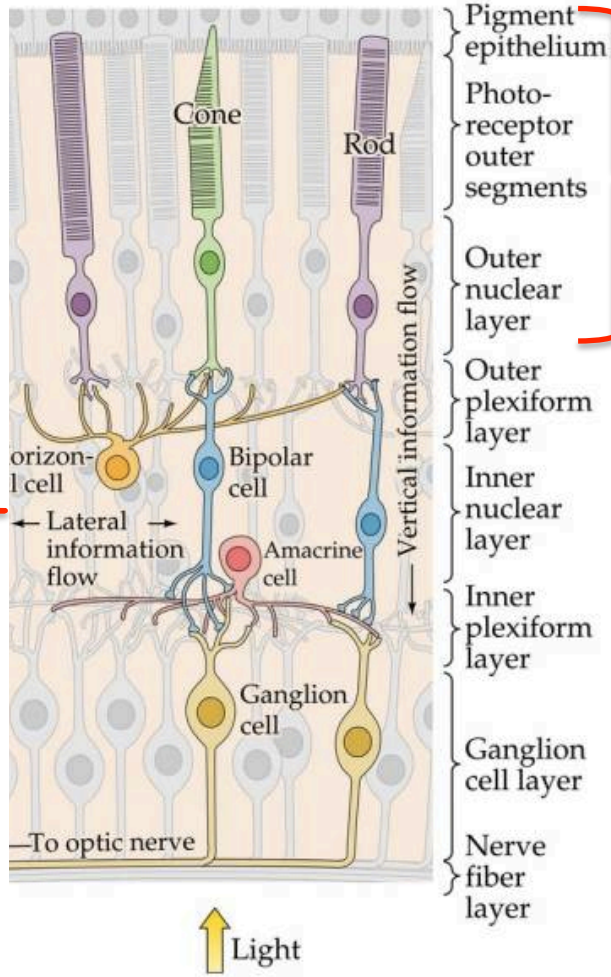


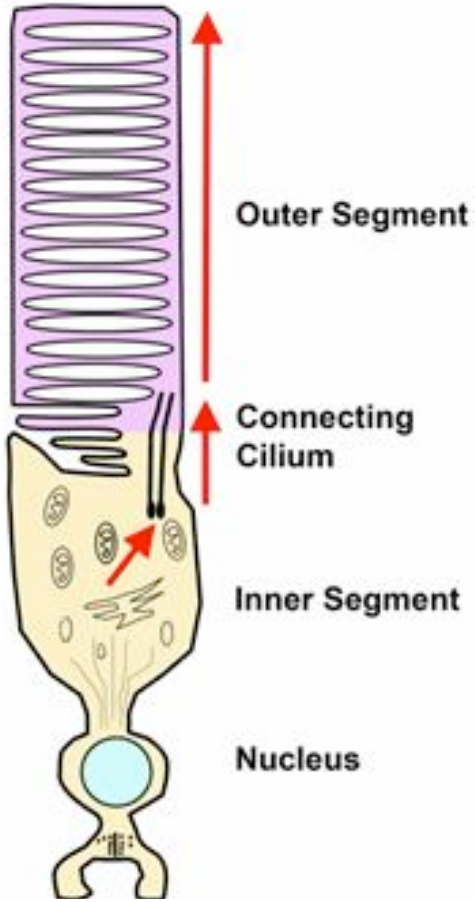
P22 Tissue



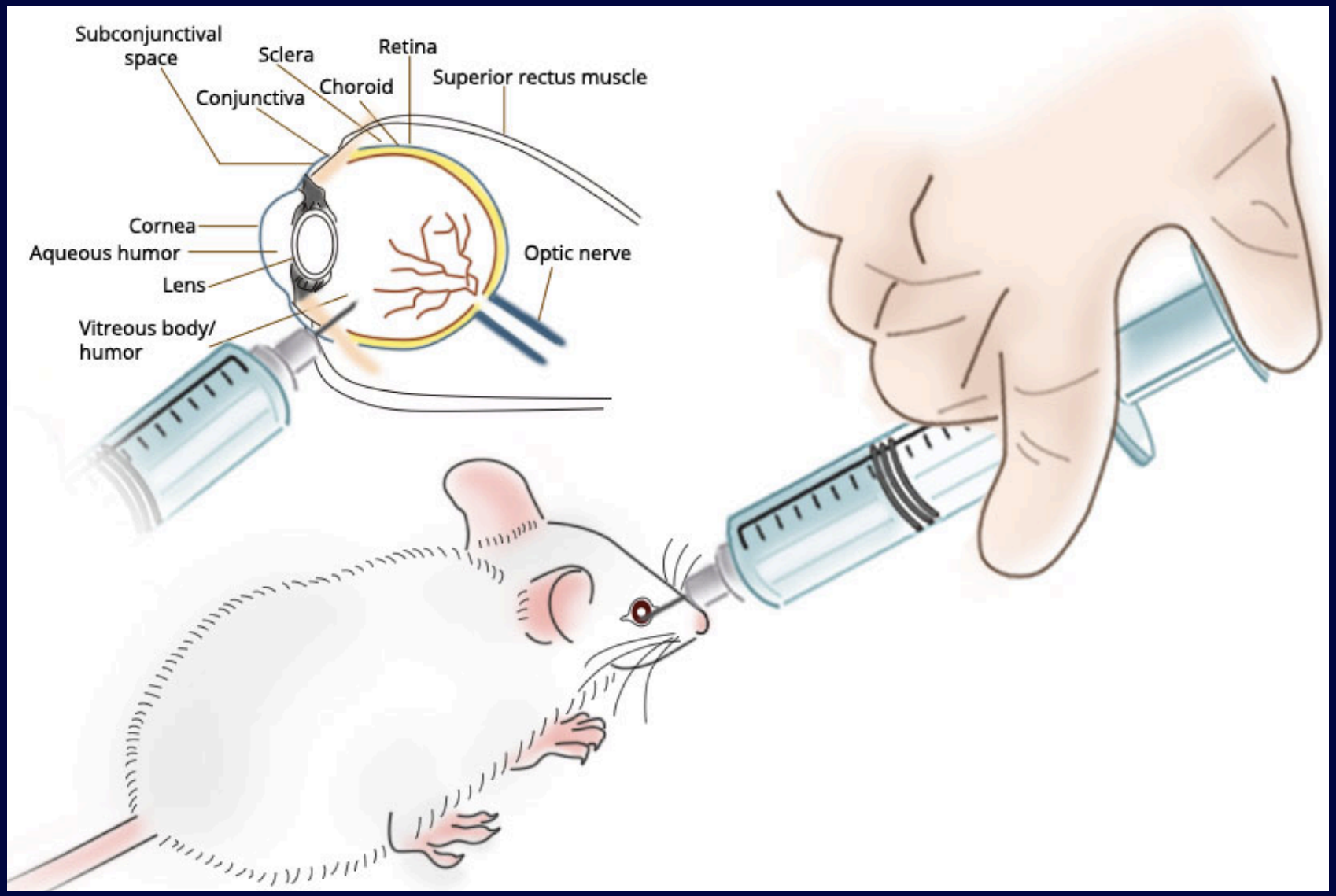
Harmonin Function in the Eye: Photoreceptor Maintenance







Intravitreal Eye Injections



SUMMARY

- ASOs target the cochlea, vestibule and retina, making them a good drug platform for treatment of conditions affecting these systems.
- One dose of ASO early in life rescues balance and hearing for more than 6 months in mice.
- *In utero* treatment with ASOs can be achieved via injection to the amniotic cavity.
- ASOs preserve visual function in Usher mice

The Future

- ASO Treatments for Additional Forms/Causes of Hearing Loss
- ASO Treatment Optimization
- ASO-29 Therapeutic Discovery and Development

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- Frank Rigo

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