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A maturing view of cochlear calcium

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Charles Darwin was exercised to explain how a structure as complex as the human eye could have evolved. Had he been conversant with the mammalian cochlea, in his time just starting to be examined by the microscope, he would have had an even harder time. The cochlea contains an intricate arrangement of sensory hair cells that detect and encode the amplitude, the frequency and the timing of sound entering the ear for more or less the whole lifetime of the animal. The taxing question is how such a complex structure develops embryologically. Our inner ear is more less completely developed by 25 weeks in utero. But in the mouse the whole process takes only 30 days from conception. Of these 12 days proceed postnatally.

Such a timetable has its advantages. The newborn cells are accessible. The soft tissues can be cultured. The signals they generate can be recorded and imaged. The downside is that the developmental processes often involve temporary structures and the steps that build the final cochlear cells must be demarcated from the final adult result. Once such transient structure is Kolliker's organ, a ridge of cells that transforms into the lining cells of the cochlear sulcus before maturation and provides synchronization signals for the auditory wiring. A second example is the sound transducing channel of hair cells where gene expression (Kawashima *et al.*, 2011) and the channels themselves (Beurg *et al.*, 2018) show conversion from a pair, TMC1 and TMC2, in the immature cell to TMC1 alone in the mature cell.

A further confound is that cells along the length of the cochlea do not develop at the same time. A wave of cells exiting the cell cycle, judged by a marker protein $p27^{KIP1}$, progresses from the cochlear apex to the cochlear base (the future high frequency end) prenatally, and then the cells differentiate over the next few days around birth (P0 in the mouse) in the opposite direction, from base to apex. Thus basal cells express many of their identifying markers before apical cells but at least ten distinct types of cell can be recognised in the cochlea, two of which are the hair cells: inner hair cells (IHCs), making the sensory input to the nerve, and the outer hair cells (OHCs), part of the sound amplification process.

The paper by Jing-Yi *et al* describes a further orchestration of cell maturation and identifies another developmental prerequisite for the distinction between these two hair cell types. They have used a mouse deficient in a cochlear calcium channel CaV1.3 – which accounts for the majority of the Ca^{2+} channels in hair cells - and where it is known that the OHCs degenerate progressively from apex to

base from the onset of hearing (around postnatal day P12). Indeed, the CaV1.3 knockout mouse is deaf (Platzer *et al.*, 2000). The surprise is that that there is an intimate connection between Ca^{2+} signalling in the first week (P0-P7) and the loss of K^+ channels (Figure 1). The most overt early sign of any deficiency is that the OHCs lose spontaneous Ca^{2+} action potentials, a necessary but not sufficient signal which is thought to contribute to the correct neural wiring of the cochlea to the brain. Other markers of the OHC maturation, in particular the motor protein prestin, seem to be unaffected and IHCs, although surviving, never quite form their adult quota of K^+ channels.

Why should a Ca^{2+} signal determine K^+ channel expression in the hair cells? Although other critical factors are required, including the serine protease Tmprss3 (Molina *et al.*, 2013), the hair cells' Ca^{2+} dependent K^+ channels are certainly affected. These include the BK/KCNMA1 and the SK2/KCNN2 channels, the latter being involved in the efferent innervation of the OHCs. But the most prominent result of the CaV1.3 deletion is to remove a hair cell K^+ channel, KCNQ4, from basal cochlear cells but leaving a residual expression further along towards the apical end, a distinction which may partly reflect the developmental timing differences along the cochlea. KCNQ4, which underpins a characteristic hair cell current I_{Kn}, is certainly required for OHC survival as it forms the pathway for intracellular K^+ homeostasis; without it the cells depolarise and die. But perhaps equally puzzling is that the Ca^{2+} entry is determined by a *voltage* gated channel. Could it be that cell potential plays a critical role in protein translocation to membrane? Or is the temporal patterning of the early Ca^{2+} signals the trigger for gene translation? Unravelling all the causal links is a challenging problem for the future.

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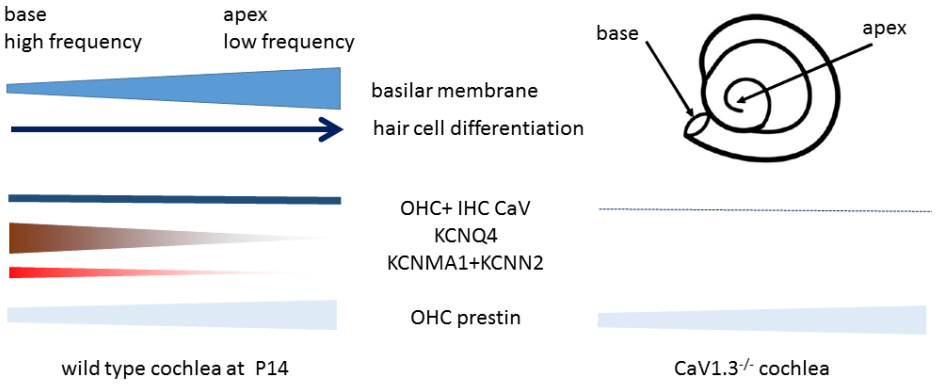

Figure legend:

Organisation of the base-apex gradient of cell maturation in the mammalian cochlea. Deletion of the CaV1.3 channel results in failure to express any of the Ca^{2+} activated channels as well as the major OHC K^+ channel, KCNQ4, although most prominently at the basal end of the cochlea.