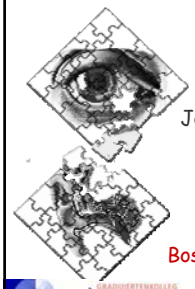
Gene-based therapy strategies for human Usher syndrome



Uwe Wolfrum,
Department of Cell & Matrix Biology,
Institute of Zoology,
Johannes Gutenberg University of Mainz
Germany
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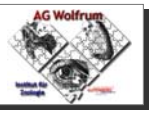
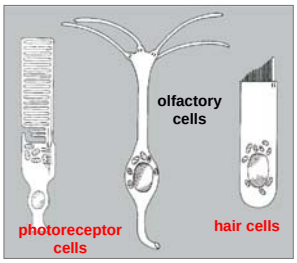
Boston, MA June 19, 2009, ~ 30 minutes

Research projects



Ciliated sensory cells in vertebrates



Uwe Wolfrum, Cell & Matrix Biol., Institute of Zoology, Johannes Gutenberg-University of Mainz, Germany

Molecular & cell biology of ciliated sensory cells

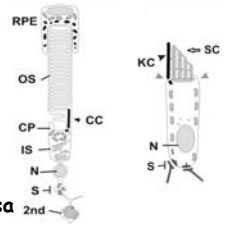
- Identification of novel molecules of the cytoskeleton in ciliated sensory cells.
- Centrins in sensory cells.
- Intracellular translocations in sensory cells
- Protein networks related to the Usher syndrome
- Pathomechanisms in senso-neuronal degenerations.

&

Evaluation of therapeutic strategies

Human Usher syndrome (USH)

- USH is the most common form of combined hereditary deaf-blindness.
- Prevalence: 5-10/100,000
- Autosomal recessive disorder
- Symptoms:
  - Hearing impairment
  - Vestibular dysfunction
  - Vision loss - Retinitis pigmentosa
- 3 clinical types (USH1-3) are based on severity, age of onset and progression of symptoms.
- There are at least 13 genetic heterogeneous subtypes.
- 10 USH causing genes are identified, so far.



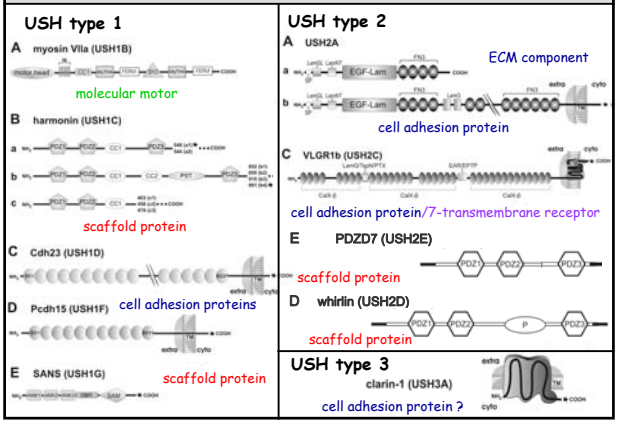
Human Usher syndrome (USH), genes

Type	Gene locus	Gene	Protein	Function	Mouse model
1A = 1B					
1B	11q13.5	*MYO7A*	myosin VIIa	molecular motor	*Shaker-1 (sh1)*
1C	11q15.1	*USH1C*	harmonin	scaffold protein	*Deaf circler (dfcr)*
1D	10q21-q22	*CDH23*	cadherin 23	cell-cell adhesion	*Waltzer (v)*
1E	21q21	--	--	--	--
1F	10q11.2-q21	*PCDH15*	protocadherin 15	cell-cell adhesion	*Ames waltzer (av)*
1G	17q24-25	*SANS*	SANS	scaffold protein	*Jackson shaker (js)*
1H	15q22-23	*USH1H*	--	--	--
2A	1q41	*USH2A*	USH2A (usherin)	ECM, cell adhesion	k.o.
2C	5q14.3-21.3	*VLGR1b*	GPR98/VLGR1b	GPCR, cell adhesion	*Mass1 (frings)*
2D	9q32	*DFNB31*	whirlin	scaffold protein	*Whirler (wi)*; k.o.
2E	10q24.3	*PDZD7*	PDZD7	scaffold protein	
3A	3q21-25	*USH3A*	clarin-1	cell adhesion	k.o. in prep.
3B	20q	--	--	--	--

UW 2009

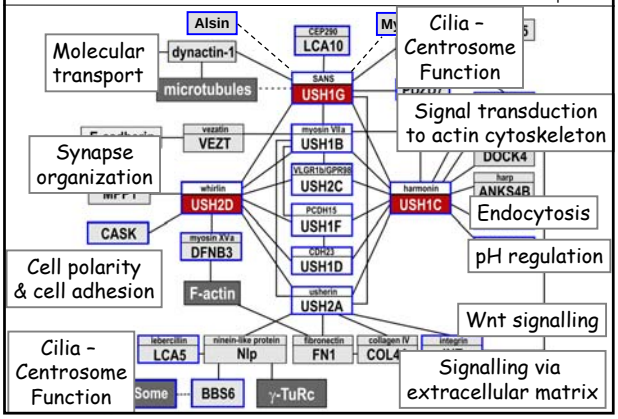
modified from Reiners et al. 2006 ExpEyeRes

Diversity of Usher syndrome proteins

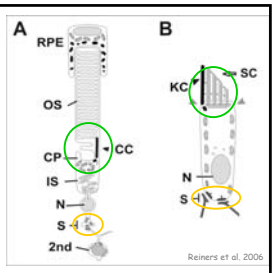


USH protein interactome Feb. 2009 *

*selected proteins



Conclusions



USH protein networks are integrated and linked to the cytoskeleton by the USH scaffold proteins: harmonin, whirlin and SANS.

In the ear, USH networks participate in kinocilia/stereocilia differentiation during hair cell development, but also in ribbon synapse function, and in signal transduction.

In photoreceptor cells, USH networks are found at the synapse and in the ciliary region. They may contribute to the intracellular transport and to the ciliary import and delivery.

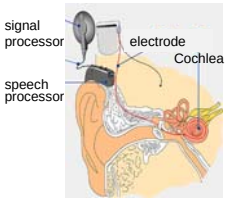
Defects in one protein of these networks may cause dysfunction of the entire networks leading to USH.

UW

Therapy strategies for the Usher Syndrome

Ear: USH is an developmental defect – prenatal diagnoses and molecular genetic treatment are necessary.

- Accessibility is very difficult.
- Cochlea implants are efficient.



Eye:

The is progression in retina implant development, but they are still at early development stages.

Retina provides very good accessibility for molecules/agents – neuroprotection and molecular genetic interventions.
~ Gene based therapies

Gene addition - replacement

Viral gene addition - replacement:

Lentivirus:

- single stranded RNA-Virus, member of *Retrovirus* family,
- maximum insert size: 7.5 kb,
- integration into genome, long term expression,
- low immune response, infect both dividing and non-dividing cells

Adeno-associated virus (AAV):

- DNA virus, belongs to *Parvovirus* family, maximum insert size: 7 kb;
- no/low rate integration (preferentially chromosome 19) into genome;
- long term expression, non pathogenic, no immune response;
- infect both dividing and non-dividing cells;
- depend on a helper virus to replicate (*Dependovirus*).

Non-viral gene addition – replacement by nanoparticle:

- unlimited size for delivery, very poor integration,
- no immunological problems; expression termination ?
- low efficiency in transfection of both dividing and non-dividing cells
- alternative "carriers": liposomes, DNA-protein conjugates …

Gene addition - replacement

Vector	Host cells/ efficiency	Gene expression	Integration into genome	Immune-response
Viral				
Lenti-virus	dividing and non-dividing cells/ high efficiency	long term/ years	yes	low
rAVV	dividing and non-dividing cells/ high efficiency	long term/ years	no/ ? under discussion	no
Non-viral				
DNA only	dividing and non-dividing cells very low efficiency	short term	no	no
PEG nano-particle	dividing and non-dividing cells/ high efficiency	up to 3 months analyzed	stays episomal	no

Gene addition via rAAVs in *retinitis pigmentosa*/LCA2 patients

The New England Journal *of* Medicine, 2008
Brief report
Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis
Albert M. Maguire ….. Jean Bennett, Philadelphia, U.S.A.

The New England Journal *of* Medicine, 2008
Brief report
Effect of Gene Therapy on Visual Function in Leber's Congenital Amaurosis
James W. B. Bainbridge ….. Robin R. Ali, London, England

Human Gene Therapy, 2008
Phase I Trial of Leber Congenital Amaurosis due to RPE65 Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results
William W. Hauswirth……. Samuel G. Jacobson, Florida and Pennsylvania, U. S. A.

Gene addition projects for USH genes

USH1B (myosinVIIa) - Consortium – Welp/Brown families
David Williams, San LA, U.S.A. - Lentivirus successful transfer into mouse RPE
Alberto Aurichio, Univ. of Naples, Italy - recombinant adeno-associated virus (modified rAVV5) …

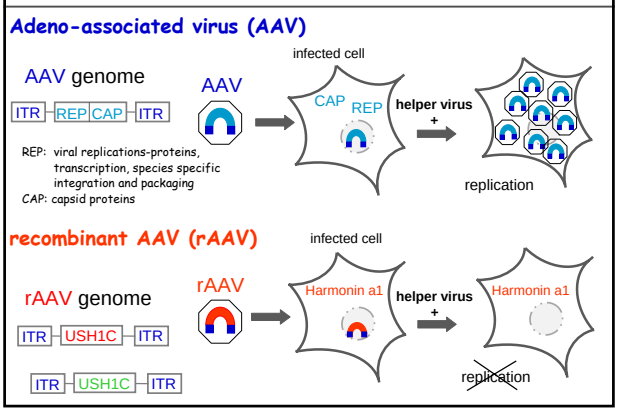
USH1C (harmonin) – Consortium planned Suchert family
Uwe Wolfrum, Mainz,* Germany; - recombinant adeno-associated virus (rAVV5); PEG nanoparticle

USH2A - Consortium ?
W. Kimberling, Omaha, Peter Francis, Portland, U.S.A.* …

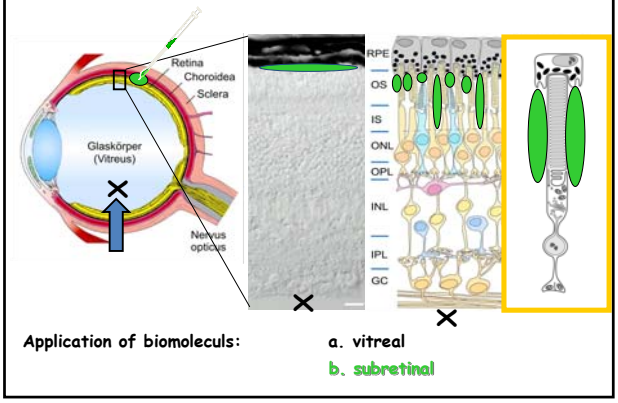
USH3A (clarin-1) - Consortium – Alexander family – "Hope for Vision"
John Flannery, Berkeley, U.S.A.*; Eeva-Marja Sankila, Helsinki, Finland;
Kris Palczewski, Cleveland; David A. Saperstein, Seattle, U.S.A.
/QLT Inc., Vancouver, Canada …
- recombinant adeno-associated virus (rAVV5)

*Cooperation with W.W. Hauswirth, Gainesville, U.S.A.

Recombinant adeno-associated virus (rAAV)



Application of biomolecules in the eye



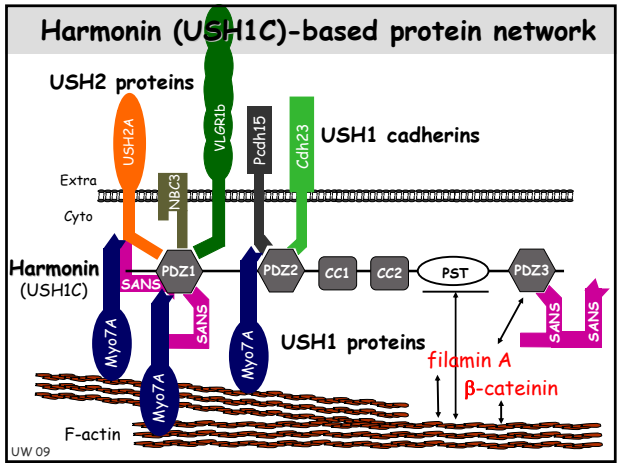
Application of biomoleculs: a. vitreal

b. subretinal

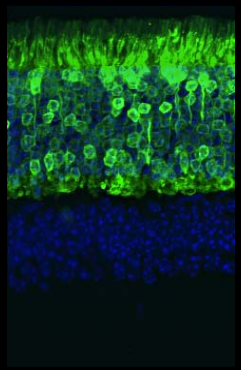
Strategies for treatment of USH1C in the retina – Wolfrum Team Mainz

- **gene addition** using recombinant adeno-associated virus (rAAV),
  Collaboration: W.W.Hauswirth, Gainesville, USA
- **gene addition** using polyethylen glycol (PEG) nanoparticles,
  Collaboration: M. Naash, Oklahoma City, USA
- **gene repair** by homologous recombination with zinc finger nucleases (ZFN) delivered by nanoparticles,
  Collaboration: (D. Carroll, Utah, USA)
- **translational readthrough** with modified aminoglycosides
  Collaboration: T. Bassov, Haifa, Israel
  T. Ben-Yosef, Haifa, Israel

Harmonin (USH1C)-based protein network



Gene addition via recombinant adeno-associated viruses (rAAV5)



- photoreceptor cell specific

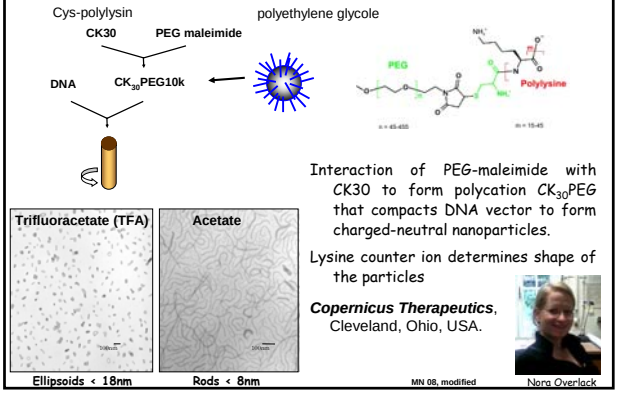
ITR USH1C ITR

rAAV5 Opsin:: GFP harmonin a1 delivered by subretinal injected into *Ush1c* -/- mouse retina

GFP/ DAPI-fluorescence image of mature mouse retina

Dr. K. Nagel-W

Non-viral gene addition by DNA PEG nanoparticles



Interaction of PEG-maleimide with CK30 to form polycation $CK_{30}PEG$ that compacts DNA vector to form charged-neutral nanoparticles.

Lysine counter ion determines shape of the particles

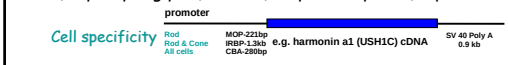
Copernicus Therapeutics, Cleveland, Ohio, USA.

MN 08, modified

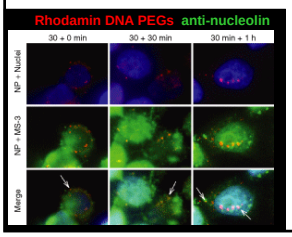
Nora Overlack

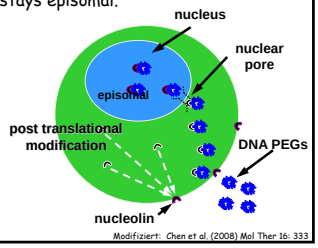
Mol. characteristics of DNA PEG nanoparticles

- PEG (Polyethylenglycol), CK30 (30 lysins - cystein) + plasmid DNA



- ~ 8 nm; neutral charge; no toxicity; no immunoreactions; no cellular degradation; high *in vivo* transfections efficiency.
- bind to cell surface exposed Nucleolin – internalization and transport into the nucleus (~ 15 min) & stays episomal.



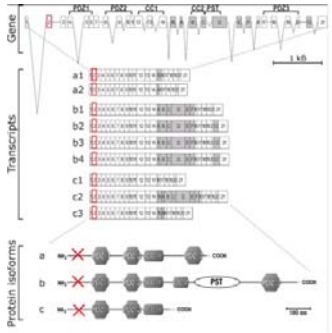


Modifiziert: Chen et al. (2008) Mol Ther 16: 333

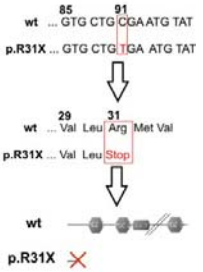
Alternative molecular genetic therapy strategies for USH ?

USH1C, Harmonin, p.R31X

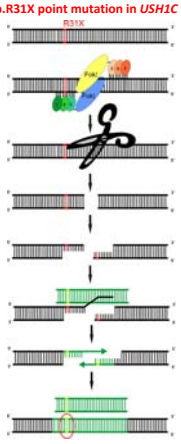
Harmonin slice variants



USH1C mutation p.R31X



p.R31X point mutation in *USH1C*



Gene repair by homologous recombination mediated by zink finger nucleases (ZFN)

ZFN as molecular scissors

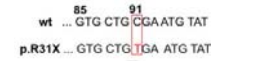
ZFN induced double-strand break

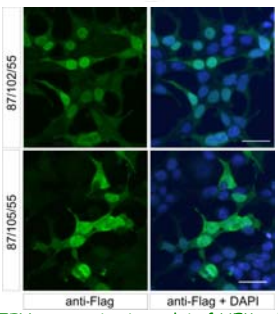
ZFNs induced double-strand breaks increase frequency of homologous recombination 10^3-10^4-fold (1 in 1000/100).

repaired *USH1C* gene

Nora Overlack

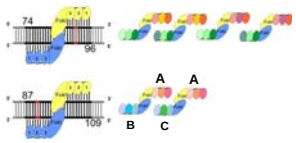
Characterization of ZFN for 91C-T/p.R31X mutation in *USH1C*



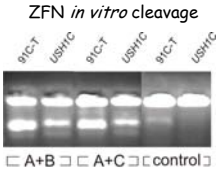


ZFN expression in nuclei of HEK cells.

Designed and cloned ZFN combinations

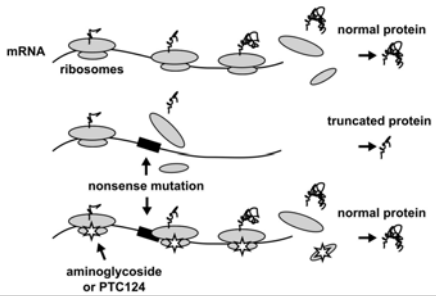


ZFN *in vitro* cleavage



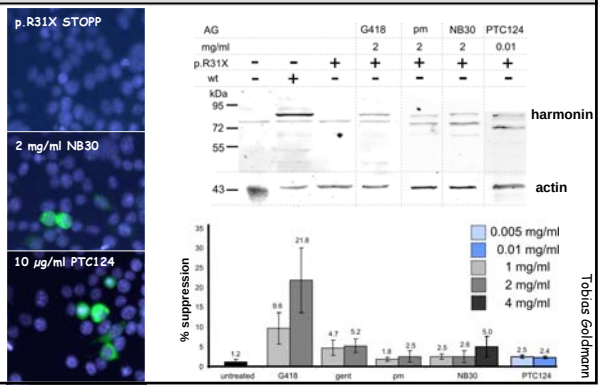
Aminoglycoside/PTC124 pharmacogenetic therapy

- **Aminoglycoside** are broad-spectrum antibiotics and inhibit prokaryotic protein synthesis.
- In eukaryotic cells, they interfere with protein synthesis and suppress of nonsense mutations (= **readthrough**).
- **PTC124** is a synthetic compound with readthrough activity.



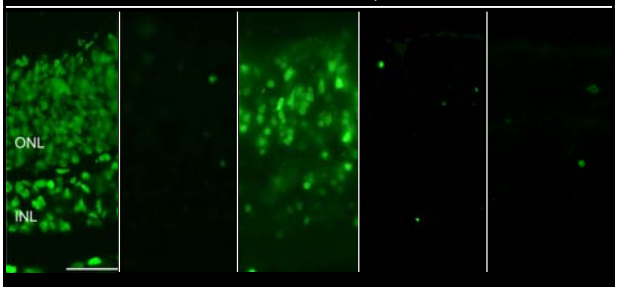
T. Goldmann

Aminoglycoside/PTC124 mediated readthrough leads to expression of full length harmonin



Toxicity of aminoglycosides in the retina

Toxicity of aminoglycoside/PTC124 in treated retina explants

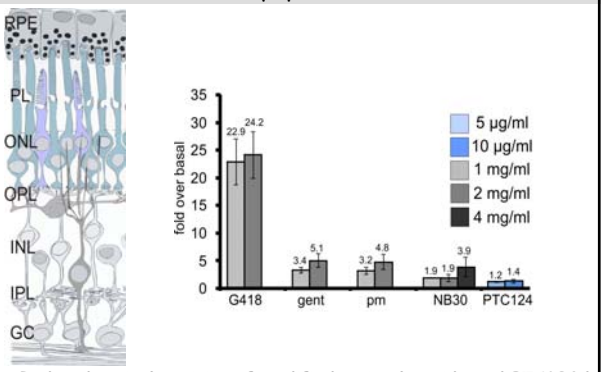


green: TUNEL stained apoptotic nuclei

Very low retinal toxicity of modified aminoglycoside and PTC124 !

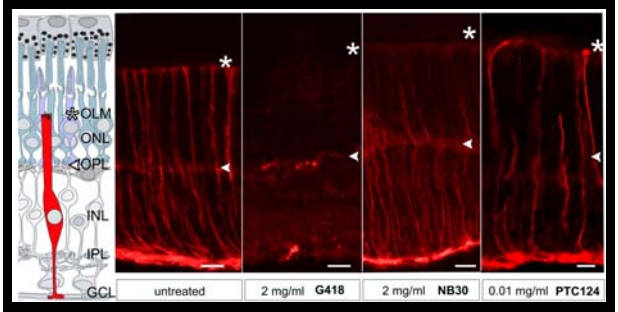
Goldmann *et al.*, in prep

Quantification of aminoglycoside/PTC124 induced apoptosis



Reduced retinal toxicity of modified aminoglycoside and PTC124 !

Toxicity in aminoglycoside treated retina explants - Müller glia cell integrity



GFAP: glial fibrially acidic protein

Reduced glial toxicity of modified aminoglycoside and PTC124 !

Research prospects, gene-based therapy strategies for USH1C in the retina

- rAAV2/5 harmonin a1 and b4 – in primates
- Subretinal application of rAAV2/5 harmonin in Ush1C knock-out and knock-in mice.
- Evaluation of nanoparticle mediated transfer of zinc finger nucleases and rescue plasmids.
- Evaluation of gene repair *in vitro*, in cells and in R31X transgenic mice.
- Validation of results obtained by modified aminoglycosides and PTC124 in R31X transgenic mice.
- Evaluation of delivery protocols for aminoglycosides and PTC124 – systemic – local administration.
- Success - Transfer outcome to other USH genes and other hereditary retinopathies.

Support: FAUN, Nuremberg; Foundation Fighting Blindness