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- Call 800-753-2160 (M-F, 8 AM-8 PM ET)
- Email customerservice@AudiologyOnline.com



21st Century Audiology: Hair Cell Regeneration, presented in partnership with Salus University

Brenda M. Ryals, PhD

Radhika Aravamudhan, PhD



21st Century Audiology Education:

Challenges and Opportunities

Radhika Aravamudhan, Ph.D

Dean, Osborne College of Audiology



Audiology as profession:

- Evolving from a Master's degree to Doctoral degree
- Allied Health to Independent Practitioner
- The goal for the profession to become a doctoring profession and become a patient point-of-entry for the hearing healthcare system.
- What were the necessary steps?



From a Curricular perspective:

- With this change, the new "doctoral" level education needed to be defined and implemented.
- What does a doctoral curriculum look like when the scope of practice has not changed, but the responsibility for patient outcomes will change?

What to learn for an unknown future?

- The challenge of “what to learn for an unknown future?” pedagogically is not a new one for higher education.
- But, when the end point is unknown, “what should be changed” in the curriculum is a challenge all academic programs face.

Challenges faced when bench marked with other doctoral professions

- The challenges faced in this model range from increased workload.
- Foundational sciences knowledge that cannot be directly applied within the existing clinical settings.
- Allied health model of Audiology does not implement such in-depth knowledge of biomedical sciences,
- Evaluation of relevance of this new content also proved to be difficult.



Opportunities

- Gives us the opportunity to include various areas of content that were not a part of the original program.
- Also gives us the ability to expand both the breadth and depth of the academic and clinical content.



FOCUS: Does Education truly precede Legislation?

- If education truly precedes legislative changes in a profession, we believe the transformation should start from the curricular content and levelling of academic programs.
- The outcomes of the programs should also be assessed using a rather intense mechanism that covers the projected and future role in public health and not just the current scope of practice.
- The challenges and opportunities we met while designing a curriculum that we believe will drive a change in the profession that is heading to an unknown future.



Some challenges other than curriculum and education:

- The flat applicant pool for the profession
- This reduces the total number of qualified audiologists in the field
- Unable to meet the demands of the population

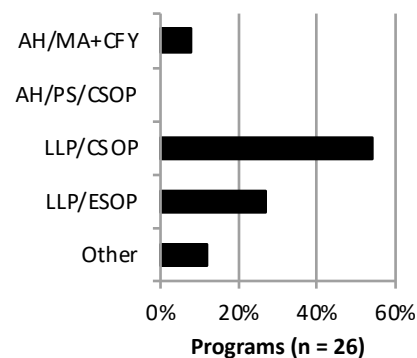


Ideal Au.D. Preparation

Code for Graph

- MA+CFY = Old Model
- AH = Allied Health
- PS = Physician Supervision
- CSOP = Current Scope of Practice
- LLP = Autonomy from Physician Supervision
- ESOP = Expanded Scope of Practice

Where Do We Want to Go?



Data from survey conducted by Aravamudhan, R., Bray, V. and Owen, J (2016-17)



Survey Findings

- Ideal for Au.D. Program Directors
 - 54% would prepare graduates for LLP status under current scope-of-practice
 - 27% would prepare graduates for LLP status under expanded scope-of-practice
 - 8% of programs would prefer Allied Health status and the prior Masters + CFY training model



What does this mean to us?

- In the following lecture you will hear Dr. Ryals talk about 21st Century advances and what do we have to look forward.
- Think about, are we preparing future and current audiologists for these developments?
- What is the role of clinical supervisors/preceptors in audiology education?
- How can programs prepare the students better?

continued

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Hair Cell Regeneration – 21st Century Audiology

Brenda M. Ryals, Ph.D.
October 16 , 2019

ARC

continued

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Why a talk about regeneration when it isn't
currently a human therapy?



Your patients may be reading these.....

continued



- Patient Counseling:
 - Parents want to know their options:
 - Are these pharmaceutical therapies appropriate for my child?
 - When?
- Professional Expertise
 - Who will determine candidacy?
 - Is this therapy appropriate for genetic deafness?
 - Is this therapy appropriate for infants/children?
 - Is this therapy appropriate for the elderly? Or those with long-standing hearing loss?



Goals

- What have we learned from birds?
- How does development fit in to therapy for hair cell regeneration?
- What are the current strategies for regenerating/restoring hair cells?

How do we translate current strategies to humans?

- Cell cycle –
 - Stem cells
- Cell fate –
 - Gene Therapy
- Clinical Trials
- Challenges for Audiology

Dogma prior to 1988 –loss is Permanent

- When hair cells die all that remains is a phalangeal scar
- Auditory nerve synapses are swollen and die
- These anatomical changes result in permanent sensorineural hearing loss

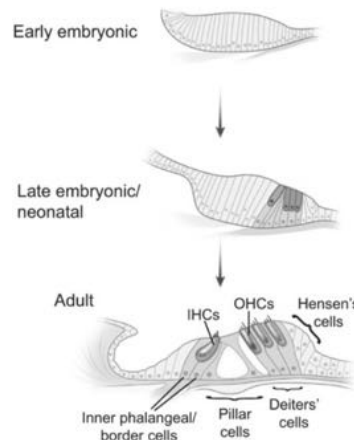
Why question this? Where do birds come in?

- If you really want to understand development.....

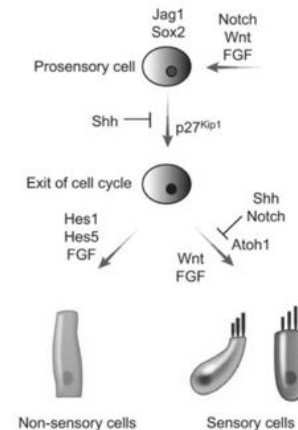


Avian inner ear develops similarly to mammalian inner ear

A Cochlear development



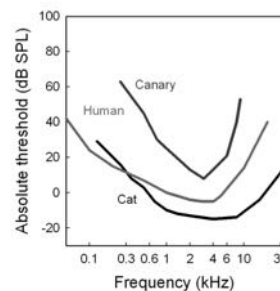
B Cellular differentiation



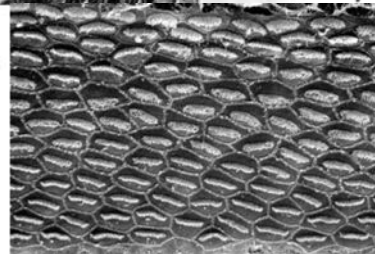
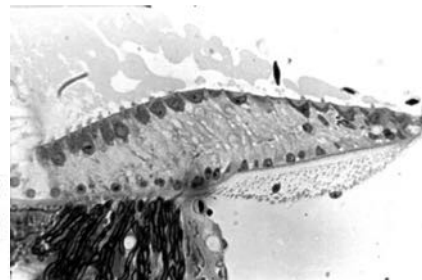
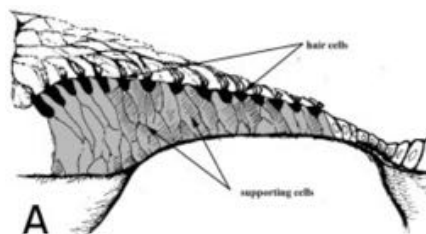
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Development; 142(9):1561-71
<https://www.biologists.com/development/article/10.1242/dev.114926>

Hearing and “speech” in birds – can we use it to inform regenerative effects in humans?

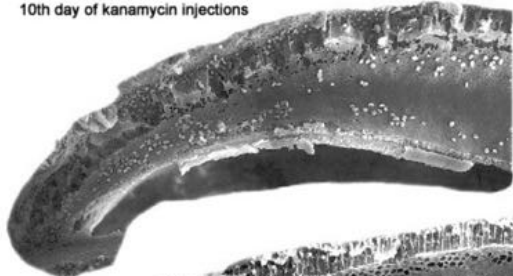
- Hearing thresholds are similar in the narrow bandwidth from 1,000-4,000
- Comparable to human best frequency hearing
- Avian vocalizations are important for breeding and socialization
- Comparable to humans!



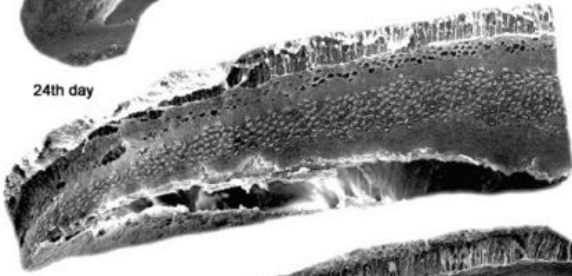
Similarities and Dissimilarities Avian vs Mammalian



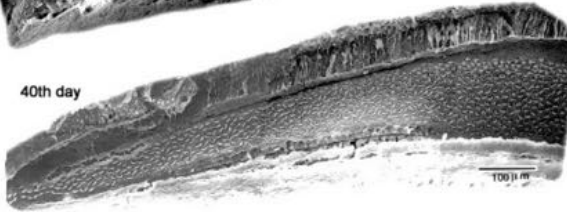
10th day of kanamycin injections



24th day



40th day



100 μ m


Do birds respond the same as mammals to acoustic trauma or ototoxicity?

NO!


continued

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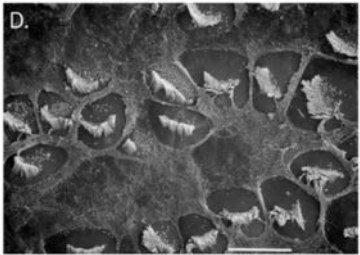
B.



C.



D.



Budgie hair cell counts

Hair cells can be replaced through mitosis/cell division

...what we learned from birds...

Precursor cells within the avian inner ear restart the developmental mechanism to form hair cells!

Ryals and Rubel, 1988; Corwin and Cotanche 1988

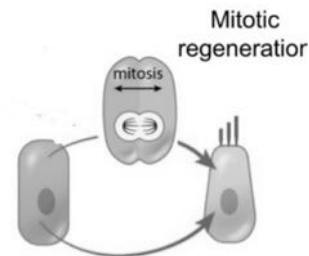
...what we learned from birds...

- Endogenous precursor cells (supporting cells) exist within the avian inner ear
- When damaged by noise or drugs these cells restart development! - they begin to divide

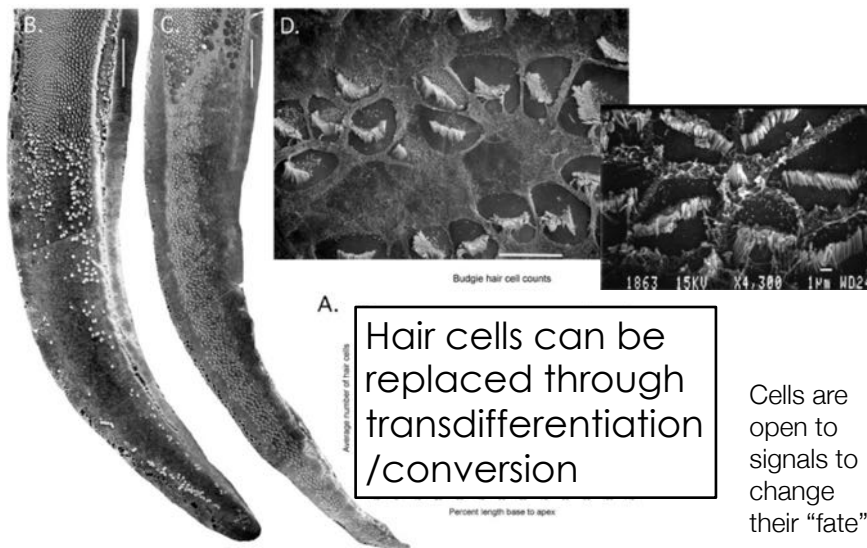


▪ Mitosis (cell division)

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Development; 142(9):1561-71
<https://www.biologists.com/development/10.1242/dev.114926>



What we learned from birds...

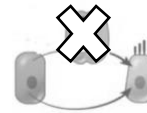


...what we learned from birds...

- Endogenous precursor cells (supporting cells) exist within the avian inner ear
- When damaged by noise or drugs these cells restart development -some of them transform to become hair cells



- **Transdifferentiation** (conversion)



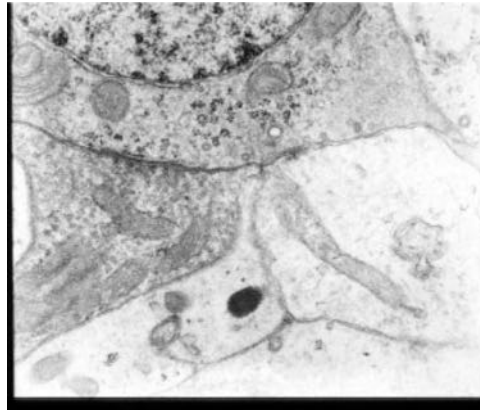
reproduced/adapted with permission; Atkinson et al (2015) *Development*; 142(9):1561-71 <https://www.biologists.com/development/10.1242/dev.114926>

So here's what we know about hair cell regeneration in birds:

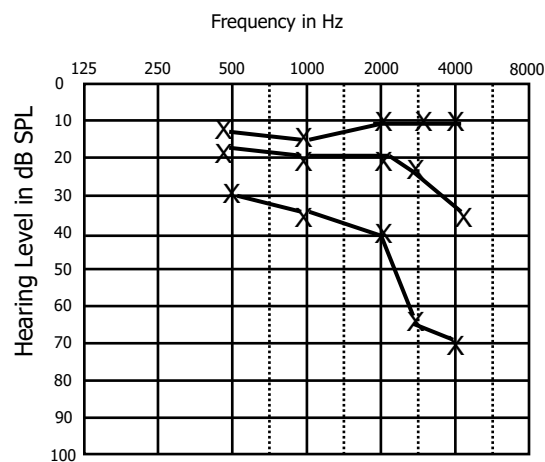
- Precursor cells (supporting cells) are triggered to either re-enter the cell cycle or dedifferentiate and convert to hair cells when mature hair cells are damaged or destroyed

So here's what we know about hair cell regeneration in birds:

Newly formed hair cells are re-innervated and are functional.



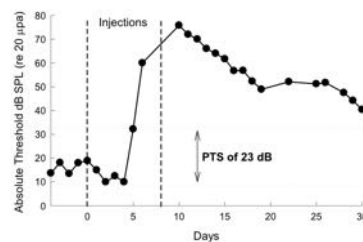
Hearing Sensitivity Recovers to near normal within 8 weeks



X = pre-injection

X = 2 weeks post injection

X = 8 weeks post injection



Dooling, Ryals and Manabe 1997

If precursor cells regenerate to form hair cells in birds - Are there precursor cells in the mammalian organ of Corti?

- All cells in the mammalian sensory epithelium CAN be induced to become hair cells. IF....
 - Immature – only up to post-natal 7-10 days
 - Cells are released from local signals – genetic inhibition
 - Presence of genetic signal for hair cell differentiation

Factors involved in regulating Cell Cycle

- Genetically controlled **tumor suppressor proteins** can inhibit or stimulate cell cycle and **Growth factors** generally stimulate re-entry into cell cycle.

Why don't these cells regenerate in mammals?

Tumor suppressing genes

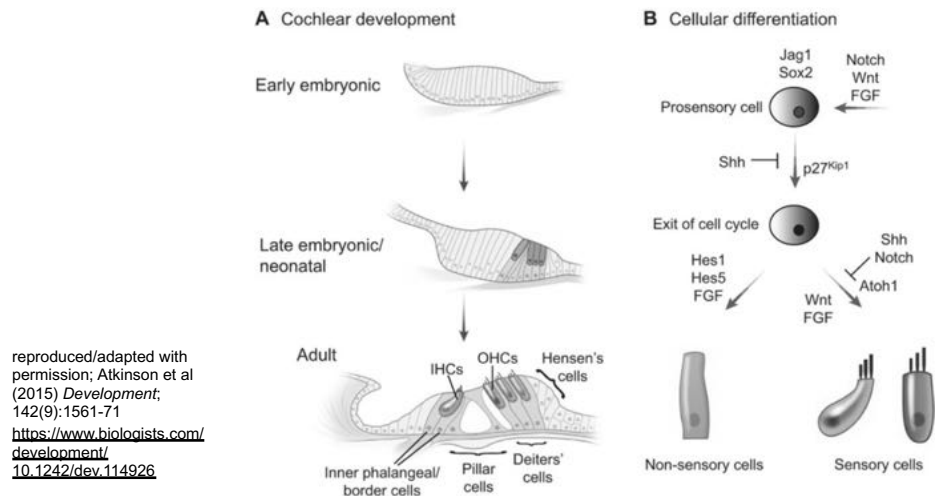
- ▶ The first tumor suppressing gene/protein associated with hair cell regeneration was p27 kip1
- ▶ Mice deficient in the gene that regulates this protein developed too many hair cells (Segil et al 1999)

What we learned from birds – translated to mammals

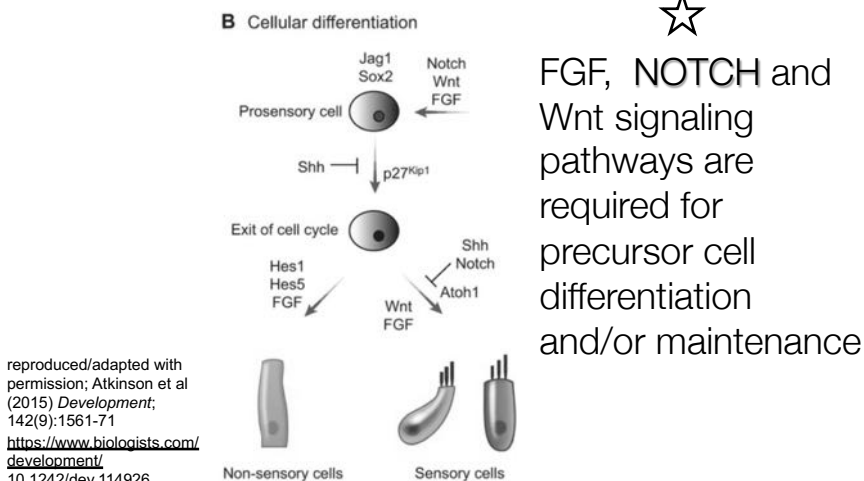
Endogenous precursor cells (supporting cells)

- ✓ BUT –Mitotic hair cell regeneration is prevented in mammals by:
 - Local factors = cell cell interactions
 - Tumor suppressor genes
 - p27kip1
 - pRb1
 - p19ink4d

Hair cell regeneration in birds essentially recapitulates development

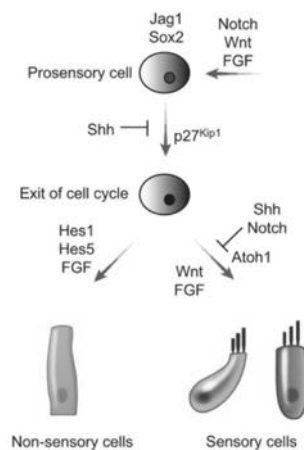


Hair cell regeneration in birds essentially recapitulates development



Hair cell regeneration in birds essentially recapitulates development

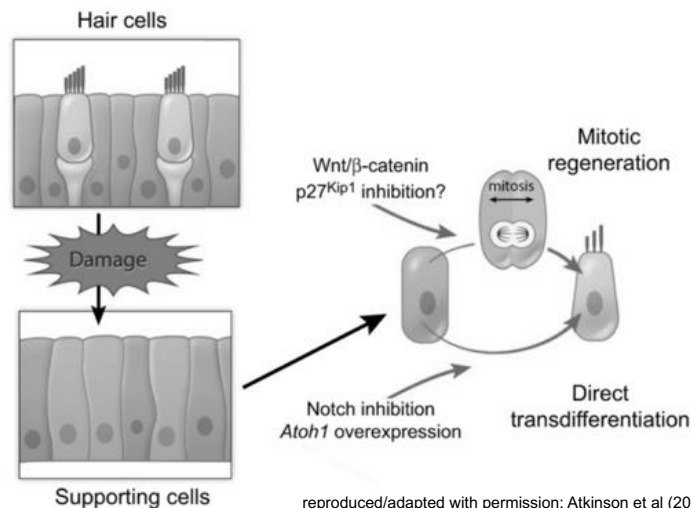
B Cellular differentiation



Atoh1 is both necessary and sufficient for precursor cells to specify differentiation as a HAIR CELL

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142(9):1561-71
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Proposed mechanisms of endogenous hair cell regeneration



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<https://www.biologists.com/development/10.1242/dev.114926>

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Hair cell regeneration: The next step – translating this to the mammalian ear

All of the cells in the mammalian cochlea are precursor cells under the right circumstances

Released inhibition – to restart cell division – mitosis

Introduction of Morphogenetic signals – to change cell fate - conversion

Strategies:

1. Factors regulating cell cycle (mitosis)
Stem Cell Therapy
2. Factors regulating cell fate
(transdifferentiation)
Gene Therapy
Molecular Therapy

B. Declan (2008) Nature
453:840-842

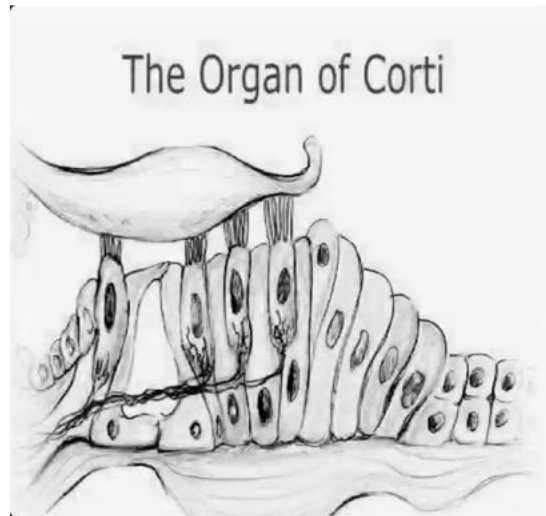
Strategies:

1. Stem Cell Therapy – introduce **exogenous** cells not inhibited from cell division
2. Gene Therapy – introduce morphogen or control transcription to stimulate an **endogenous** cell to change fate
3. Molecular Therapy - small molecule targeting of developmental pathways/genes to change cell fate

continued

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Animation – Stem cells and Gene therapy



continued

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Stem Cells

Definition: Stem cells are characterized by their capacity to self-renew and their ability to differentiate asymmetrically to form cell types other than their own.

continued



Pre-cursor cells – Stem Cells – translation?

Exogenous –

Hair cell-like cells (with stereocilia bundles and mechanosensory currents) can be derived from ES and from iPS cells

(Induced Pluripotent Stem Cells – 2012 Nobel Prize in Physiology or Medicine to Gurdon and Yamanaka)



The Promise of iPS cells

- Available and no problem with immuno-reaction
- Abundant
- Can be made from humans with the disease/disorder
- Investigators have been successful in using iPS cells to form auditory neurons (Nishimura et al 2009) and hair cells (Oshima et al 2010)



Stem Cell Therapy in the Cochlea

External application of human ES-cell-derived otic progenitors provides restoration of ABR in auditory neuropathy model.

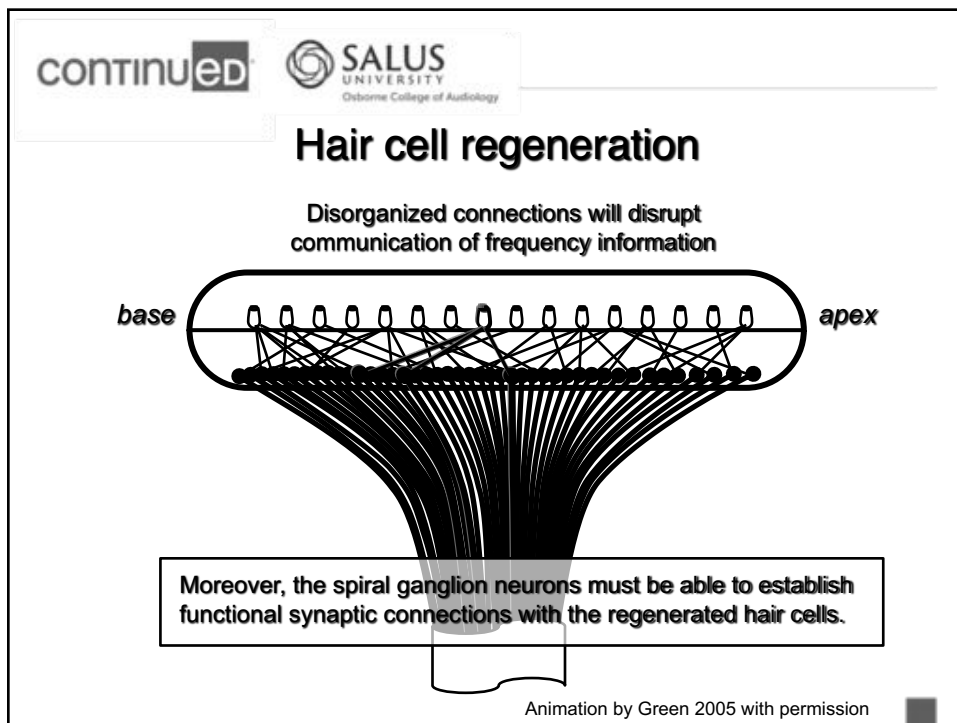
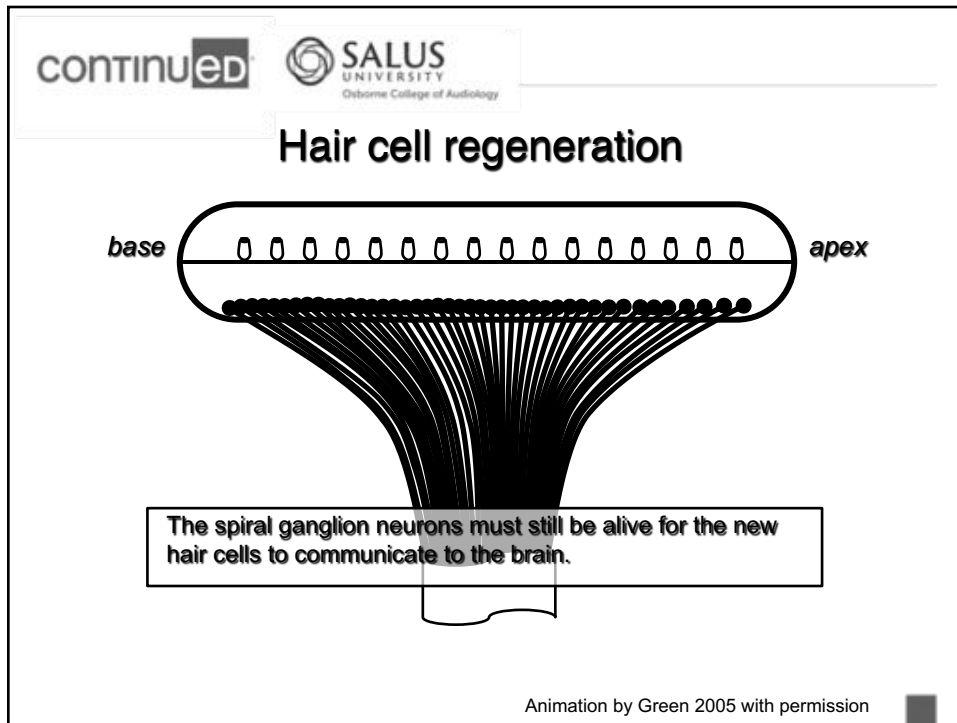
Chen et al 2012 Nature 490:278-282;

Chen et al 2017 Act-Otol didn't replicate in a SNHL model

As a general rule, stem cells transplanted into the cochlea tend to go to the modiolus
– appear driven to a neural fate



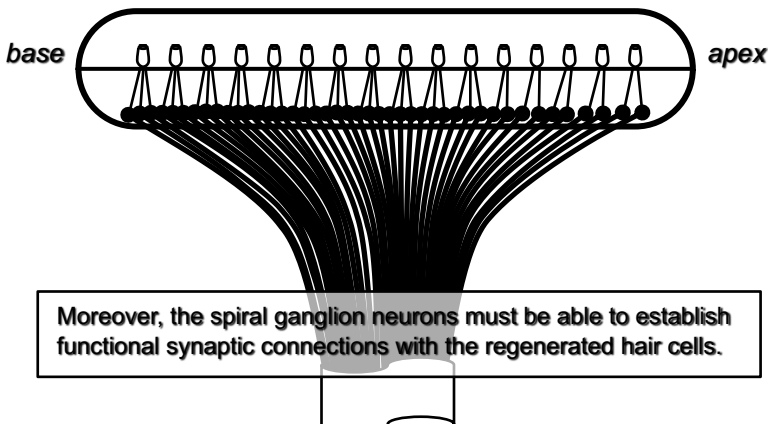
Using Cochlear Implants as a vehicle to induce neural growth/grow new neurons



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Hair cell regeneration

Functional connections restore hearing



base apex

Moreover, the spiral ganglion neurons must be able to establish functional synaptic connections with the regenerated hair cells.

Animation by Green 2005 with permission

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Challenges for the use of stem cells for hair cell replacement

- Availability*
- Delivery**
- Integration into site of lesion – endolymph is toxic to HC
- Driven to neural fate – likely more successful for neural regeneration

*iPS cells likely make this much less problematic.
 **McCall et al Drug Delivery for Treatment of Inner Ear Disease: Current State of Knowledge, Ear and Hearing. 31(2):156-165, April 2010.

Gene Therapy

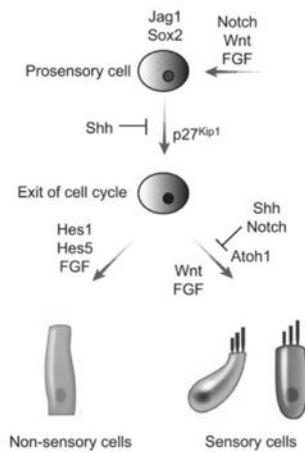
- Gene therapy is an experimental treatment that involves introducing genetic material into a person's cells to fight disease
- A gene can be delivered to a cell using a carrier known as a "vector." The most common types of vectors used in gene therapy are viruses.

How Does Gene Therapy Work?

- <http://ghr.nlm.nih.gov/handbook/therapy/procedures>

A new gene is injected into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein.

What kind of genetic signaling would help to restore hair cells to a damaged cochlea?



- *Morphogens and Transcription factors – hair cells*
 - *Inhibit Notch/Delta and Shh signaling*
 - *Enhance Wnt, FGF and Atoh1*
- *Morphogens and Transcription factors – support cells*
 - *Enhance Hes1, Hes5 and FGF*

Breuskin et al 2008 Hearing Research –
Review paper Genes and Regeneration

reproduced/adapted with permission; Atkinson et al (2015)
Development; 142(9):1561-71
<https://www.biologists.com/development/10.1242/dev.114926>

Gene Therapy in the Cochlea

- ☆ Yehoash Raphael and colleagues (2003) were the first to report success with gene therapy for regenerating hair cells. They injected Atoh1 into damaged guinea pig cochleae and saw new hair cells develop in damaged regions. In their second experiment they confirmed functionality of hair cells with ABR (2005)

Kawamoto et al., J. Neurosci 23:4395, 2003
Izumikawa et al Nat.Med. 2005



Repeat ATOH1 Gene Therapy in different lab
(Atkinson, et al PLOS, 2014)

Results

1. ATOH1 treated resulted in increase in HC post injury
2. ABR didn't improve



Challenges for Gene Therapy in the Inner Ear

- Depletion of important endogenous cell types (supporting cells, pillar cells, etc.)
- Electrical environment of hair cells/stria vascularis
- Immune response
- Problems with viral vectors
- Innervation
- Timing



Clinical trial in humans delivering ATOH1

- ▶ Novartis trial is currently (2014) recruiting participants (<https://clinicaltrials.gov>) The trial involves injecting a viral vector loaded with ATOH1 into the inner ear via a hole surgically drilled in the footplate of the stapes. The vector carries a promoter gene that confines ATOH1 expression to support cells of the cochlea.
- ▶ Enrollment began 2014 completion 2021. Target enrollment 45. Study sites: University of Kansas Medical Center, Johns Hopkins School of Medicine, Columbia Med. School NY. Target population patients 18-75 years old with severe to profound hearing loss
- ▶ Primary outcome: serious adverse events and change in audiometry.
- ▶ Secondary outcome: ABR, vestibular tests and speech audiometry



Molecular Therapy – Small molecule Progenitor Cell Activation

Mitzutari et al (Neuron 2013) showed that they could use genes important for cell fate (Notch, Sox, etc.) to **inhibit a cell fate** genetic signal after damage and **turn on the transcription factor** that stimulated *Atoh 1* – this resulted in endogenous cells converting into hair cells in the damaged cochlea. And threshold shift improvement via ABR

From Fuioka, Okano and Edge (2015)

Video abstract:
Neuron volume 77
Issue 1: 58-69



Based on this work,
Audion Therapeutics is developing a drug
(LY3056480) to inhibit Notch signaling. ★

For more info see EU Horizon 2020 funded
project Regain*:

<https://www.regainyourhearing.eu/>

*patient recruitment completed 2019



Challenge for this approach: Notch
signaling declines in first post-natal
week in mice and only persists in the
apex

If Notch isn't available how does
blocking it work in adults?

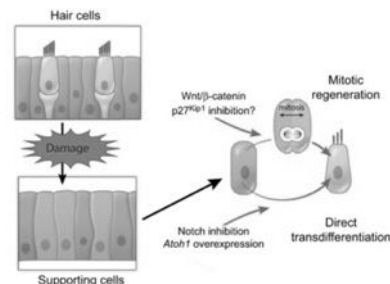
Maass et al 2015
Frontiers in Cellular
Neuroscience

Molecular Therapy - Small Molecule Progenitor Cell Activation – if Notch isn't available can we activate an existing precursor?

Lgr5+ supporting cells in the cochlea give rise to hair cells in normal development (McLean et al 2016; 2017)

Molecular Therapy - Small Molecule Progenitor Cell Activation

Instead of forcing conversion of cells that don't have ATO1 available, Lgr5 positive supporting cells through a Wnt/Notch pathway to proliferate (McLean et al 2016; 2017)



This could solve the challenge of cell depletion

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Development; 142(9):1561-71
<https://www.biologists.com/development/10.1242/dev.114926>



Clinical trial in humans based on activation of Lgr5 cells

- Frequency Therapeutics is testing safety of their drug – FX-322
- Double-blind, Placebo-controlled, Single-dose Study of FX-322 Administered by Intratympanic Injection in Adults
- July 2018 - Active but not currently recruiting (Phase 1-2)
- Targeted Enrollment – 24 patients with stable sensorineural hearing loss associated with noise exposure or sudden sensorineural hearing loss



Summary Clinical Trials – hair cell regeneration

(10/16/19)

ID	Title	Status	Intervention	Sponsor	Age	Phase	Enrollment
2016-004544-10 (REGAIN)	Notch inhibitor LY3056480	Active	Drug: LY3056480	Audion Therapeutics BV	18-80	1/2	62(target)
NCT02132130	Viral vector Atoh1	Active/recruiting	Drug: CGF166	Novartis	18-75	1/2	45 (est)
NCT03616223	Small molecule	Active/not recruiting	Drug: FX-322	Frequency Therapeutics	18-65	1/2	24 (est)

Phase 1 – safety Phase 2 – efficacy Phase 3 – compare with current treatment Phase 4 – larger group, how well it works, what are the possible side effects, risk benefit



Summary Clinical Trial (10/16/19)

- The gamma secretase inhibitor LY3056480 is a Notch inhibitor. The REGAIN consortium reported positive results from Phase I in 15 patients on 5 Feb 2019.
- CGF166 is a recombinant adenovirus 5 (Ad5) vector containing the human Atonal transcription factor (Hath1) cDNA for administration via intra-labyrinthine infusion. Part A and Part B enrollment is complete. Part C enrollment is open.
- FX-322 is a undisclosed combination of small molecule drugs, and will be given by intratympanic administration of a slow-release gel. None of the first 9 patients injected suffered significant side effects.



General Information about Clinical Trials Success Rate

- About 60% of clinical trials advance from Phase I to Phase II
- But, only about 10% of Phase I trials advance to approval

Phase I – safety Phase II – efficacy Phase III – compare with current treatment Phase IV (NDA/BLA approval) – larger group, how well it works, what are the possible side effects, risk benefit



Factors Affecting Trial Success (BIO Study)

Utilization of selection biomarkers increases success (up from 10% to about 25%)

- ▶ Identify promising drug candidates early
- ▶ Stratify patients and develop diagnostics




So translation from animal model –chick – to animal model mammal is progressing rapidly.
We are working to overcome challenges presented to both exogenous approaches (stem cells) and endogenous approaches (gene therapy)

We know that, at least for birds where regeneration occurs naturally and within days/weeks of hair cell loss, auditory perception recovers or at least remains plastic and vocal production is only temporarily affected.


What is the role of the Audiologist??

What is the Audiologist's Role?

- Appropriate Counseling: 
 - When will hair cell regeneration be a reality for my patients?
 - Will hearing aids or cochlear implants continue to be necessary in the face of hair cell regeneration?

Stem cells to cure deafness – You Tube

Specifics: The current state of diagnostic Audiology

- Determination of Candidacy: 
 - Where is the site of lesion?? What is the underlying pathology??
 - Do we need Audiology or will a gene chip do?
 - Need a test to differentiate IHC from VIII nerve (synapse?)
 - Need a test for endocochlear function

What is the Audiologist's Role?



- Quantifying therapeutic success:
 - Determine appropriate measure (OAE? ABR? Pure tone audiogram? OTHER?)
 - Define and quantify patient determined parameters of success

We've come a long way in 30 years
Where will we be in the next 30?

“The mind once expanded to
the dimensions of larger ideas,
never returns to its original
size” Oliver W. Holmes