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

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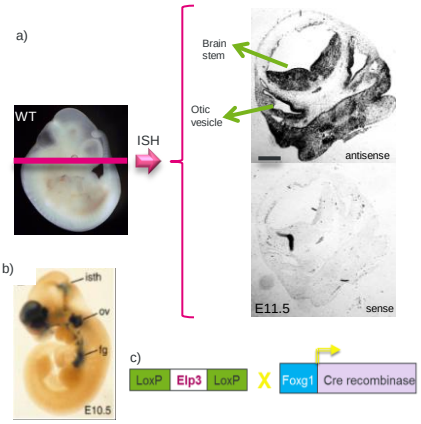
Introduction

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



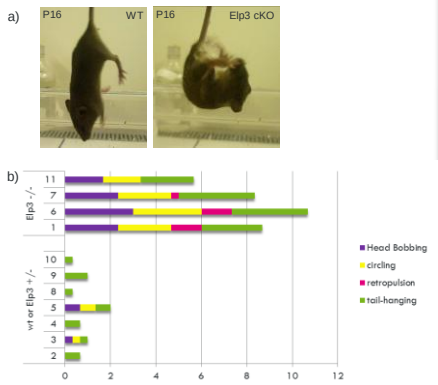
a) In situ hybridisation (ISH) with both antisense and sense Elp3 probes performed on transversal cross sections at embryonic stage E11.5 on wild type (WT) mice. b) Cre recombination in FoxG1-Cre embryo at E10.5 by LacZ staining (*Hébert et McConnell, 2000*); isth = isthmus, ov = otic vesicle, fg = foregut. 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

Elp3 cKO mice show:

- 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)



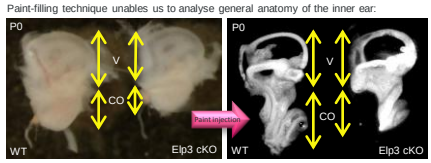
a) Tail-hanging test with wild-type and Elp3 cKO young adults, 16 days after birth (P16): whereas wild-type mice tends to occipital landing, Elp3 cKO mice shows an absence of the reaching response and crawls up. b) Behavioural tests to evaluate vestibular defects of Elp3 cKO: behaviour of wild-type and 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.

Elp3 cKO mice seem to be insensitive to sound stimuli; unfortunately, premature death of Elp3 cKO mice before the onset of hearing unables us to perform accurate evaluation of audition.

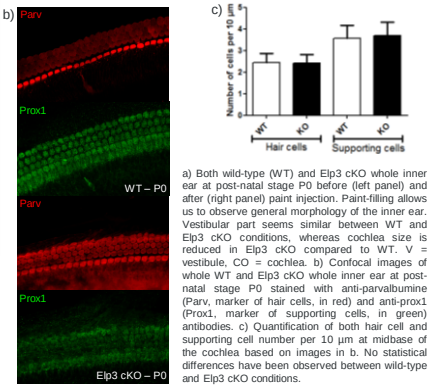
Elp3 is implicated in balance.

3 Inner ear morphology and cell specification in Elp3 cKO mice

Paint-filling technique unables us to analyse general anatomy of the inner ear:



Immunostaining with parvalbumine and Prox1 was performed to identify hair cells and supporting cells, respectively:

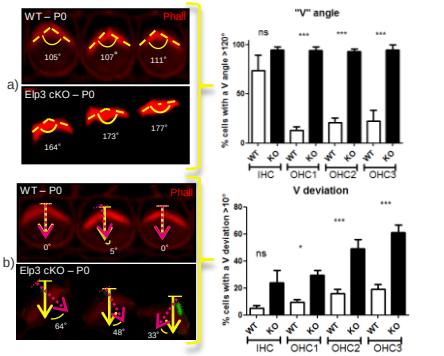


a) Both wild-type (WT) and Elp3 cKO whole inner ear at post-natal stage P0 before (left panel) and after (right panel) paint injection. Paint-filling allows us to observe general morphology of the inner ear. Vestibular part seems similar between WT and Elp3 cKO conditions, whereas cochlea size is reduced in Elp3 cKO compared to WT. V = vestibule, CO = cochlea. b) Confocal images of whole WT and Elp3 cKO whole inner ear at post-natal stage P0 stained with anti-parvalbumine (Parv, marker of hair cells, in red) and anti-prox1 (Prox1, marker of supporting cells, in green) antibodies. c) Quantification of both hair cell and supporting cell number per 10 µm at midbase of the cochlea based on images in b. No statistical differences have been observed between wild-type and Elp3 cKO conditions.

Loss of Elp3 induces cochlea size reduction but does not impair cell specification in the cochlea.

4 Elp3 and ciliogenesis

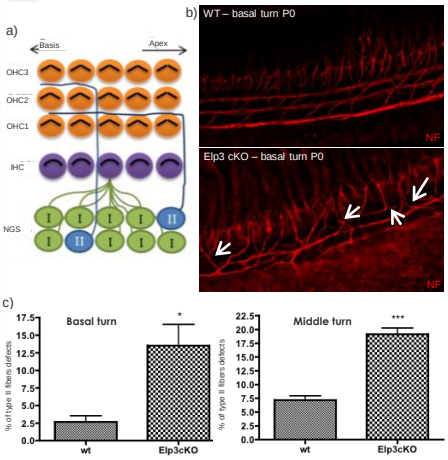
Defects concerning the hair bundle of cochlear hair cells have been observed:



Confocal images (left panel) of both wild-type and Elp3 cKO cochlear hair cells at stage P0 stained with phalloidin (phall, in red) in order to label hair bundles. a) In the WT, hair bundles are disposed in a V-shaped structure (yellow dotted lines). Angles determined by these V-shaped structures have been measured for fifteen hair cells per row and percentages of cells with a V angle superior to 120° (corresponding to an abnormal shape) have been calculated (n=4, right panel). b) In the KO, some hair bundles are misoriented regarding the mediolateral axis of the cochlea (yellow arrow). Angles formed between the mediolateral axis and the symmetrical axis of the V-shaped structure were measured. Percentages of cells with a V deviation superior to 10° (corresponding to an abnormal orientation) have been calculated (n=4, right panel). IHC = inner hair cell row; OHC1, 2, 3 = outer hair cell row 1, 2, 3.

Elp3 is implicated in shape and position of hair bundle of the cochlear hair cells .

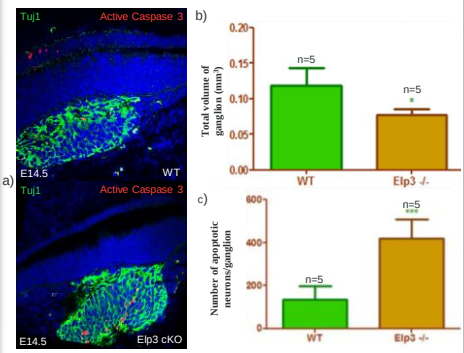
5 Elp3 and cochlear hair cell innervation



a) General situation 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 basis of the cochlea thereby contacting many outer hair cells. NGS = neurons of spiral ganglion, I = type I neurons, II = type II neurons, IHC = inner hair cells row, OHC1, 2, 3 = outer hair cells row 1, 2, 3. b) Confocal images of whole wild-type (WT) and Elp3 cKO cochleae (basal turn) at post-natal stage P0 stained with neurofilament (NF, marker of fibers from neurons, in red). Axonal guidance and innervation defects (white arrow) have been observed in Elp3 cKO condition compared to the WT. c) Quantification of these defects regarding type II fibers in the basal and the middle turn of the cochlea.

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

6 Elp3 and neuronal survival



a) Confocal images (left panel) of both wild-type and Elp3 cKO cochleae cross-sections at embryonic stage 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 both wild-type (WT) and Elp3 cKO spiral ganglia (n = 5). Volume of spiral ganglion in Elp3 cKO is reduced compared to WT. c) Number of apoptotic neurons per ganglion in both wild-type (WT) and Elp3 cKO (Elp3-/-) mice (n = 5). Apoptotic level is increased in Elp3 cKO spiral ganglion compared to the wild-type.

Loss of Elp3 increases neuronal apoptosis in the spiral ganglion

Conclusion & Perspectives

In conclusion, we have confirmed the expression of Elp3 in the inner ear and pointed out a role for this acetyl-transferase in balance function and probably in audition. 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. Until now, two substrates of Elp3 have been discovered: histone H3 and the alpha-tubulin. The latter being enriched in the kinocilium that serves as guidepost for the hair bundle formation in both vestibular and cochlear hair cells. We plan therefore to establish an eventual link between the lack of acetylation of the alpha-tubulin by Elp3 and these ciliogenesis defects in Elp3 cKO mice. In order to identify Elp3-regulated genes that could be involved in axonal guidance or neuronal survival, microarray analysis will be performed with wild-type and Elp3 cKO cochleae.

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