Summary: Cell and Molecular Biology of the Usher Syndrome

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Human Usher syndrome (USH)

• USH is the most common form of combined hereditary deaf-blindness.
  ~ 1:10,000 to 1:6,000
  - Hearing impairment
  - Vestibular dysfunction
  - Vision loss – *Retinitis pigmentosa*
  - Olfactory impairment?

• USH is a complex disease:
  - 3 clinical types (USH1, USH2, USH3);
  - > 15 genetic subtypes.

• 10 USH causing genes are identified, so far.

• USH proteins belong to diverse protein families
  molecular motors, cell adhesion & transmembrane molecules, scaffold proteins …
Deciphering the molecular and cellular function of disease/USH molecules is essential for the development of an effective therapy.
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- USH proteins belong to diverse protein families: molecular motors, cell adhesion & transmembrane molecules, scaffold proteins …

All USH proteins are integrated into protein networks - the USH interactome.
Scanning electron microscopy of cochlear hair cells

- outer hair cells
- inner hair cells
Hair cells of the inner ear

Organ of Corti

Hair cell

Stereocilia

Synapses

OHC

IHC

TM

Reiners et al. HMG 2005
Auditory hair cell function

Depolarization of hair cell

Hair cell

Stereocilia

Synapses

Hearing

Reiners et al. HMG 2005
Usher protein expression in hair cells

Kinocilium

Stereocilia

**USH1 proteins**
- Myosin VIIa (USH1B)
- Harmonin b (USH1C)
- Cadherin-23 (USH1D)
- Protocadherin-15 (USH1F)
- Sans (USH1G)

**USH2 proteins**
- Usherin (USH2A)
- Vlgr1 (USH2C)
- Whirlin (USH2D)

**Labels and Colours**
- **F-actin**
- **Microtubules**
- **ELL**: Early lateral links
- **AL**: Ankle links
- **KL**: Kinociliary links
- **TC**: Top connectors
Usher protein expression in hair cells

**Kinocilium**
- Tip-link
- ELL

**Stereocilia**
- **USH1 proteins Clarin-1 (USH3)**
  - Myosin VIIa (USH1B)
  - Harmonin b (USH1C)
  - Cadherin-23 (USH1D)
  - Protocadherin-15 (USH1F)
  - Sans (USH1G)
- **USH2 proteins**
  - Usherin (USH2A)
  - Vlgr1 (USH2C)
  - Whirlin (USH2D)

- KL: Kinociliary links
- TC: Top connectors
- ELL: Early lateral links
- AL: Ankle links

0...1...2...3...4...5...21 days...Adult

El-Amraoui and Petit 2014
Auditory hair cell function

Differentiation:
  Defective organization
  hair bundles (sterocilia)

Wild type

Myo7a deficient

Disordered stereo cilia

Mature hair cells:
  Defective signal transduction

Depolarization of hair cell

Hearing
Hair cells of the inner ear

Organ of Corti

Hair cell

Stereocilia

OHC

IHC

TM

Clarin-1 (USH3A)

Aziz El-Amraoui

VLGR1 (USH2C)

Synapses

Dulon et al. J Clin Invest 2018

Reiners et al. HMG 2005
Impact of the Usher syndrome on touch

Touch deficits in USH2A patients.

- Higher finger vibration detection thresholds

Touch and vibration receptors in the skin

- USH2A protein is expressed in glabrous and hairy skin terminal Schwann cells, associated with Aβ-low threshold mechanoreceptors (Meissner´s corpuscles).
- Ush2a knock out mice show reduced vibration sensitivity.

USH2A/Usherin is involved in vibration and gentle touch in the skin.
Expression of USH molecules in olfactory epithelium/cells
e.g. Wolfrum et al. 1998

Controversy on olfaction deficiency in Usher syndrome patients:
Zrada et al. 1996 - Seeliger et al. 1999

Analyses of olfaction in USH mice

... reveal olfactory deficiency.

Jansen et al. 2016

Collaboration: H. Hatt lab/RU Bochum & U. Wolfrum lab JGU Mainz
USH proteins in the intestine

intestinal tube

enterocytes

Brush border microvilli


Matt Tyska

Mingjie Zhang
The distal tips of stereocilia and microvilli are linked by a conserved adhesion complex.

**Stereocilia tip-link/Uscher complex**

- **CDHR2**
- **CDHR5**
- **PDZ1**
- **PDZ2**, **cs**
- **PDZ3**
- **SANS**
- **SAM**
- **CEN**
- **MF1**
- **MF2**
- **SH3**
- **Motor**
- **IQs**
- **MYO7A**

**USH1C**

**Trans** heterophilic adhesion link to shorter stereocilia

**Plasma membrane**

**Intermicrovillar adhesion complex (IMAC)**

- **ANKS4B**
- **ATD**
- **PDZ1**
- **PDZ2**, **cs**
- **PDZ3**
- **USH1C**
- **CEN**
- **SAM**
- **Motor**
- **IQs**
- **MF1**
- **MF2**
- **SH3**
- **MYO7B**

**Plasma membrane**

**Trans** heterophilic adhesion link to adjacent microvillus
Intermicrovillar adhesion complex defects cause reduced microvillar packing & decreased growth rate.

Decreased growth due to nutritional deficiency.

Matthew J. Tyska
The vertebrate eye and retina

Photoreceptors: cones & rods
USH protein expression

VLGR1/GPR98 (USH2C)

USH1:  
- myosin VIIa
- harmonin
- Cdh23
- Pcdh15
- SANS

USH2:  
- USH2A (usherin)
- NBC3 ("USH2B")
- VLGR1b (USH2C)
- whirlin (USH2D)

Wolfrum (2011)
USH protein expression

Jun Yang

Yang et al. 2010
USH adhesion complex defines the docking membrane for transport vesicles

Papermaster 2000

Maerker et al. 2008

Yang et al. 2010
USH1 mouse retinas do not degenerate.
Calyceal processes are absent in mouse

RNAseq data and qPCR revealed very low expression of USH genes in murine retinas.

Sahly et al. 2012, JCB
Wolfrum 2010, ARVO
Absence of calyceal processes in a Pcdh15 (USH1F) frogs

Xenepus

Over growth of basal outer segment disks

Pcdh15

no calyceal processes:
bend outer segments

Aziz El-Amraoui

Schietroma et al. 2017
Porcine eyes/photoreceptors are similar to human ...

- More cones
- Macula

- Eye ball size:
  - Human: 25 mm
  - Pig: 24 mm
  - Mouse: 3.5 mm

- USH proteins in CP

- Calyceal processes:
  - Pig: Several
  - Human: Numerous
Design of a transgenic pig as a valuable model for USH

Designe and generation of a humanized USH1C mutation for evaluating treatment options

- Gene therapy
- Read-through treatments
- Gene repair approaches
- Cellular and tissue replacement

Nikolai Klymiuk
Eckhard Wolf
Uwe Wolfrum
Tell me who your friends are and I´ll tell you who you are.

Proteomics
**USH protein network - functions**

- **mechanical stabilization**
- **channel regulation**
- **membrane adhesion**
- **endocytosis**
- **ciliary transport**
- **ciliogenesis / deciliation**
- **gene regulation splicing**

**Abstracts from Wolfrum lab**

- Gregory et al. 2011
- Dulon et al. 2018
- Bauss et al. 2014
Thank you!
In memory of:

H. Steffen Suchert
1945 - 2015

"Ted", Elaine, and Thomas Welp
- 2015