Usher 1B in the retina: basic science and gene therapy

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Usher Syndrome

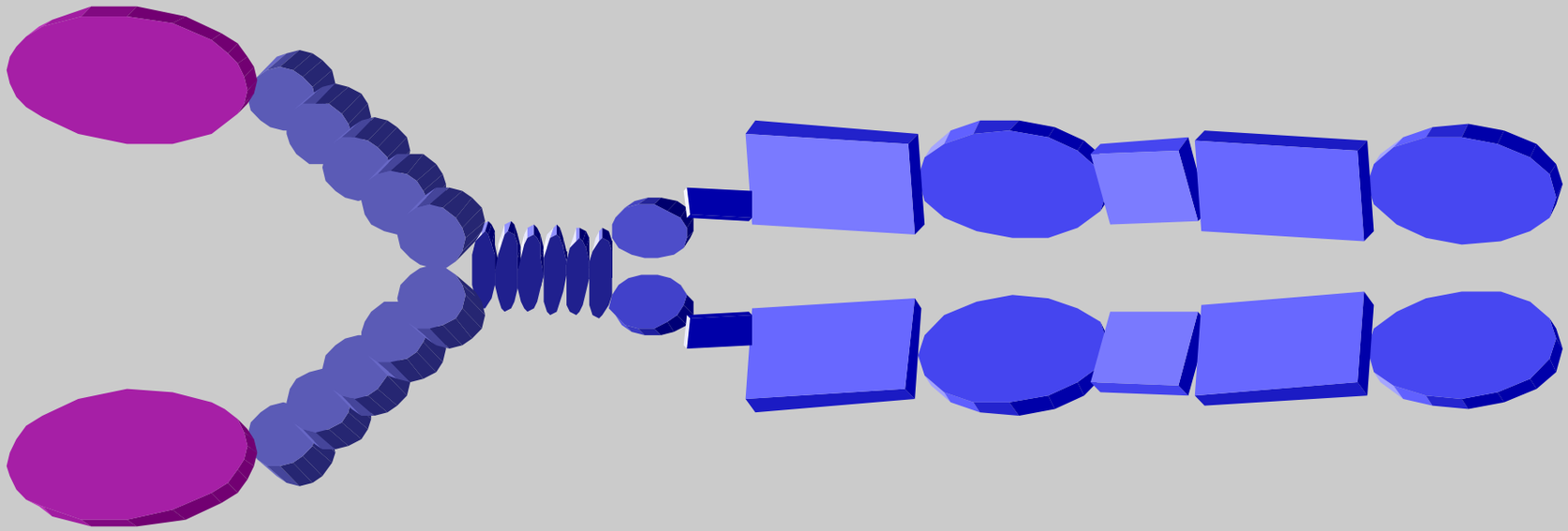
Subtype	Gene	Protein (function[1])	Animal models Mouse (zebrafish)
Usher 1B*	MYO7A	myosin 7a (actin motor)	shaker1 (mariner)
Usher 1C	USH1C	harmonin (PDZ-domain protein)	deaf circler
Usher 1D	CDH23	cadherin23 (adhesion protein)	waltzer (sputnik)
Usher 1E	12q21	unknown	
Usher 1F	PCDH15	protocadherin15 (adhesion protein)	Ames waltzer (orbiter)
Usher 1G	USH1G	sans (scaffold)	Jackson shaker
Usher 1H	15q22-23	unknown	
Usher 2A	USH2A	usherin (transmembrane linkage)	knockout
Usher 2C	GPR98	VLGR1 (G-protein coupled receptor)	Vlgr1/del7TM
Usher 2D	DFNB31	whirlin (PDZ-domain protein)	whirler
Usher 3	CLRN1	clarin (synaptic shaping)	none reported

[1]Some of the indicated functions have not been demonstrated and are merely speculations based on primary sequence.

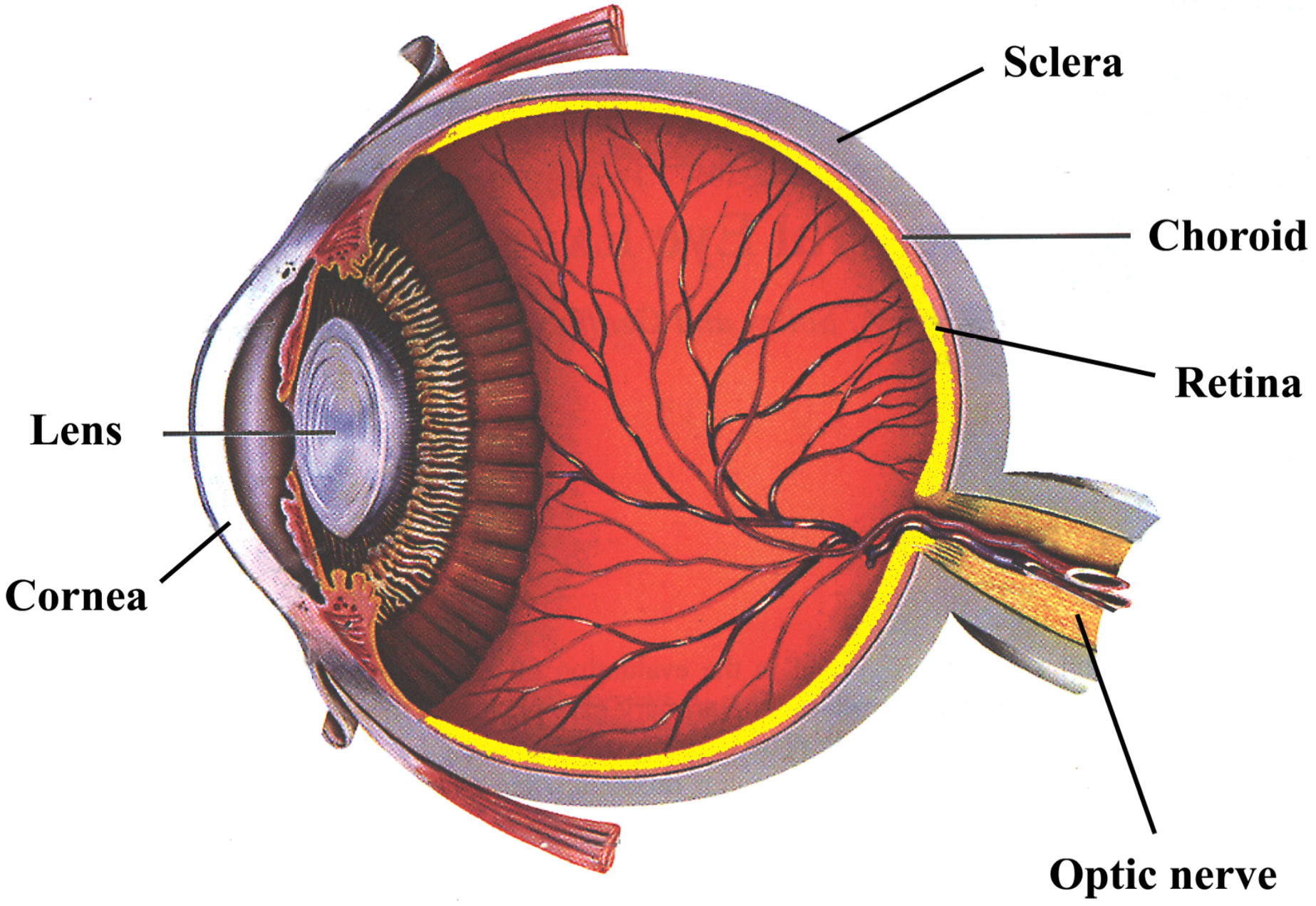
*60% of Usher1 cases.

Williams, Vision Research 48:433-441, 2008.

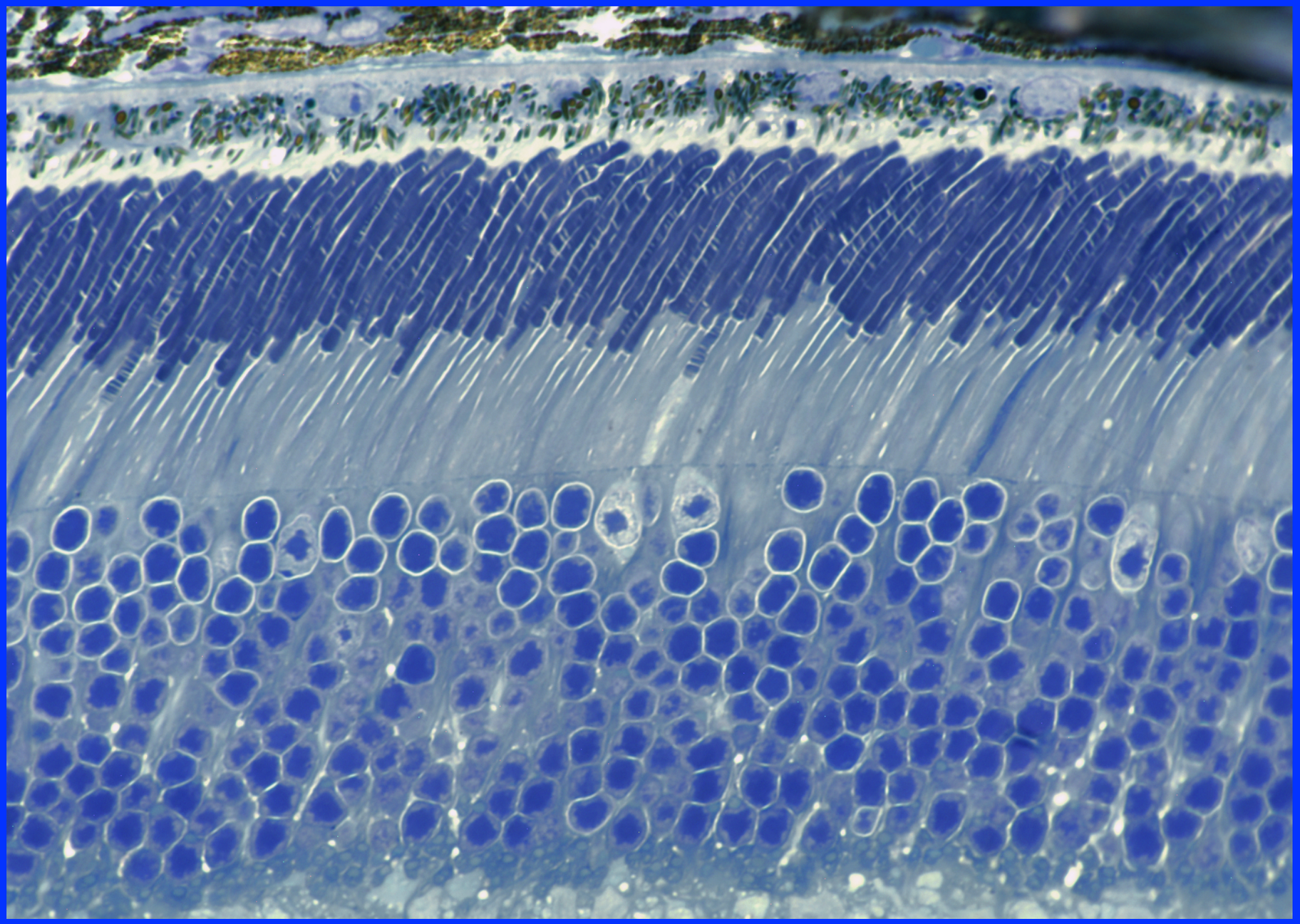
Myosin VIIa



Section of an eyeball



The photoreceptor cells in the retina



RPE

Outer segments

Inner segments

Nuclear layer

Synaptic layer

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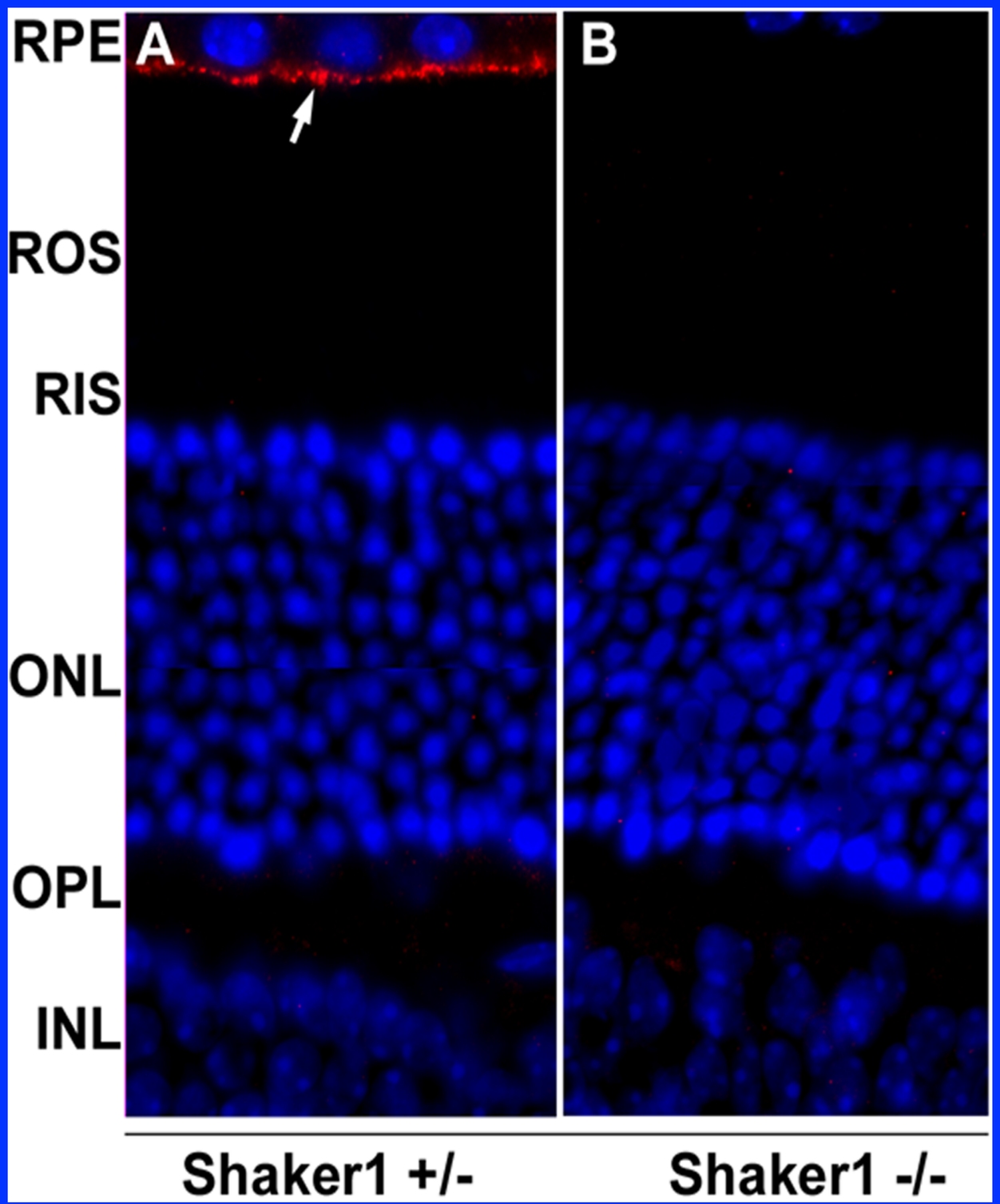
Where does MYO7A function in the retina?

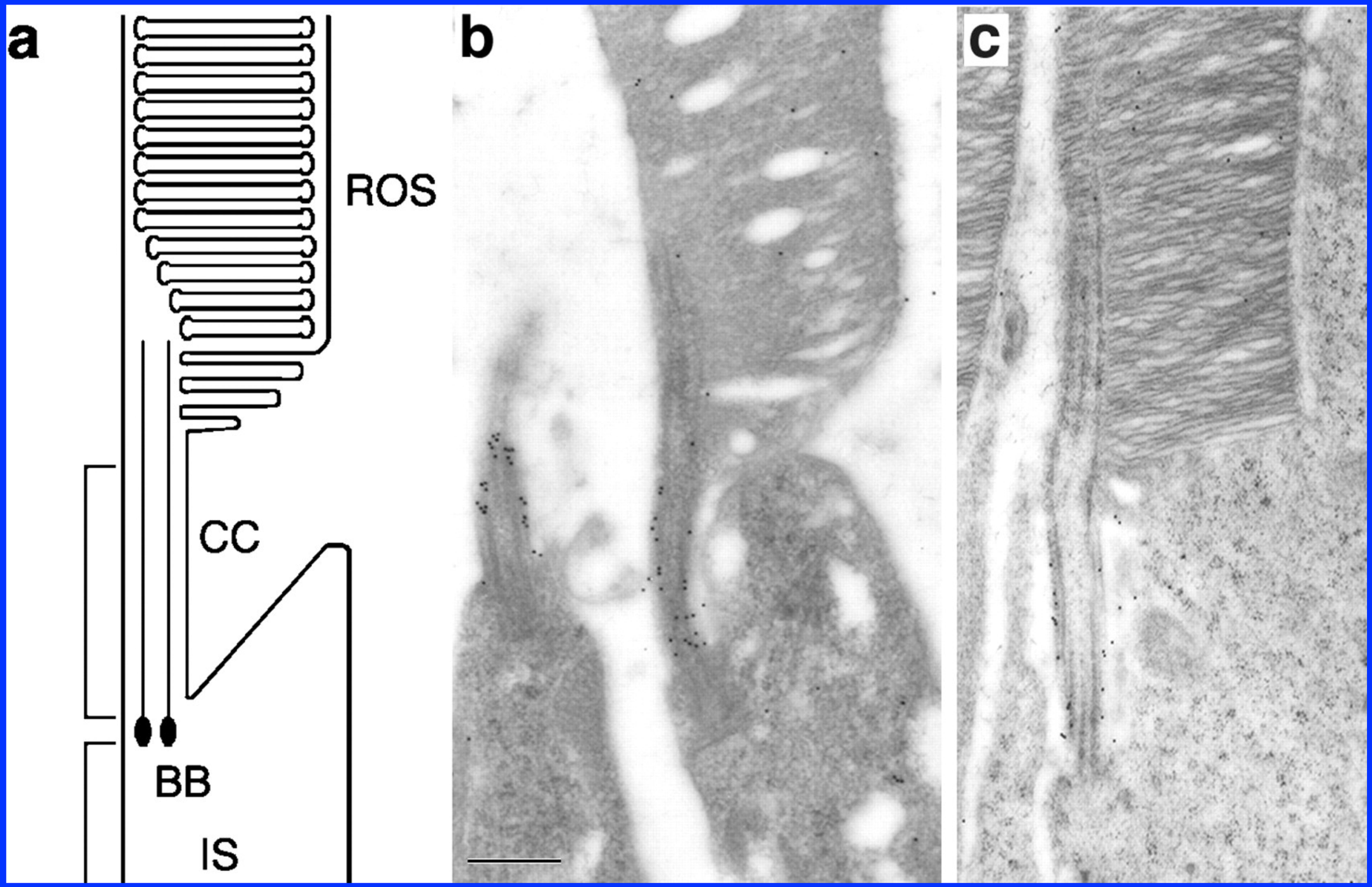
We use antibodies to detect the protein.
The antibodies are placed on a section of the retina.
By linking the antibodies to a fluorescent probe, we can thus localize the protein.



MYO7A is detected in the RPE

David Williams Lab

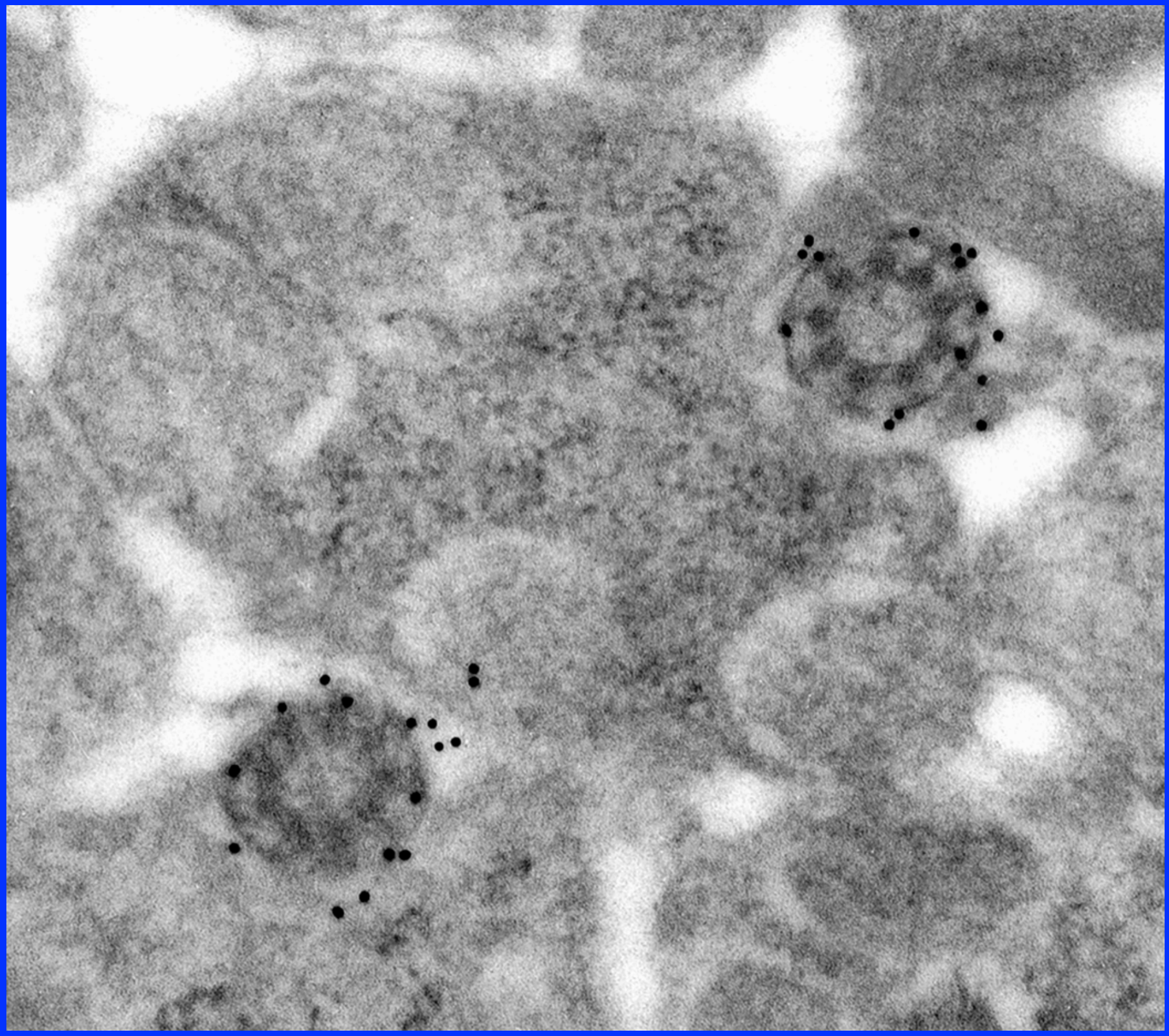
At higher resolution, using an electron microscope and gold particles to detect the MYO7A antibodies, we also detect small amounts of MYO7A in the connecting cilium of the photoreceptor cells.



Liu X et al. J. Neurosci. 1999;19:6267-6274

David Williams Lab

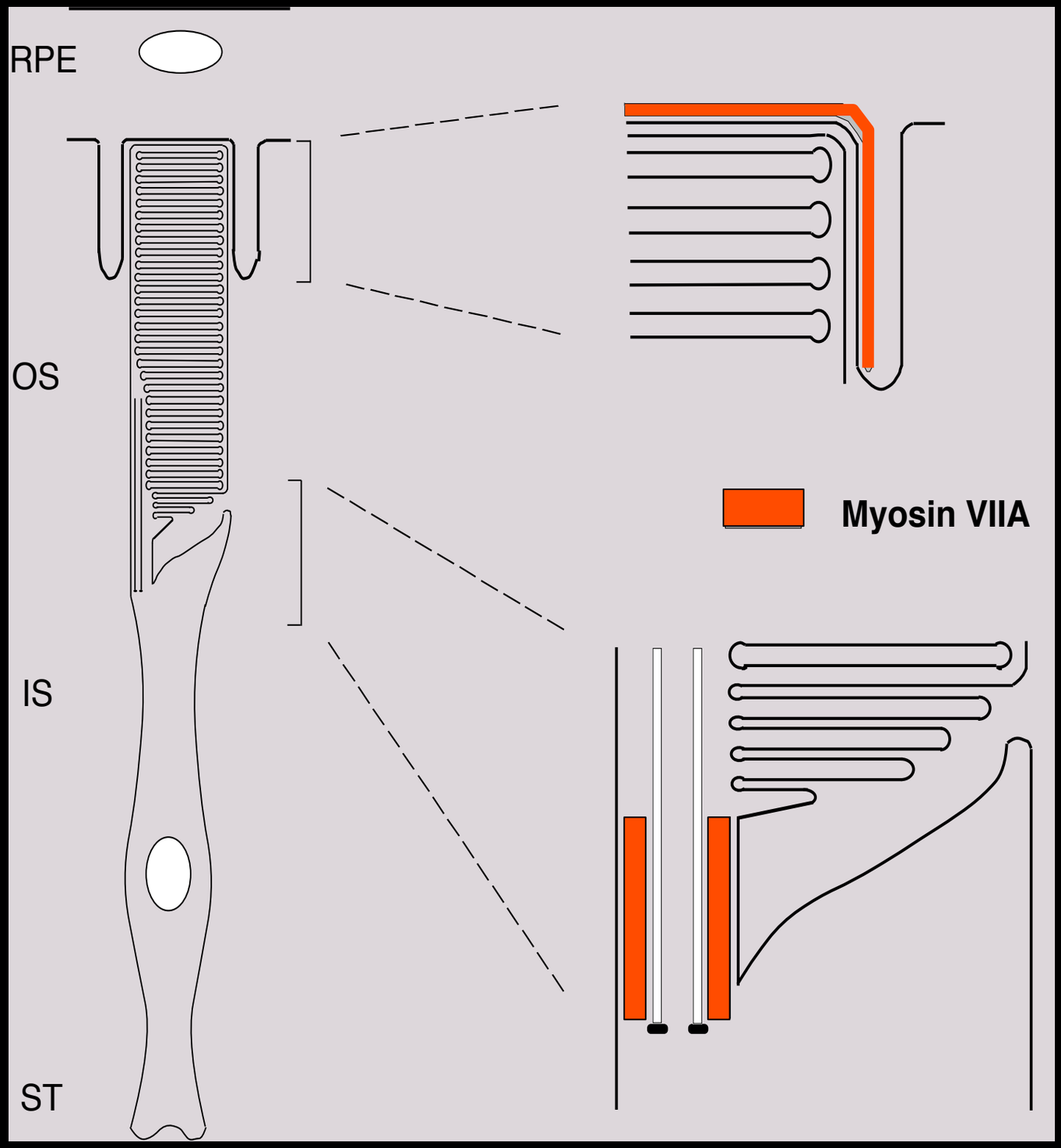
Immunogold Labeling of MYO7A in a TS of Photoreceptor Cilia



Liu et al., Cell Motility and the Cytoskeleton 37:240-252 (plus front cover), 1997.

David Williams Lab

MYO7A in present in two cell types in the retina.



RPE
apical region

Photoreceptor
connecting cilium

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Mutations in the gene encoding Myosin VIIa:

Usher syndrome type 1B — HUMAN

Shaker1 — MOUSE

Shaker1 4626SB (-/-) lack myosin VIIa.

Due to vestibular dysfunction, they have circling and head-tossing behavior.

Do not undergo retinal degeneration, but defects in the retina tell us what MYO7A does, and possible causes of retinal degeneration in Usher 1B.

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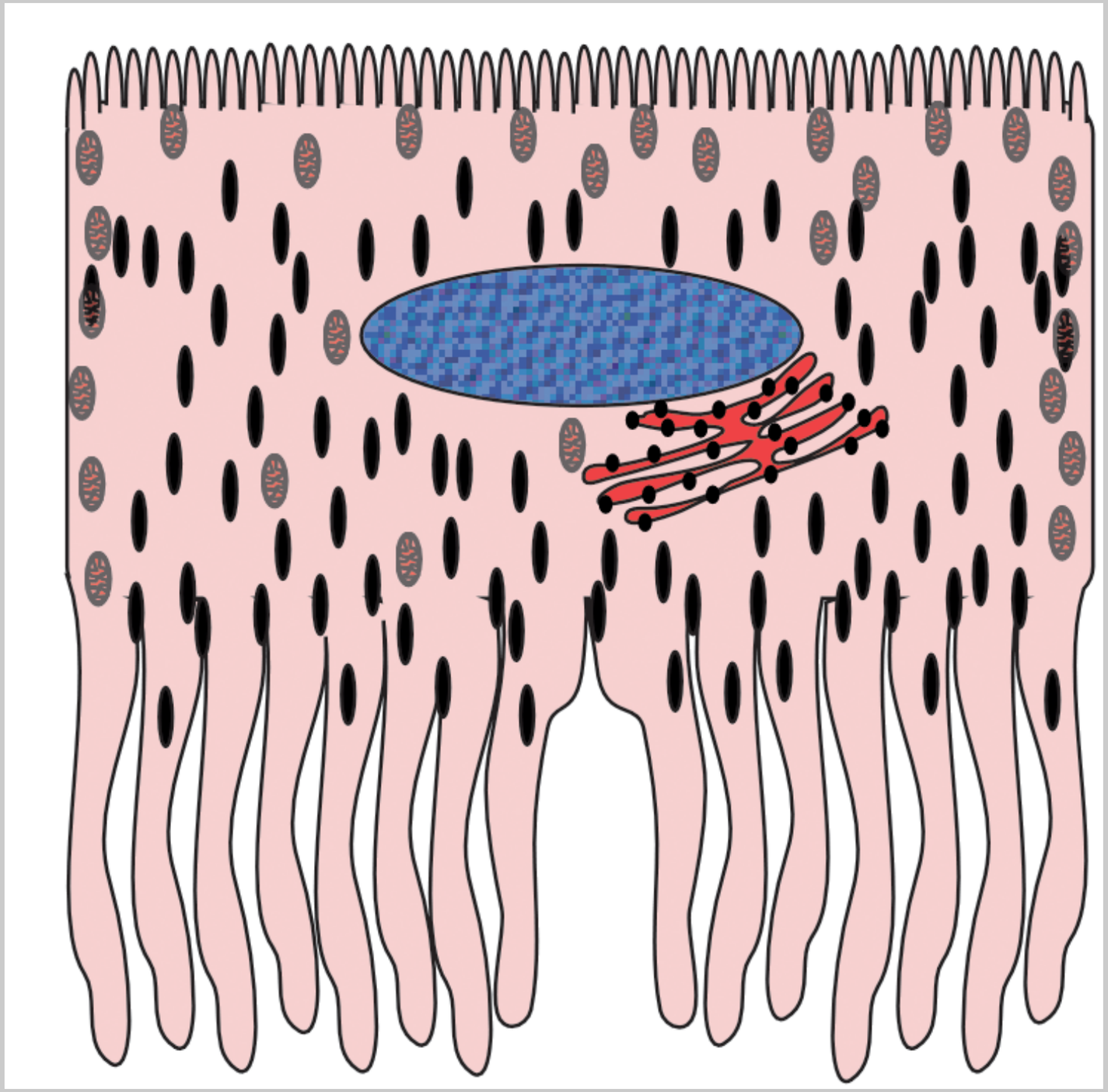
Retinal PIGMENTED Epithelium



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MYO7A is required for melanosome localization in the apical RPE

Control

OS

Mutant

Control

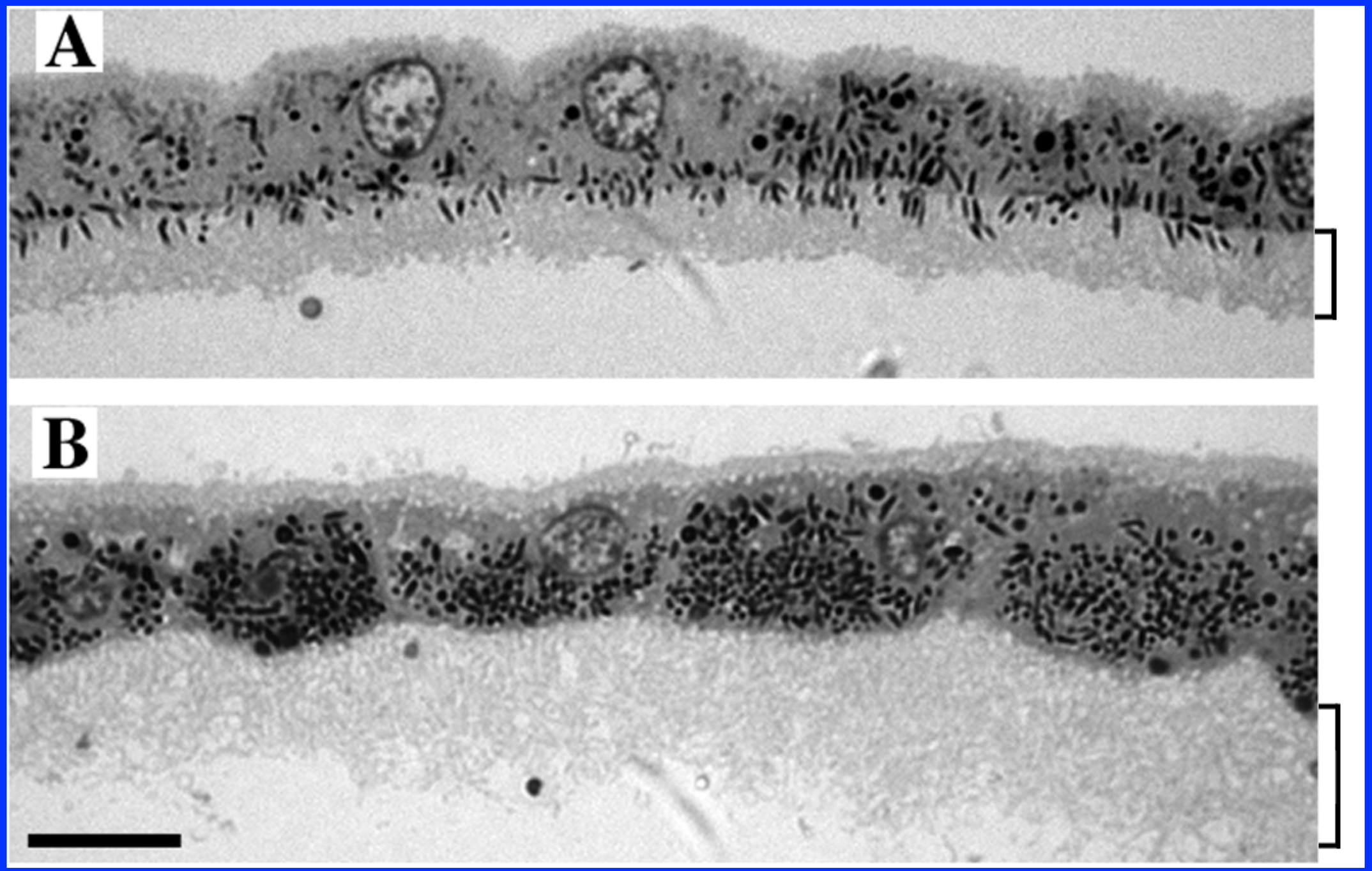
OS

Mutant

David Williams Lab

Liu et al., Nature Genetics 19:117-118, 1998.

Melanosome Distribution is recapitulated in Isolated Sheets of RPE Cells



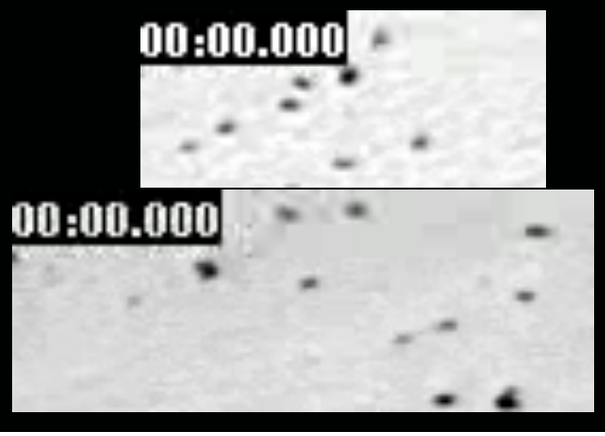
Control

Myo7a Null

David Williams Lab

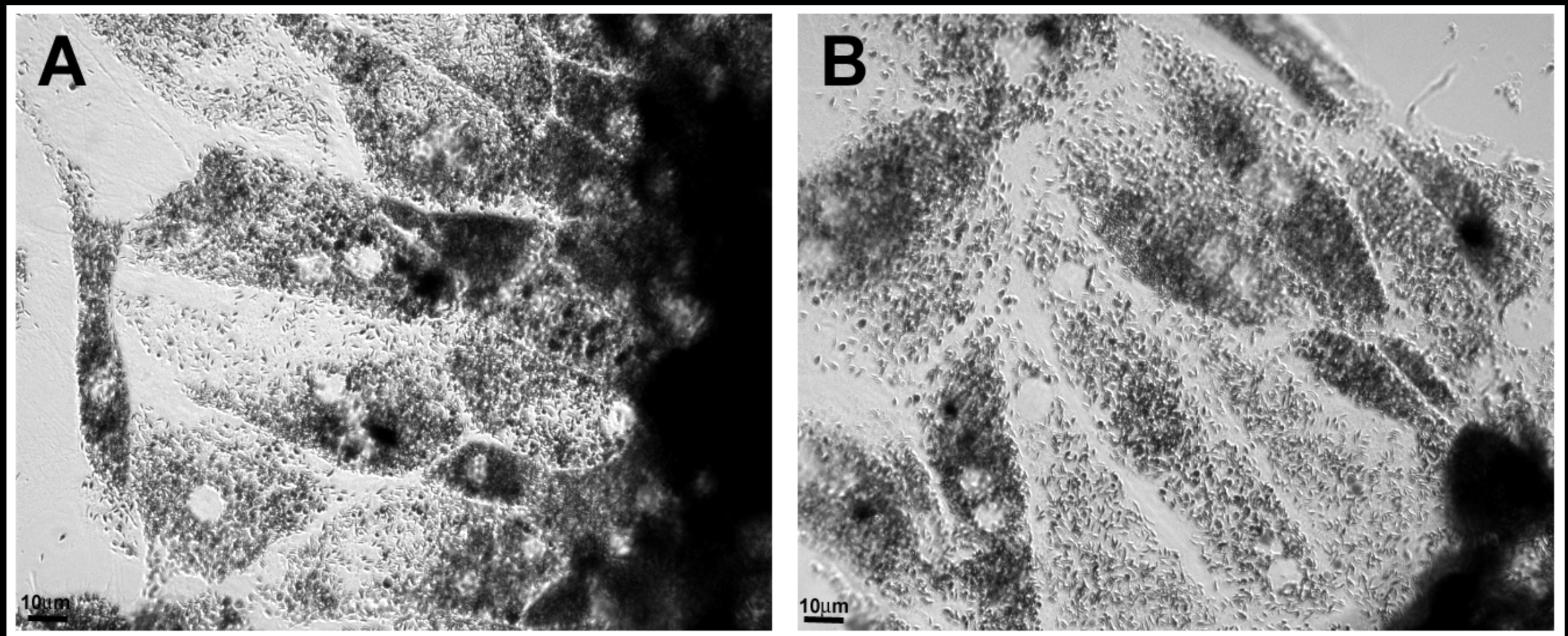
Gibbs et al. Journal of Cell Science 117:6473-6483, 2004.

Melanosomes dynamics are altered in primary cultures of MYO7A-null RPE cells



Control

Mutant



Control (Myo7a $^{4626SB/+}$)

Null Mutant (Myo7a $^{4626SB/4626SB}$)

David Williams Lab

Gibbs et al. Journal of Cell Science 117:6473-6483, 2004.

Phagocytosis and degradation

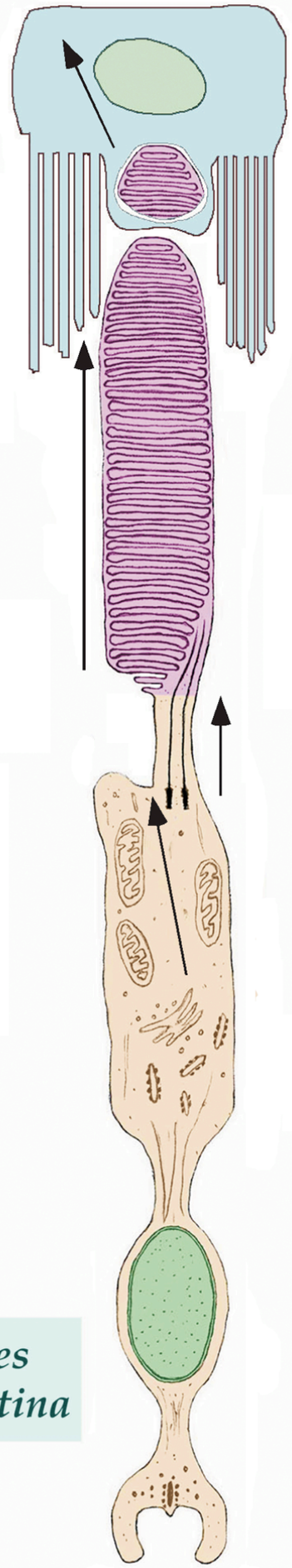
Distal displacement of disk membranes

Disk morphogenesis

Transport along CC

Vesicular transport to the cilium

Synthesis

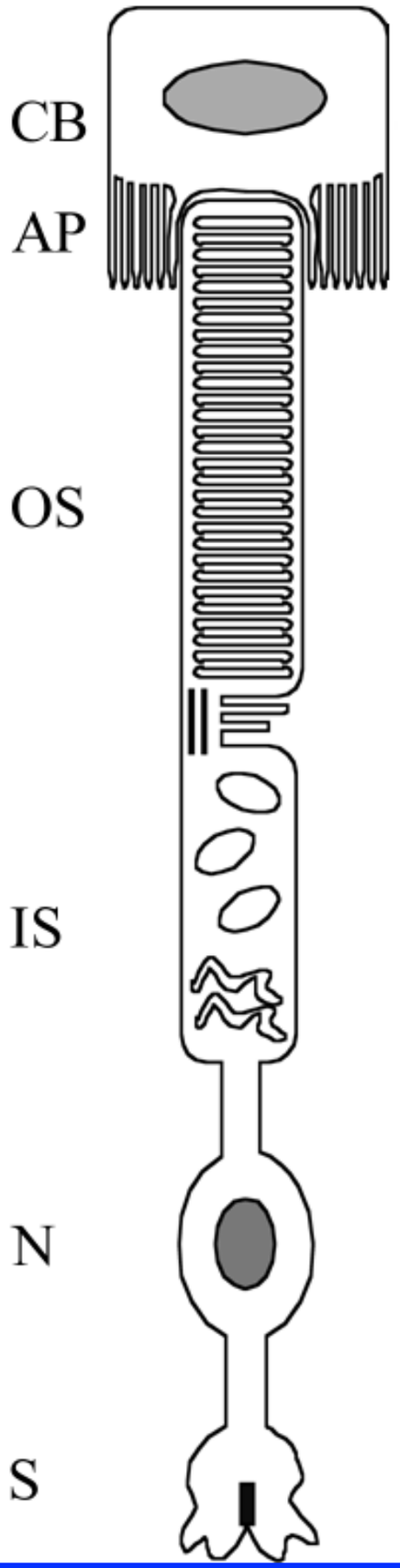


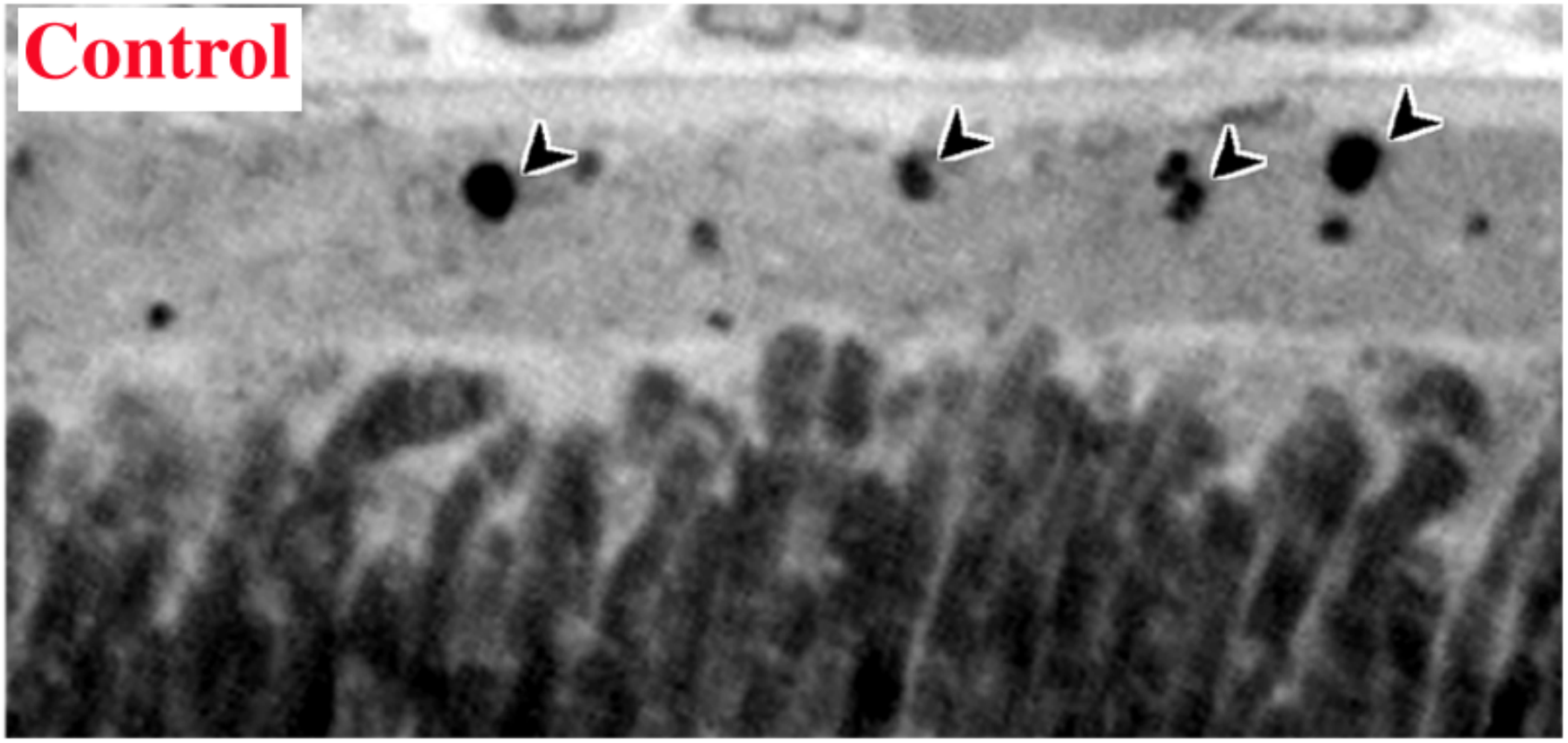
9 billion opsin molecules per sec. in each human retina

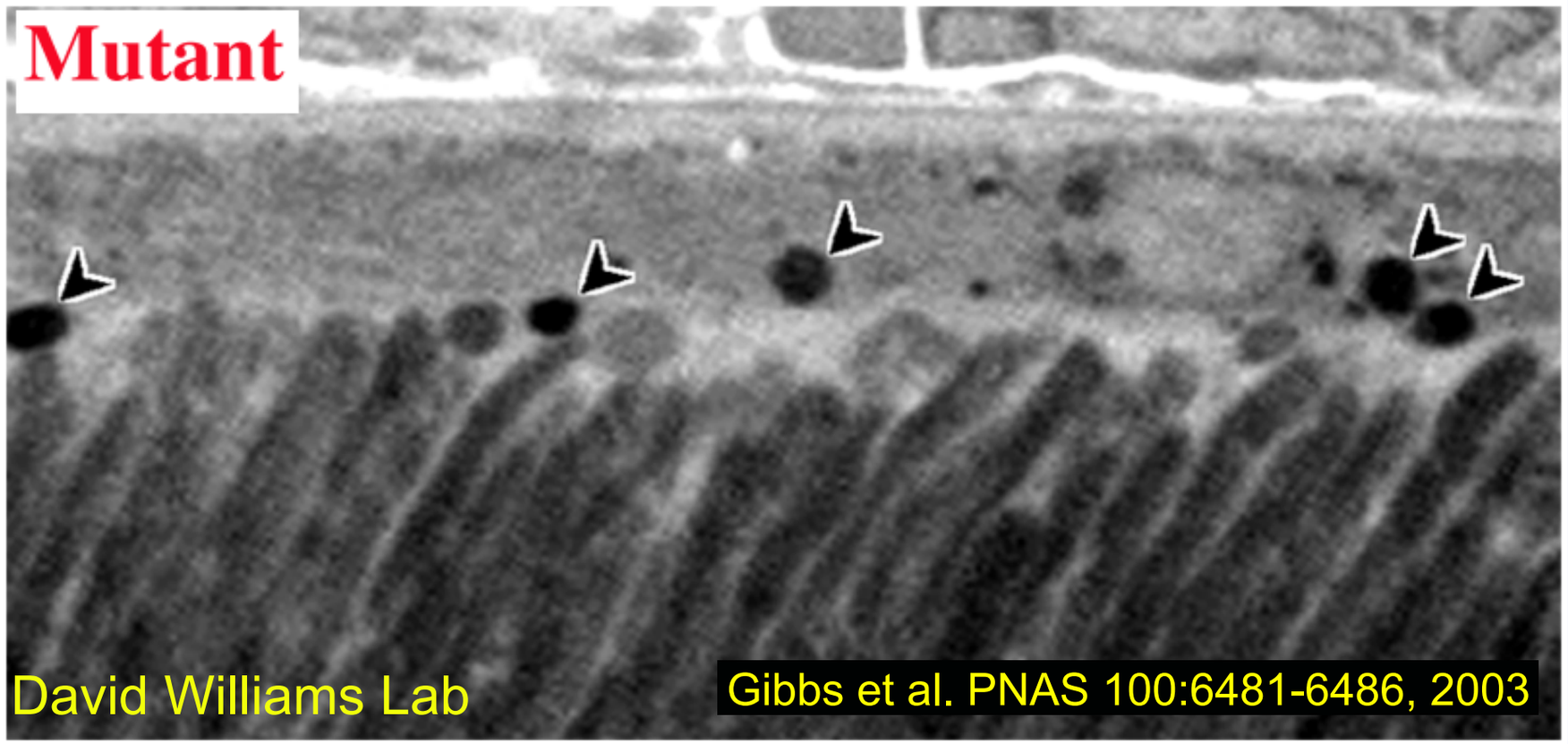
Turnover of the disk membranes of the outer segment

Williams, Vision Research, 42:455-462, 2002

Lack of MYO7A function inhibits phagosome movement into the RPE cell.

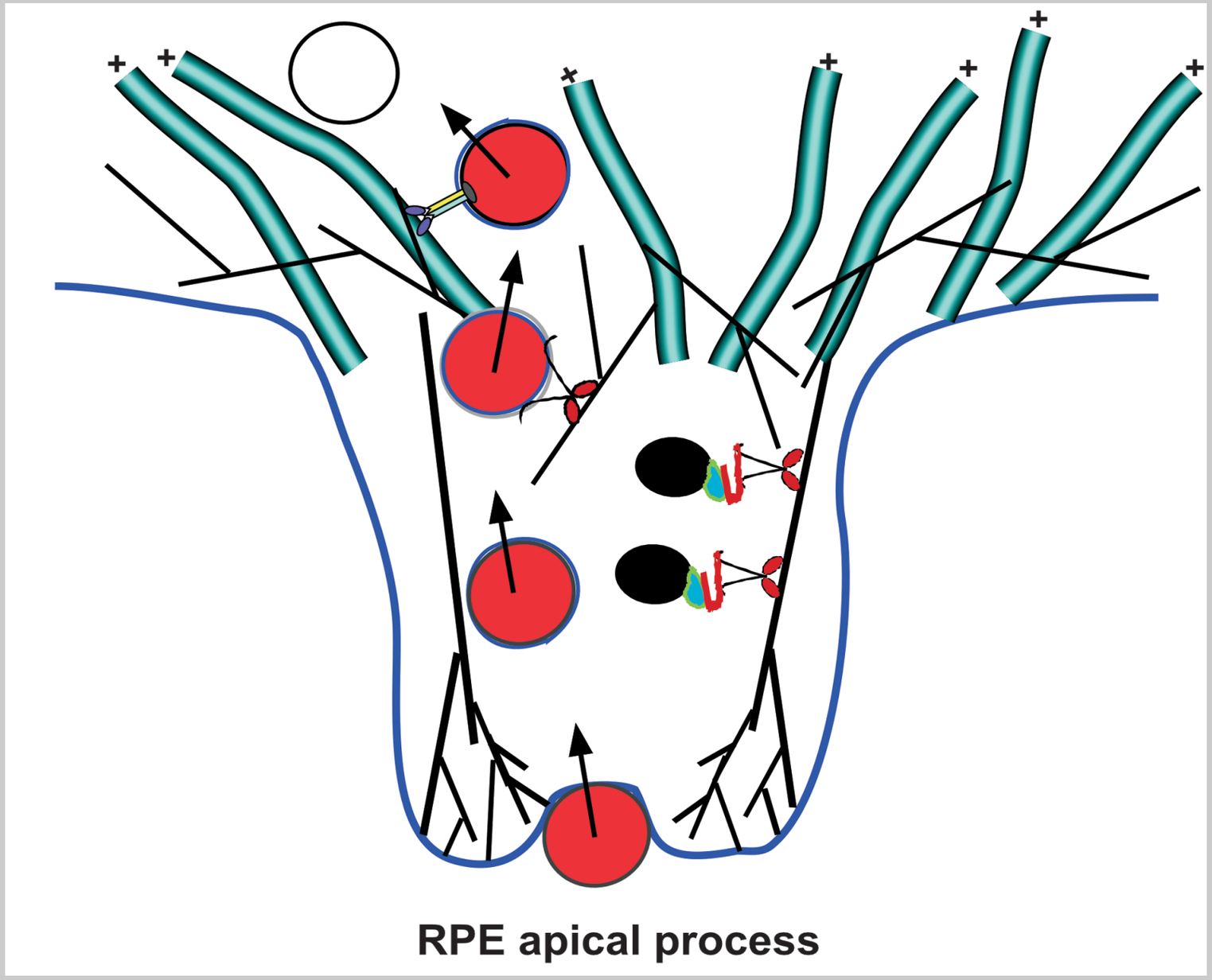


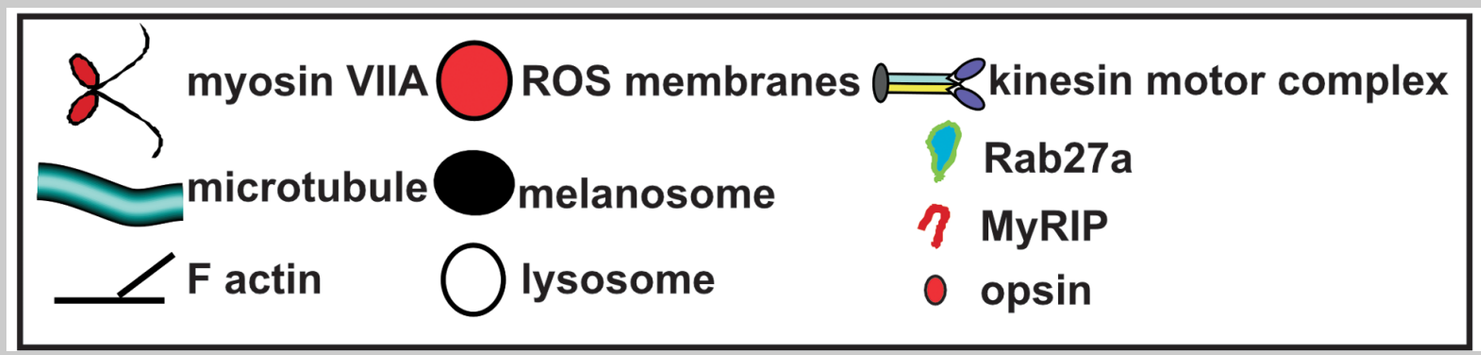




MYO7A function in the RPE

1. Moves and retains melanosomes into the apical processes.
2. Moves phagosomes out of the apical processes.

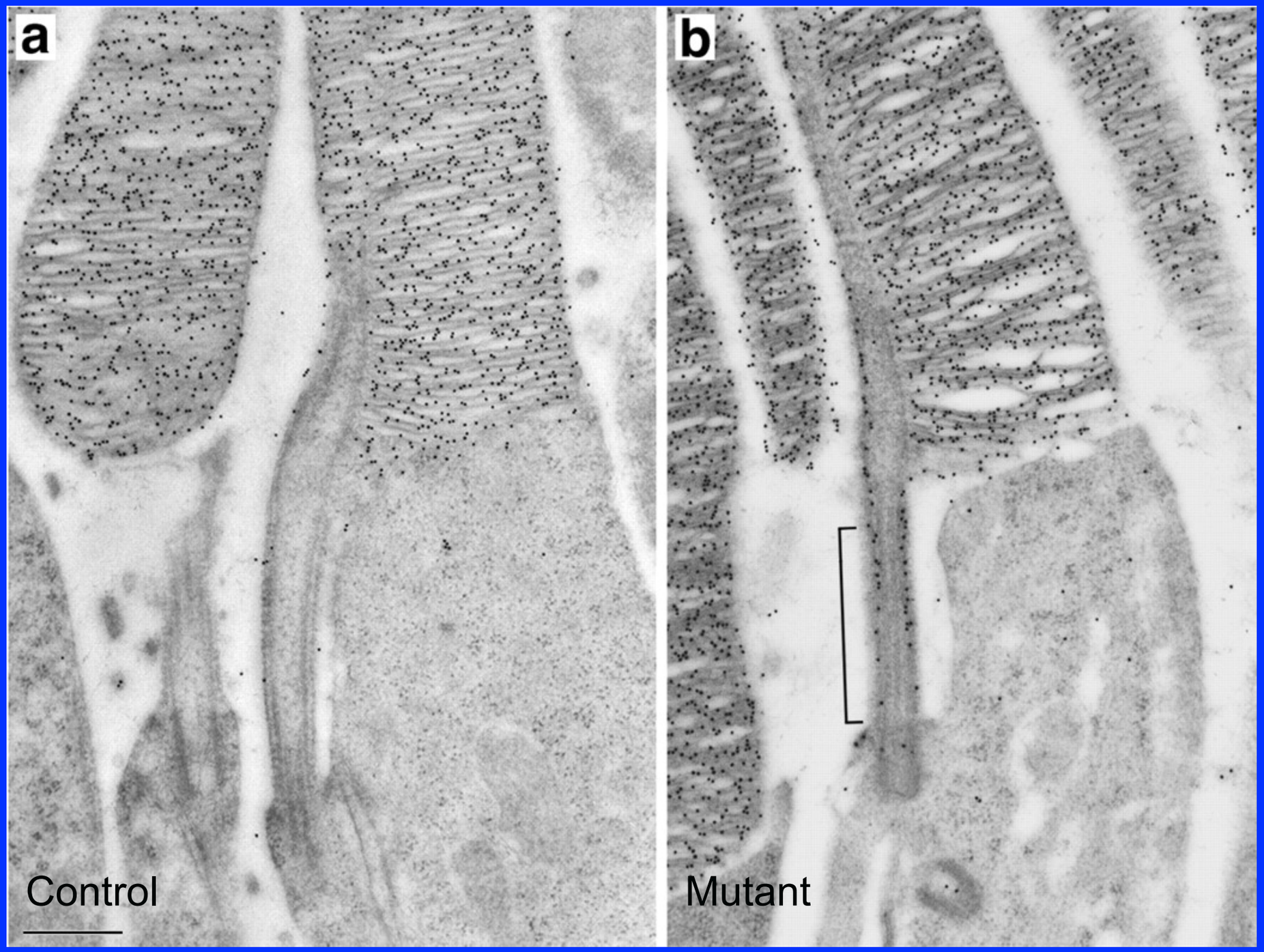




From:
Williams DS, Gibbs D: Myosin VIIa in the retina. In: Photoreceptor Cell Biology and Inherited Retinal Degenerations. Ed: Williams DS. World Scientific Publishing, Singapore, pp. 397-436, 2004.

Lack of MYO7A function impairs the passage of rhodopsin along the connecting cilium of photoreceptor cells.

Electron micrographs of immunogold labeling of rhodopsin



Liu X et al. J. Neurosci. 1999;19:6267-6274

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Summary of retinal dysfunction Function of MYO7A

1. Melanosome mislocalization in the RPE

2. Phagosome mislocalization in the RPE,
   resulting in inhibition of phagosome degradation

3. Impaired delivery of rhodopsin to the outer segment

These mutant phenotypes

- Tell us about the retinal function of MYO7A
- Provide us with tools for preclinical testing of therapies for Usher 1B blindness

Cause of blindness in Usher syndrome 1B

Mutations in the *MYO7A* gene that result in:

- No MYO7A protein synthesis (premature stop codon)
- Synthesis of an unstable MYO7A protein (that is quickly degraded)
- MYO7A protein that cannot function properly (impaired motor function or cargo binding)

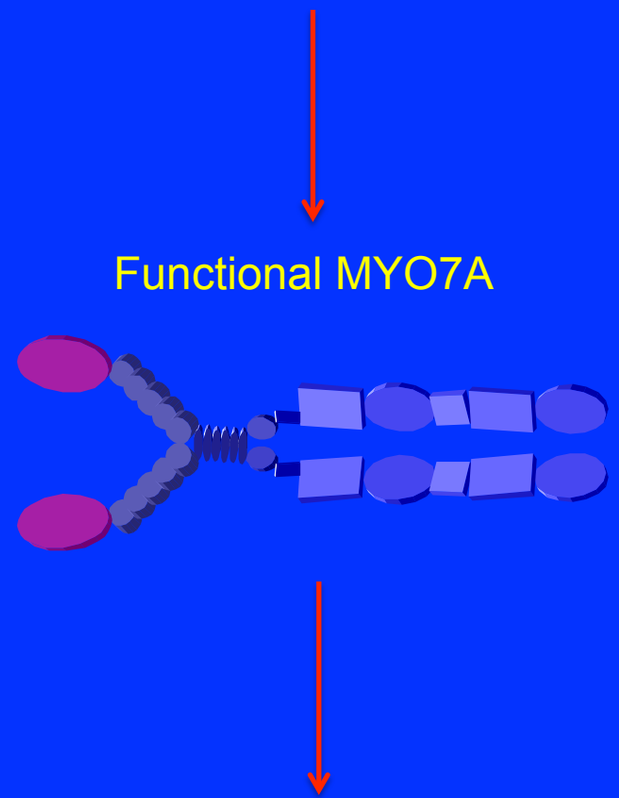
All lead to a loss of MYO7A function in the RPE and photoreceptor cells.

Therefore a potential therapy is to add a WT version of the *MYO7A* gene to the RPE and photoreceptor cells – *i.e. gene therapy*.

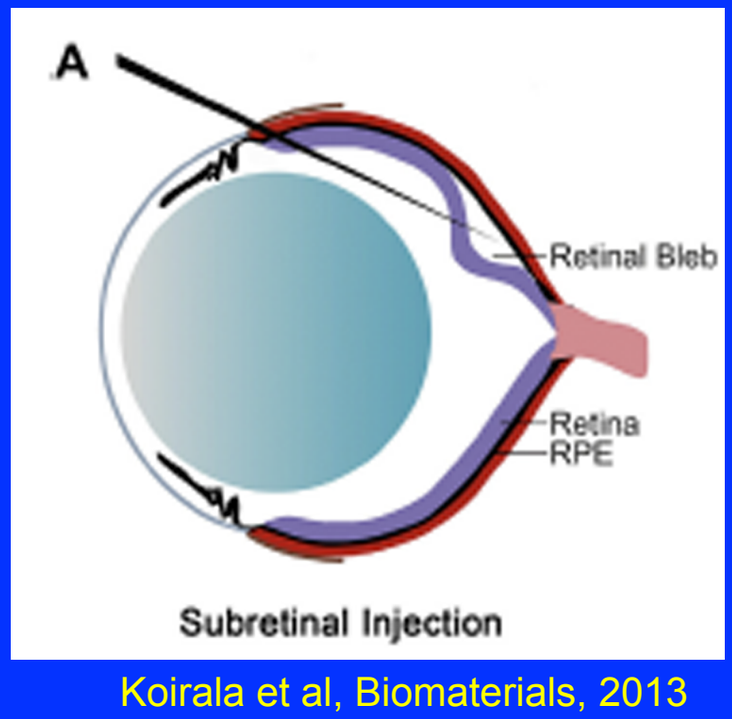
Gene Therapy Strategy for Usher syndrome 1B

Use of a virus to deliver the WT *MYO7A* cDNA to the RPE and photoreceptor cells by subretinal injection

Introduction of cDNA in the subretinal space



Correction of mutant phenotypes in the RPE and photoreceptors



Koirala et al, Biomaterials, 2013

Gene Therapy Strategy for Usher syndrome 1B

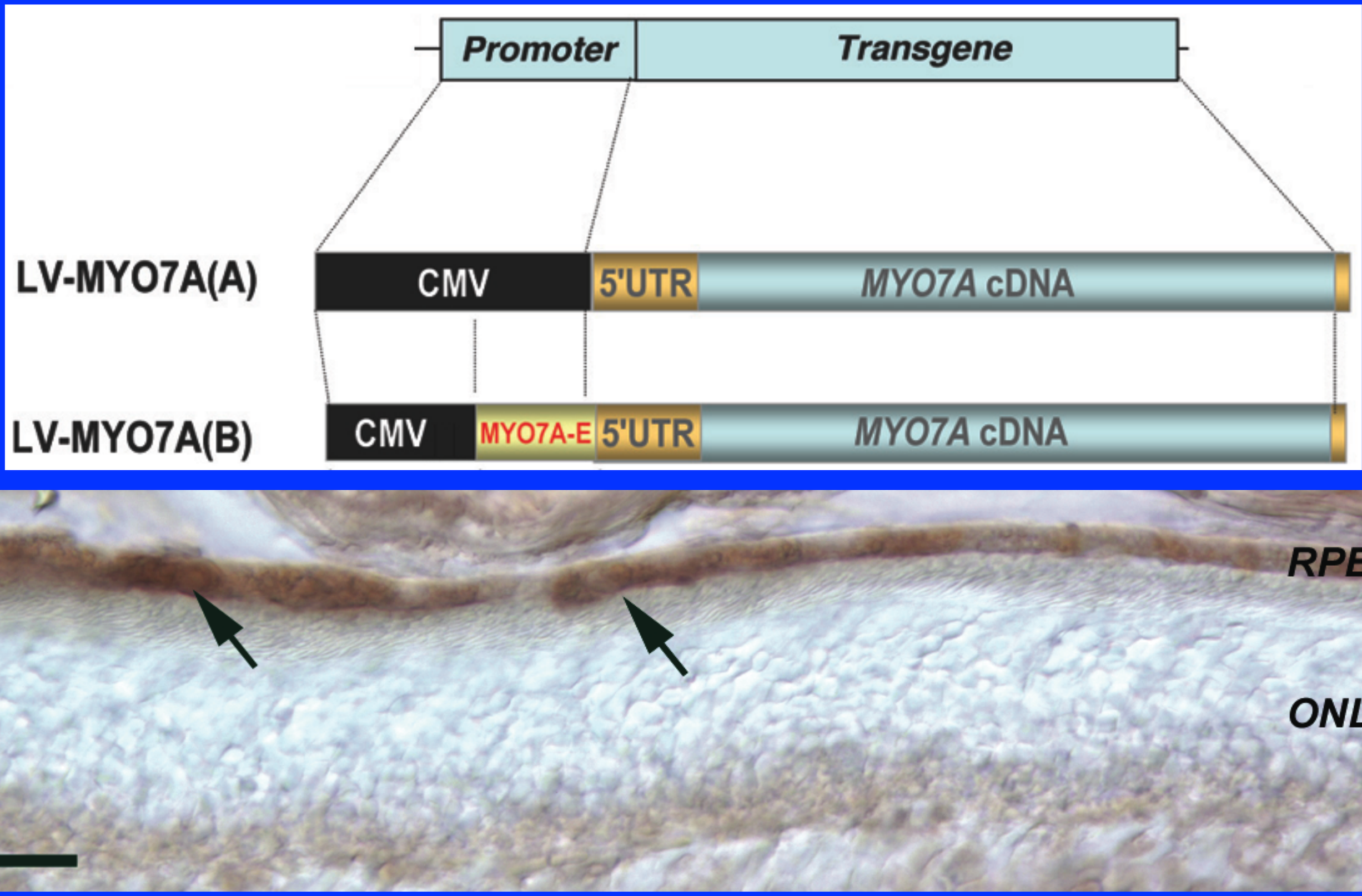
First problem is the size of the *MYO7A* cDNA.

MYO7A cDNA plus a promoter is ~7kb.

The adeno-associated virus (AAV), which has been used very successfully in the LCA2 trials, has a “nominal” carrying capacity of only ~5kb.

Potential solution: Use a lentivirus

HIV-based LV-*MYO7A*

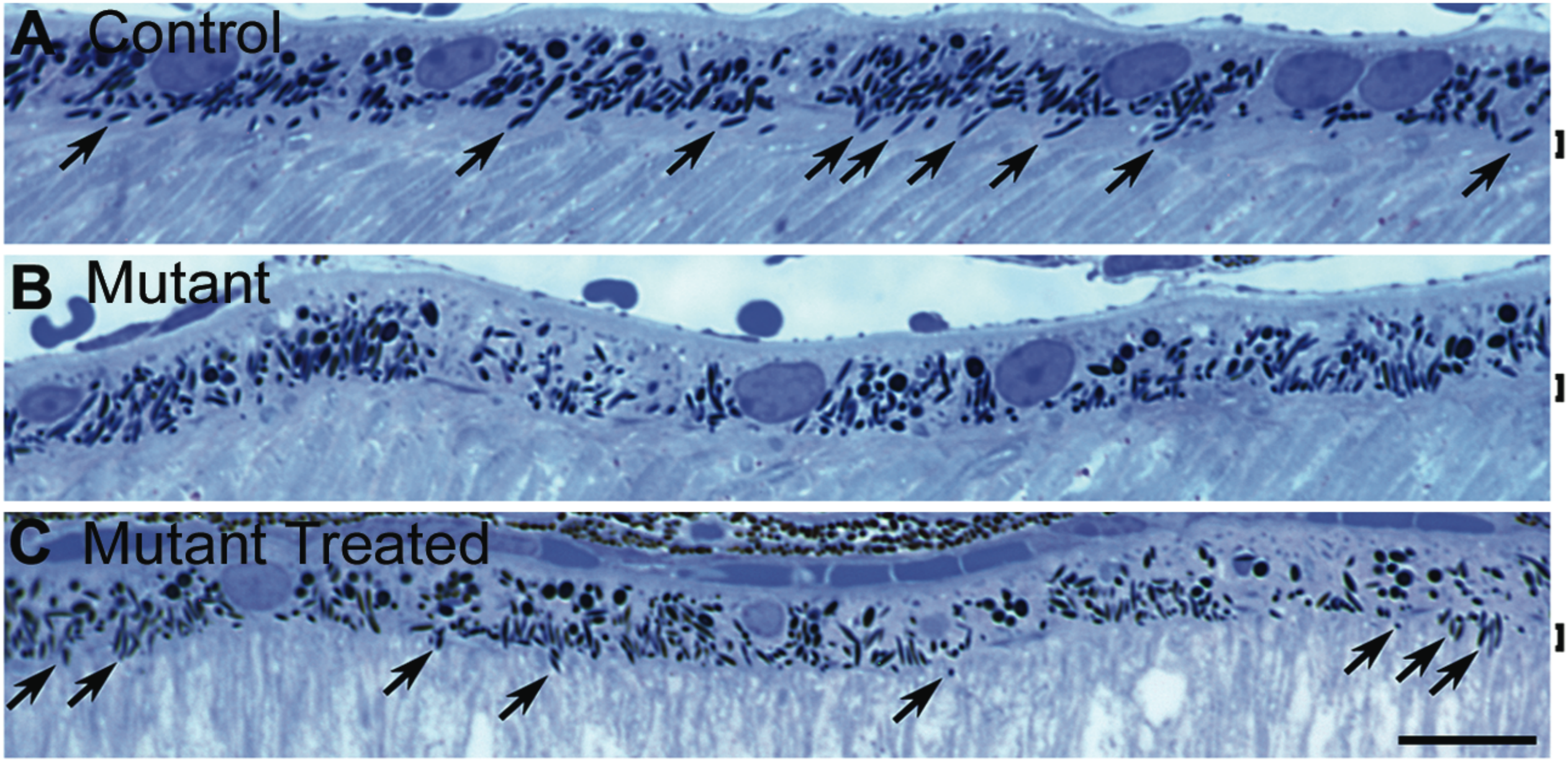


David Williams Lab

Hashimoto et al. Gene Therapy 14:584-594, 2007.

(Hashimoto et al, GT, 2007.)

Lentiviral mediated delivery of WT *MYO7A* in vivo corrects melanosome distribution in *some* null RPE cells



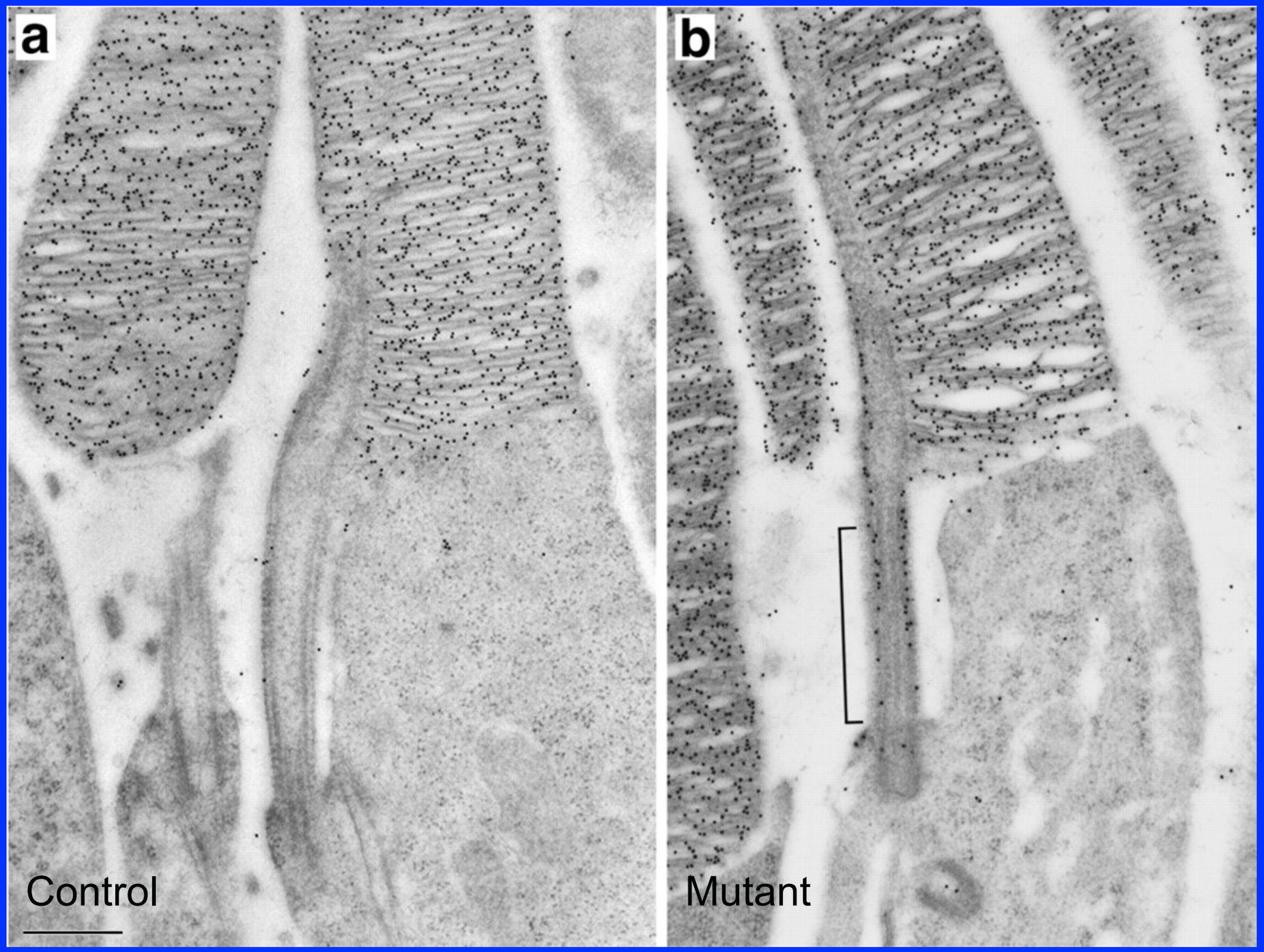
David Williams Lab

(Conchi Lillo, GT, 2007)

Hashimoto et al. Gene Therapy 14:584-594, 2007.

Test for correction of opsin distribution as a test for effective transduction of photoreceptor cells

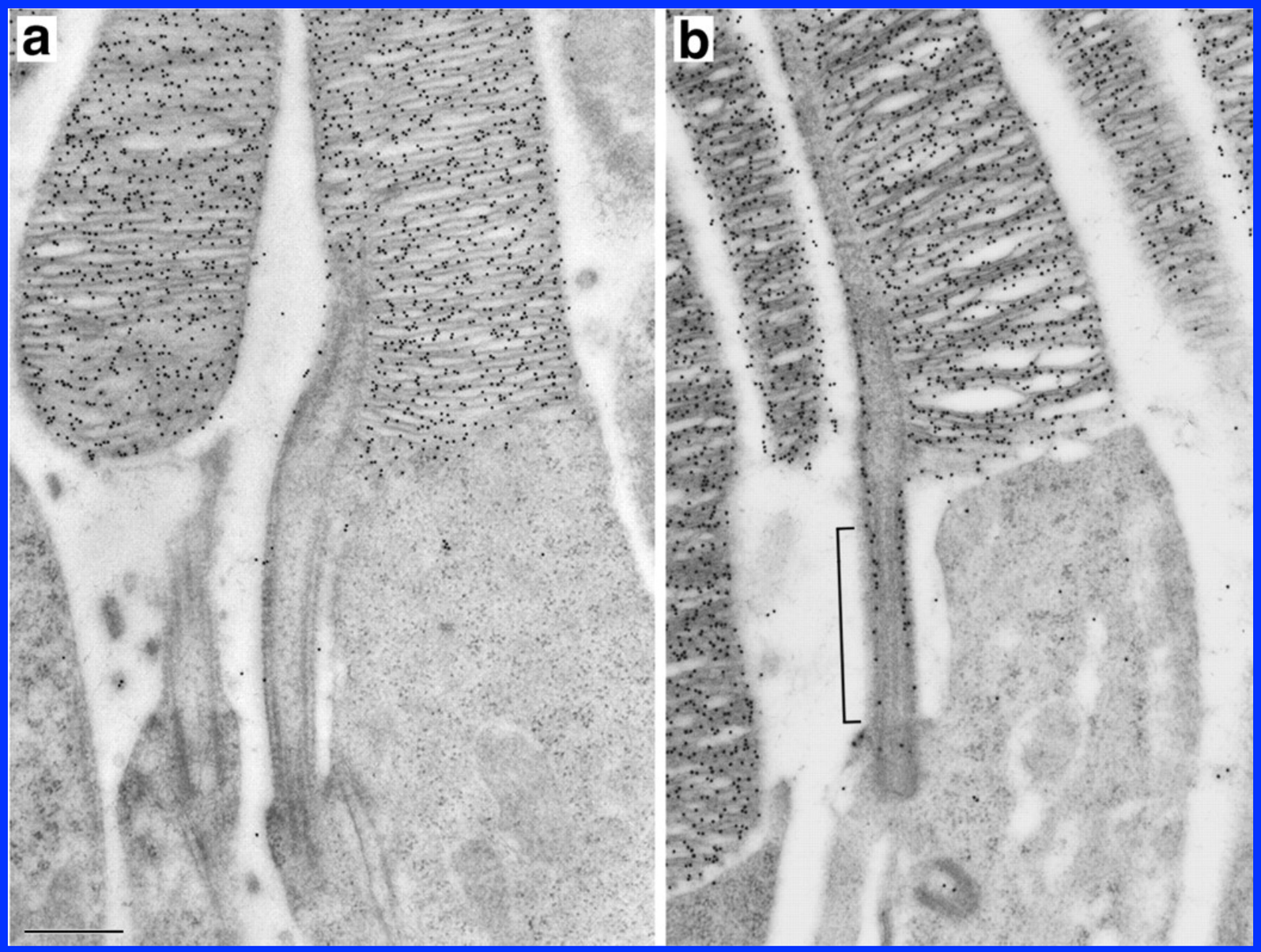
Electron micrographs of immunogold labeling of rhodopsin

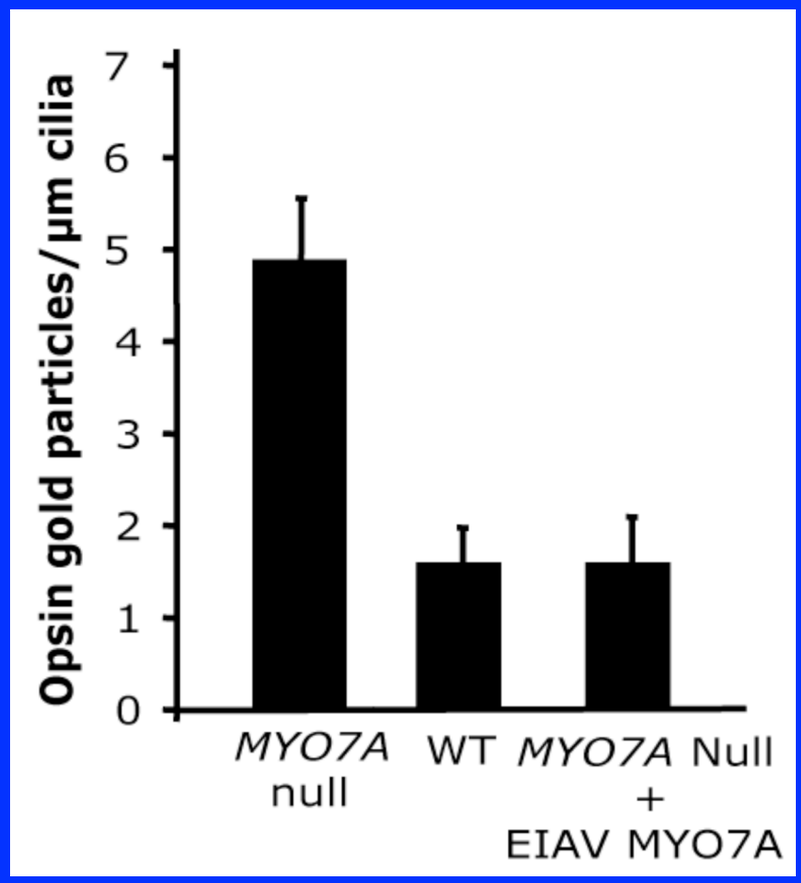


Liu X et al. J. Neurosci. 1999;19:6267-6274

David Williams Lab, UCLA

Phenotype correction photoreceptors with EIAV-*MYO7A*

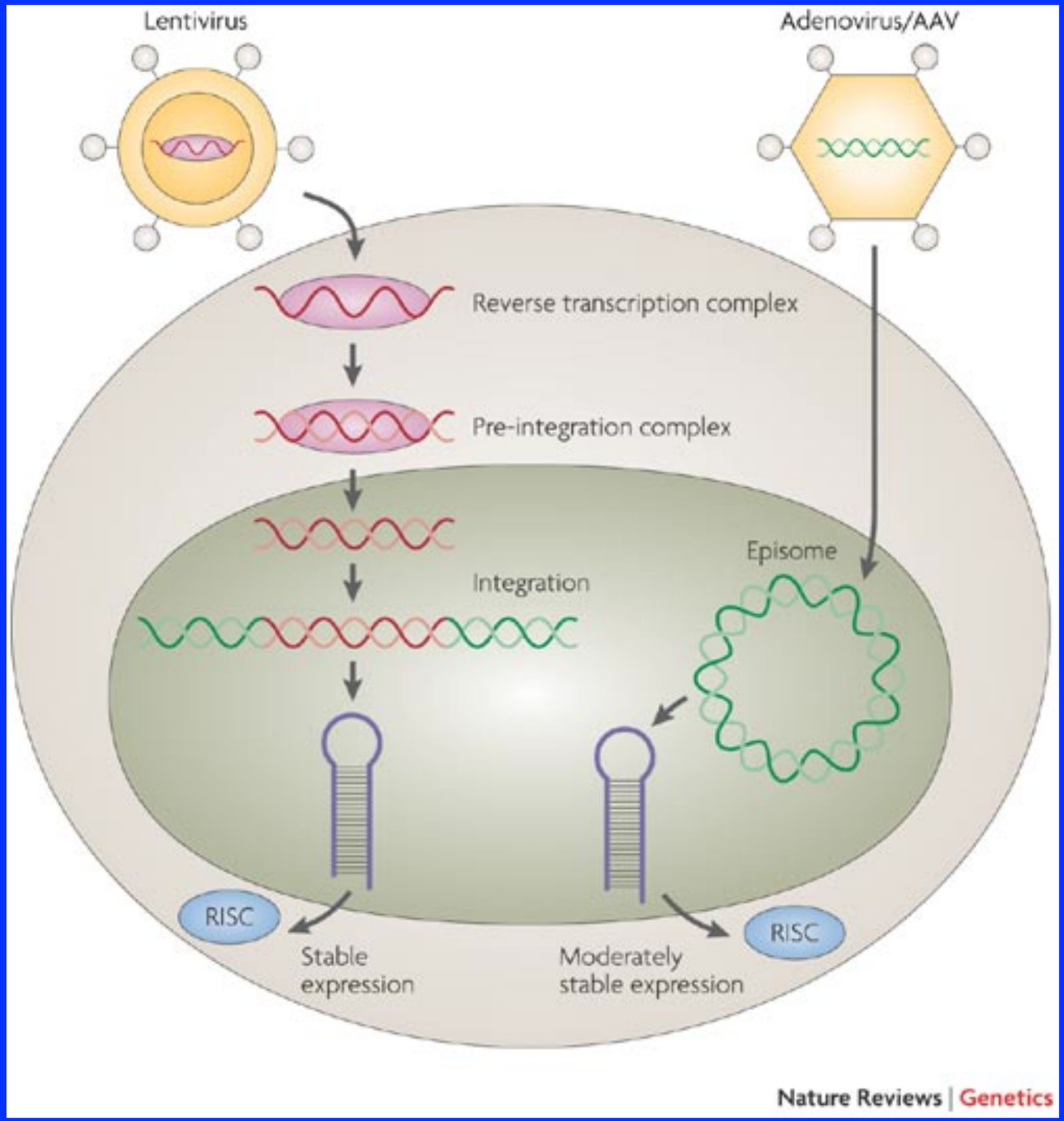




Liu X et al. J. Neurosci. 1999;19:6267-6274

David Williams Lab (unpublished)

LV vs AAV


Lentivirus
Adenovirus/AAV
Reverse transcription complex
Pre-integration complex
Integration
Episome
RISC
RISC
Stable
expression
Moderately
stable expression
Nature Reviews | Genetics

Demonstration that *MYO7A* cDNA could be delivered to primary RPE cells by AAV5

The Journal of Clinical Investigation

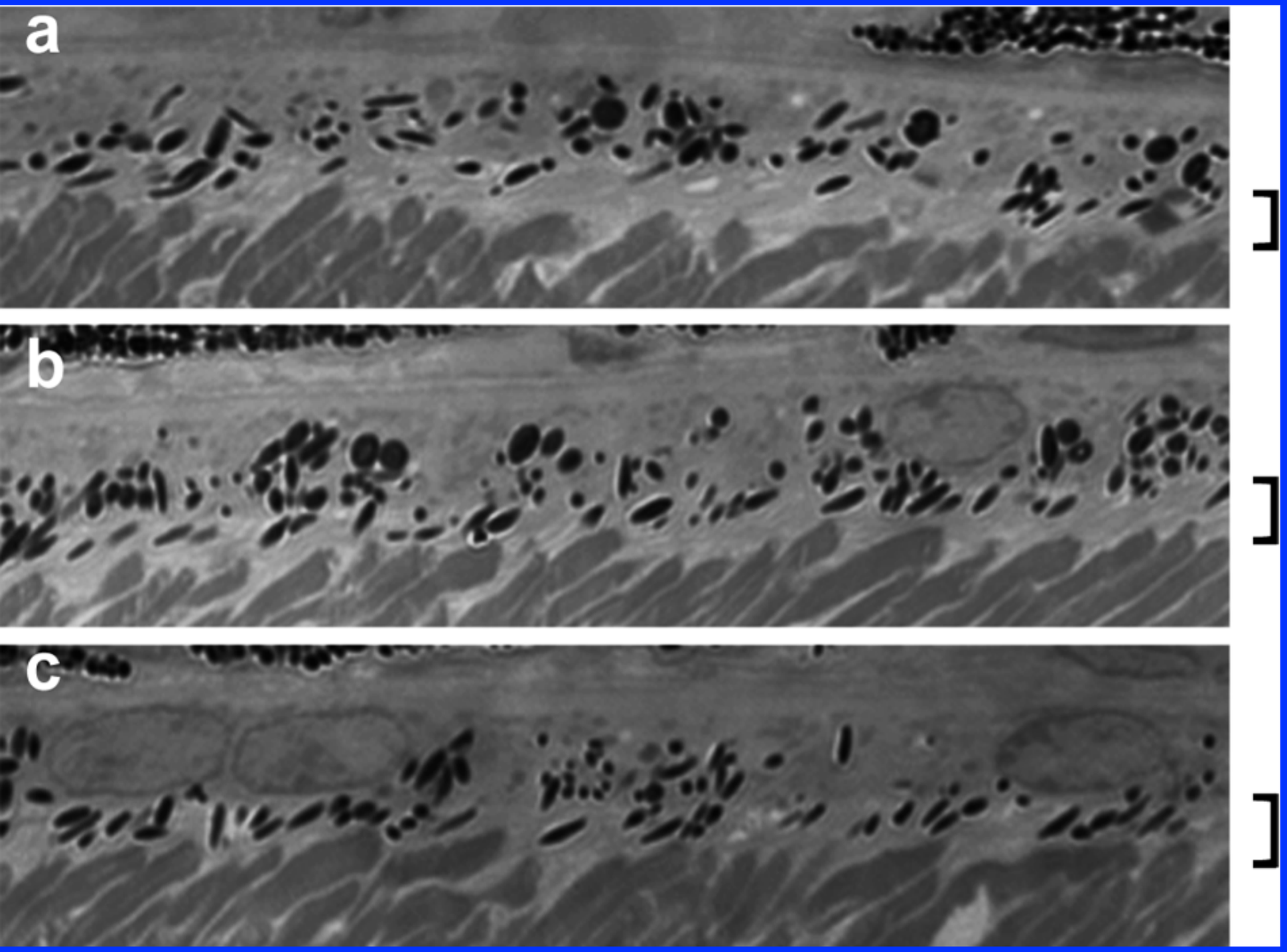
Technical advance

Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice

Mariacarmela Allocca,[1] Monica Doria,[1] Marco Petrillo,[1] Pasqualina Colella,[1] Maria Garcia-Hoyos,[1] Daniel Gibbs,[2] So Ra Kim,[3] Albert Maguire,[4] Tonia S. Rex,[4] Umberto Di Vicino,[1] Luisa Cutillo,[1] Janet R. Sparrow,[3] David S. Williams,[2] Jean Bennett,[4] and Alberto Auricchio[1,5]

[1]Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy. [2]Department of Pharmacology and Department of Neurosciences, UCSD School of Medicine, La Jolla, California, USA. [3]Department of Ophthalmology, Columbia University, New York, New York, USA. [4]F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [5]Medical Genetics, Department of Pediatrics, University of Naples Federico II, Naples, Italy.

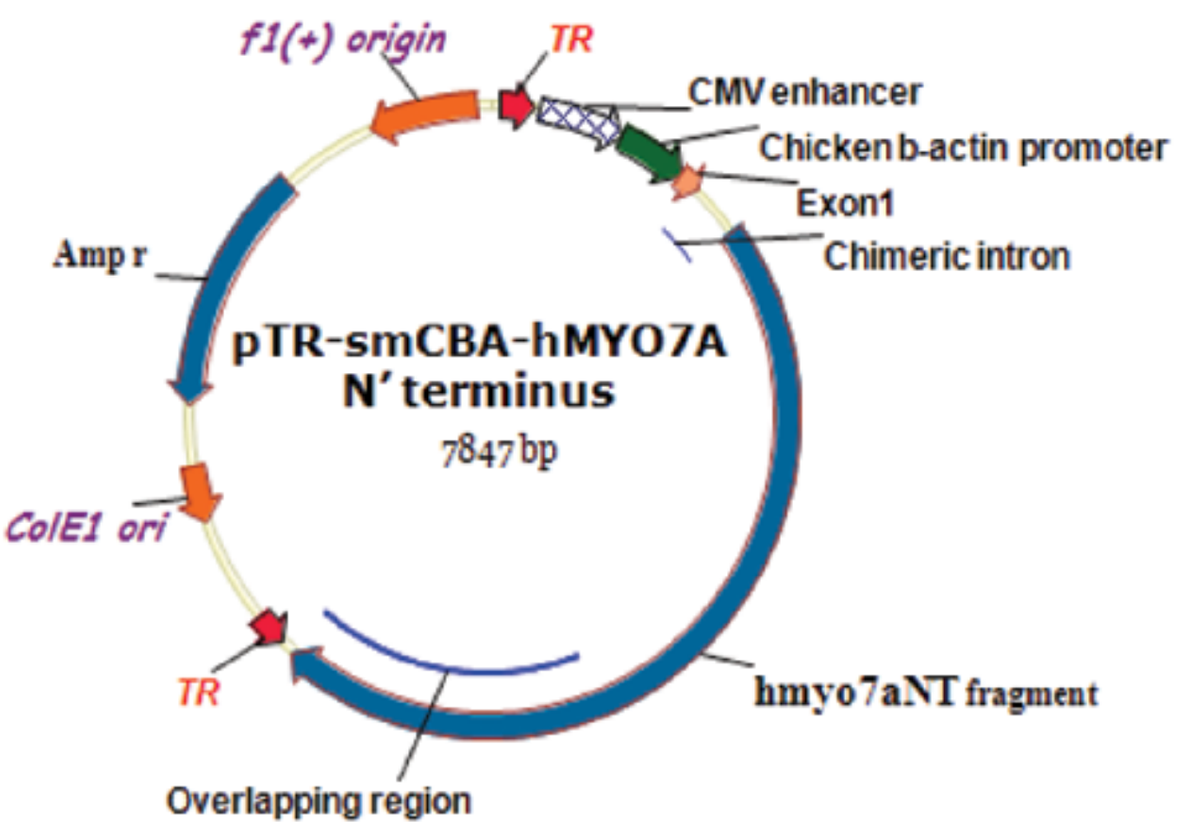
AAV2-*MYO7A* and AAV5-*MYO7A* correct mutant phenotypes

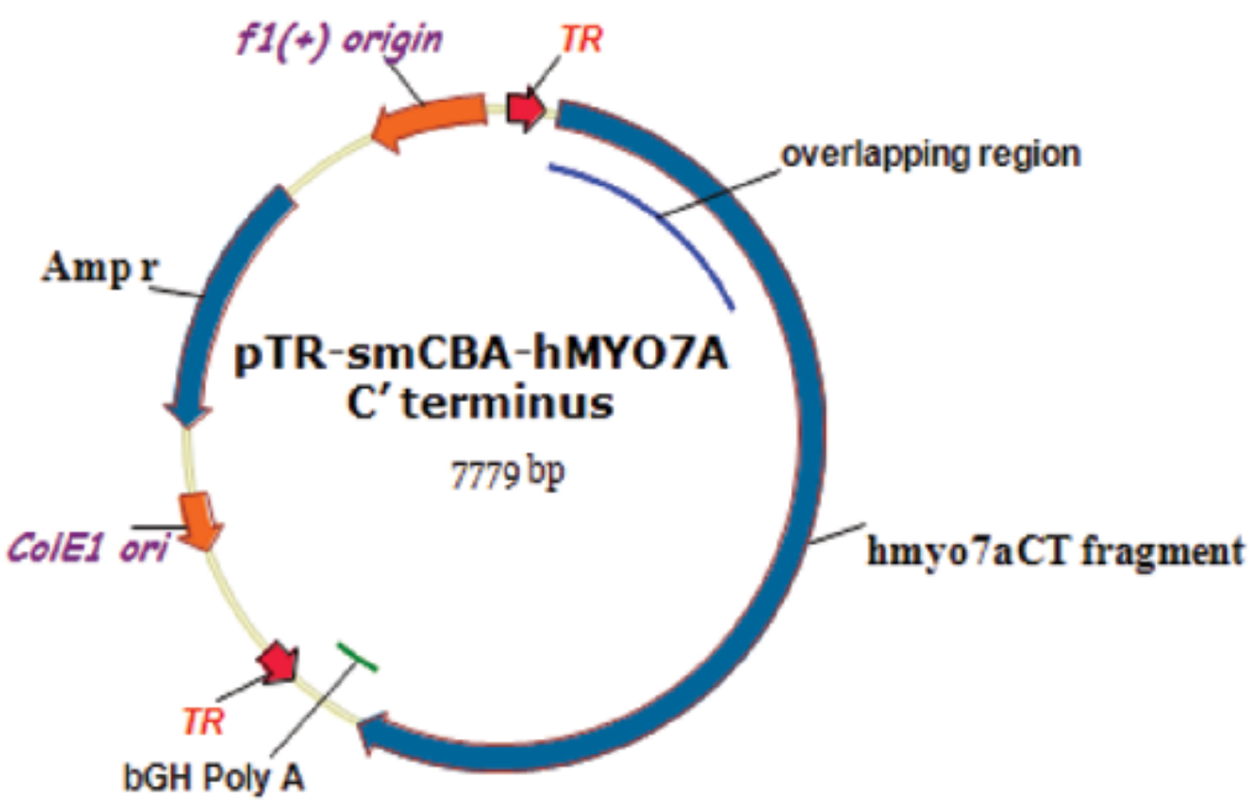


David Williams Lab, UCLA

Lopes et al. *Gene Therapy* 20:824-833 2013

AAV2-*MYO7A* Dual Vector Approach

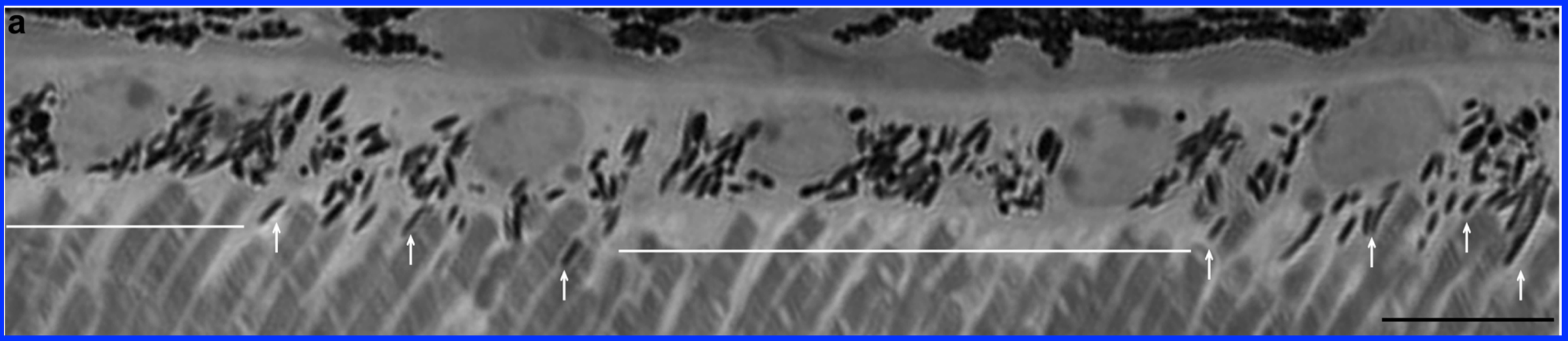




Bill Hauswirth Lab

Lopes et al. *Gene Therapy* 20:824-833 2013

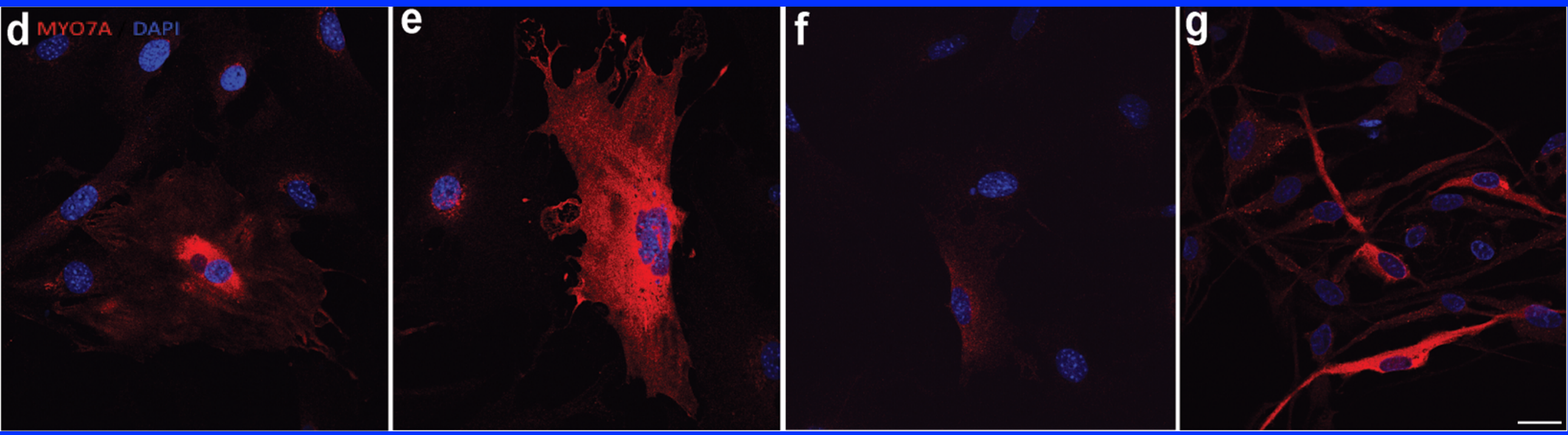
AAV2-*MYO7A* Dual Vector results in only partial correction.



David Williams Lab, UCLA

Lopes et al. *Gene Therapy* 20:824-833 2013

AAV2-*MYO7A* Dual Vector causes pathological overexpression in a few cells and insufficient expression in others.



MYO7A-null primary RPE cells

ARPE-19 cells

David Williams Lab, UCLA

Lopes et al. *Gene Therapy* 20:824-833 2013

Summary of preclinical gene therapy experiments

- Lentiviral-*MYO7A* works

  Downside:
  - photoreceptors may not be transduced well.
  - variable expression from cell to cell
  - potential for insertional mutagenesis

  Current clinical trials in Oregon and Paris

- AAV2-*MYO7A* or AAV5-*MYO7A* works

  Downside:
  - virus appears to deliver a variety of cDNA fragments.

  Used in successful clinical trials on LCA2

- AAV2-*MYO7A* dual vector

  Downside:
  - Did not provide correction in most of the cells
  - Pathology observed in overexpressing cells in culture