Unravelling the roles of lysine acetylation by Elp3 during inner ear development

*Susana Mateo Sánchez, *Laurence Delacroix, Sophie Laguesse, Sandra Huysseune, Alain Chariot, Laurent Nguyen and Brigitte Malgrange
GIGA-Research, Developmental Neurobiology Unit, Université de Liège

* = equally contributed to the work.

Introduction

The inner ear is composed of a vestibular part that controls balance, and the cochlea, which is dedicated to hearing. In both parts of the inner ear, sensory epithelia comprise supporting cells surrounding the sensory hair cells. These cells bear at their apical surface a staircase-structured hair bundle, consisting of multiple rows of actin-based stereocilia and a single tubulin-based kinocilium. This hair bundle allows the transduction from mechanical stimuli, initiated by sound or gravitational changes, to electrical signals that will then be transmitted by neurons from the spiral ganglion (innervating hair cells of the cochlea) or the vestibular ganglion. The inner ear organogenesis requires a tightly regulated transcriptional program that can be affected by post-transcriptional and post-translational modifications among which lysine acetylation. Given the importance of acetylation homeostasis in controlling developmental processes, we planned to investigate its role in inner ear formation and focused our attention on Elp3 acetyl-transferase, a member of the Elongator complex recently implicated in neurogenesis.

Results

1 Elp3 expression and Elp3 conditional KO (Elp3 cKO) generation

Figure 1

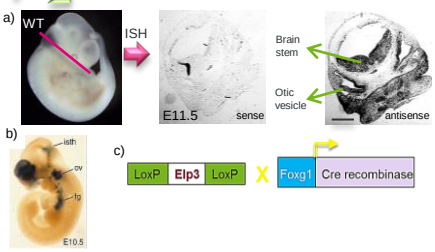

Figure 1: **a)** *In situ* hybridisation (ISH) with both antisense and sense Elp3 probes performed on transversal cross sections at embryonic stage E11.5 on wild type (WT) mice. Scale bar = 800 µm. **b)** Cre recombination in Foxg1-Cre embryo at E10.5 by LacZ staining (*Hébert et McConnell, 2000*); isth = isthmus, ov = otic vesicle, fg = foregut. **c)** Genetic strategy followed to generate Elp3 cKO mice: Cre/lox recombination allows depletion of Elp3 in early otocyst at embryonic stage E8.5.

Elp3 is expressed in the entire otic vesicle and Elp3 is depleted in the early otocyst in Elp3 cKO from E8.5.

2 Characterization of Elp3 cKO mice

Figure 2 ➔ **Evaluation of balance function in Elp3 cKO mice**

To analyse balance in mice lacking Elp3 in inner ear, we performed different tests that were shown to be associated with vestibular defects, such as:

- Stereotyped circling ambulation in both directions
- Head bobbing (intermittent extreme backward extension of the neck)
- Retropulsion (backward displacement)
- Absence of reaching response in tail-hanging test ("crawling" up toward their tails)

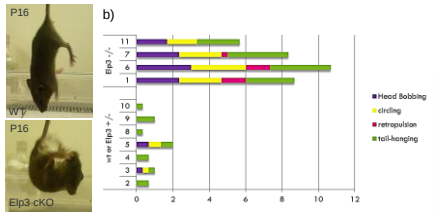

Figure 2: a) Tail-hanging test with wild-type (WT) and Elp3 cKO young adults, 16 days after birth (P16): whereas WT mice tend to occipital landing, Elp3 cKO mice shows an absence of the reaching response and crawls up. **b)** Behavioural tests to evaluate vestibular defects of Elp3 cKO: behaviour of wild-type and heterozygous mice (2, 3, 4, 5, 8, 9 and 10) and Elp3 cKO (1, 6, 7 and 11) were observed and ranked from 0 to 4 for head-bobbing, circling, retropulsion and tail-hanging.

Figure 3 ➔ **Evaluation of hearing in Elp3 cKO mice**

Because Elp3 cKO mice seemed to be insensitive to sound stimuli, we performed ABR to evaluate their audition.

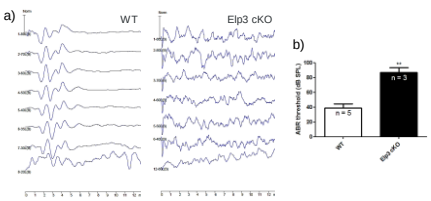

Figure 3: a) Auditory Brainstem Response (ABR) analysis was performed on wild-type (WT) and Elp3 cKO mice at different sound intensities (from 80 dB to 20 dB). Five peaks between 1-5 milliseconds after the stimulus were observed in WT mice, each peak corresponding to a specific relay of the auditory neural pathway. On the contrary, this hallmark signal was not observed in Elp3 cKO mice, suggesting deafness in these mice. **b)** ABR threshold mean (dB SPL) from 5 WT and 3 KO. SPL = Sound Pressure Level

Elp3 is implicated in both balance and hearing.

At the morphological and cellular level, we did not find any structural abnormalities nor cell patterning defects that could explain deafness or balance dysfunction in Elp3 cKO mice. However…

3 Elp3, hair bundle and ciliogenesis

Figure 4 ➔ **Evaluation of hair bundle integrity**

Hair bundles and their orientation are essential for the mechanotransduction of the signal. Defects concerning the hair bundle of cochlear hair cells have been observed:

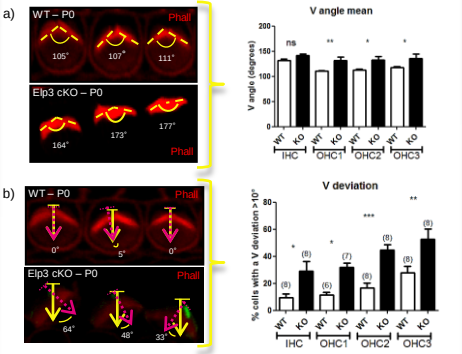

Figure 4: Confocal images (left panel) of wild-type (WT) and Elp3 cKO cochlear hair cells at birth (stage P0) stained with phalloidin (phall, in red) in order to label hair bundles. In the WT, hair bundles are disposed in a V-shaped structure (yellow dotted lines). **a)** Angles determined by these V-shaped structures have been measured for fifteen hair cells per row and the mean angle has been calculated (n=8, right panel). **b)** In the KO, some hair bundles are misoriented regarding the mediolateral axis of the cochlea. Angles formed between this mediolateral axis (yellow arrow) and the symmetrical axis of the V-shaped structure (pink arrow) were measured. Percentages of cells with a V deviation superior to 10° (corresponding to an abnormal orientation) have been calculated (number in brackets = number of animals). IHC = inner hair cells row; OHC1, 2, 3 = outer hair cells row 1, 2, 3.

Figure 5 ➔ **Evaluation of ciliogenesis**

Kinocilium is also essential for the mechanotransduction of the signal and crucial for hearing. Defects concerning the length of the kinocilium of hair cells have been observed:

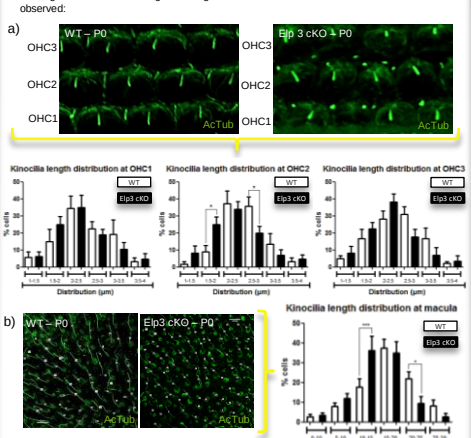

Figure 5: a) Confocal images (upper panel) of wild-type (WT) and Elp3 cKO cochlear hair cells at birth (stage P0) stained with acetylated alpha-tubulin antibody (AcTub, in green) in order to label kinocilia. Percentages of cells with a certain kinocilium length (in µm) per row (bottom panel, n=7-9). **b)** Confocal images (left panel) of WT and Elp3 cKO vestibular hair cells at birth (P0) stained with acetylated alpha-tubulin antibody (AcTub, in green) in order to label kinocilia. Percentages of cells with a certain kinocilium length (in µm) in the macula of the vestibule (right panel, n=7-9). In general, there are more cells with a shorter kinocilium and less cells with a longer kinocilium in the Elp3 cKO compared to wild-type littermates. IHC = inner hair cells row; OHC1, 2, 3 = outer hair cells row 1, 2, 3.

Elp3 is implicated in shape and position of hair bundle of the cochlear hair cells and in ciliogenesis, particularly in the vestibule.

4 Elp3 and neuronal survival

Figure 6 ➔ **Evaluation of apoptosis in the cochlea**

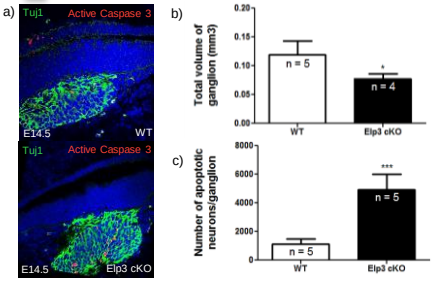

Figure 6: a) Confocal images (left panel) of wild-type (WT) and Elp3 cKO cochleae cross-sections at embryonic stage 14.5 (E14.5) stained with anti-active caspase 3 (marker of apoptosis, in red) and tuj1 (neuron-specific class III beta-tubulin, in green) antibodies. Nuclei were stained with DAPI (in blue). **b)** Total volume (mm³) of wild-type (WT) and Elp3 cKO spiral ganglia. Volume of spiral ganglion in Elp3 cKO is reduced compared to WT. **c)** Number of apoptotic neurons per ganglion in WT and Elp3 cKO mice. Apoptotic level is increased in Elp3 cKO spiral ganglion neurons compared to wild-type littermates.

Loss of Elp3 increases neuronal apoptosis in the spiral ganglion

5 Elp3 and cochlear hair cell innervation

Figure 7 ➔ **Evaluation of fibers from type II neurons**

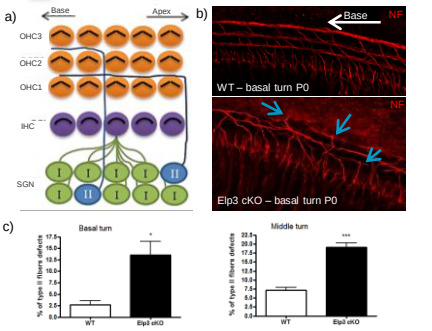

Figure 7: a) Representative scheme of cochlear hair cell innervation by the neurons from the spiral ganglion. Many fibers from type I neurons contact one inner hair cell whereas fibers from type II neurons turn all toward the base of the cochlea thereby contacting many outer hair cells. **b)** Confocal images of whole wild-type (WT) and Elp3 cKO cochleae (basal turn) at birth (stage P0) stained with neurofilament (NF, marker of fibers from neurons, in red). Misoriented fibers and innervation defects (blue arrows) have been observed in Elp3 cKO condition compared to wild-type littermates. **c)** Quantification of these defects regarding type II fibers in the basal and the middle turn of the cochlea (n=3). SGN = spiral ganglion neurons, I = type I neurons, II = type II neurons, IHC = inner hair cells row, OHC1, 2, 3 = outer hair cells row 1, 2, 3.

Figure 8 ➔ **Evaluation of synaptic ribbons**

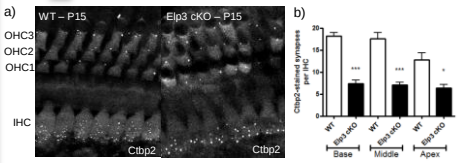

Figure 8: a) Confocal images of wild-type (WT) and Elp3 cKO cochleae at post-natal stage 15 (P15) stained with Ctbp2 (marker of synaptic ribons, in white). **b)** Counting of Ctbp2 dots per IHC from WT and Elp3 cKO cochleae at the base, middle and apex (n=3). Regarding IHC, the number of Ctbp2 dots is decreased in the KO compared to wild-type littermates. IHC = inner hair cells row, OHC1, 2, 3 = outer hair cells row 1, 2, 3.

Loss of Elp3 induces hair cell innervation defects in the cochlea.

Conclusion & Perspectives

In conclusion, we have demonstrated the expression of Elp3 in the inner ear and pointed out a role for this acetyl-transferase in both audition and balance function. Our results clearly show the implication of Elp3 in ciliogenesis, hair cell innervation and neuronal survival and we plan to go deeper in the mechanisms involved through the identification of the proteins acetylated by Elp3. In order to identify Elp3-regulated genes, RNA-Seq experiments have been performed with wild-type and Elp3 cKO cochleae at stages E14.5 and E18.5. Interesting candidates have already been identified: transcription level of several kif (kinesin member family) genes was decreased in Elp3 cKO, which could explain ciliogenesis defects, as well as Ntrk1 (Neurotrophic tyrosine kinase receptor, type 1) gene expression, which could explain increased neuronal apoptosis in the Elp3 cKO spiral ganglion compared to wild-type littermates.

Contact :
Susana.MateoSanchez@student.ulg.ac.be
ldelacroix@ulg.ac.be

We thank Philippe Compère and Professor Marc Thiry and his team for their contribution; and Patricia Ernst, Pierre-Bernard Van Lerberghe, Alice Marquet and Alexandra Pieltain for excellent technical assistance. We also thank the GIGA-Genotranscriptomics and the GIGA-Imaging and Flow Cytometry Platforms, specially Wouter Coppieters, Benoît Hennuy and Estefania Tarifeño.