Published in final edited form as:

Semin Cell Dev Biol. 2013 May ; 24(5): 438–447. doi:10.1016/j.semcdb.2013.03.010.

The role of *Atonal* transcription factors in the development of mechanosensitive cells

Andrew P. Jarman¹ and Andrew K. Groves²

Andrew P. Jarman: andrew.jarman@ed.ac.uk; Andrew K. Groves: akgroves@bcm.edu

¹Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, Edinburgh, United Kingdom

²Departments of Neuroscience and Molecular and Human Genetics, Program in Developmental Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA

Abstract

Mechanosensation is an evolutionarily ancient sensory modality seen in all main animal groups. Mechanosensation can be mediated by sensory neurons or by dedicated receptor cells that form synapses with sensory neurons. Evidence over the last 15–20 years suggests that both classes of mechanosensory cells can be specified by the *atonal* class of basic helix-loop-helix transcription factors. In this review we discuss recent work addressing how *atonal* factors specify mechanosensitive cells in vertebrates and invertebrates, and how the redeployment of these factors underlies the regeneration of mechanosensitive cells in some vertebrate groups.

1: *Atonal* genes and mechanosensory cells

Two bHLH gene families are associated with sensory cells across metazoans – the *achaete-scute* and *atonal* families. Of these, the *atonal* genes are particularly strongly linked with the specification of photo- and mechanoreceptor cells [1]. The *atonal* gene was discovered in *Drosophila* as a proneural transcription factor for mechanoreceptive neurons and photoreceptor cells [2, 3]. Jellyfish *atonal* homologues are expressed in photoreceptive and mechanosensory cells [4]. In vertebrates, these two key functions of *atonal* have been separated by gene duplication, such that *Atoh7* is required for retinal ganglion cells and *Atoh1* for mechanosensitive cells.

Invertebrates such as *Drosophila* use *atonal* to generate intrinsically mechanosensitive neurons called chordotonal (Ch) neurons. These bipolar neurons have a dendrite terminating in a sensory cilium that harbors the mechanoreceptive machinery (Fig. 1A). In some parts of the fly's body these are proprioceptive, while in the antenna they are auditory. In contrast, some vertebrate sensory systems have split the function of the mechanosensitive neuron into a sensory receptor cell (such as hair cells and Merkel cells) that makes synaptic connections with a sensory neuron that is no longer mechanosensitive (Fig. 1B). The vertebrate *Atoh1* genes are expressed in many mechanosensitive progenitor cells and are necessary for the development of hair cells of the inner ear and lateral line [5–7], and touch-sensitive Merkel cells in the skin [8, 9]. Despite obvious structural differences, there are persuasive indications that Ch neurons and hair cells are derived from an ancestral *atonal*-specified mechanosensitive cell type [1, 10, 11]. A parallel argument is that *atonal* genes (*atonal*/*Atoh7*) are also anciently connected to photoreceptive cell development [12]. Indeed, it has been proposed that photoreceptive and mechanosensitive cells are linked even further back in an *atonal*-dependent proto-sensory organ [13]. To this could be added chemosensation, since *Drosophila atonal* and its in-paralogue *amos* are also required for olfactory neurons [14]. Similarly, the *C. elegans* homologue of *atonal*, *lin-32*, is required for pairs of

mechanosensitive and chemosensitive neurons in the male tail [15]. Comparisons of the sensory transduction apparatus also hint at molecular connections between these sensory modalities. For instance, Ch neurons express visual rhodopsins and olfactory ionotropic channels, both of which contribute to auditory sensory transduction [16].

The degree of conservation in the mechanosensory function of *atonal/Atoh1* is striking: *Drosophila atonal* can fully rescue *Atoh1* null mutant mice [17]; conversely, mouse *Atoh1* can partially rescue *atonal* mutant flies [8]. In this review, we focus on these mechanosensory roles.

2: *Drosophila Atonal* and the development of sensory neurons

Ch neurons form part of internal sense organs that mediate proprioception (Ch organs are typically located to respond to joint or body movement), and hearing and gravitaxis (the large Ch neuron array of Johnston's Organ in the antenna) [18]. During the formation of the precursors of these neurons within the ectoderm (sense organ precursors or SOPs), *atonal* functions as a 'typical' proneural gene in that its expression is necessary and sufficient for SOP specification [2]. It is transiently expressed in ectodermal groups of cells (proneural clusters or PNCs) and then is restricted to a subset – the SOPs – by Notch-mediated lateral inhibition. After commitment, each SOP divides several times asymmetrically to give the 4–6 cells of a unit Ch organ, 1–2 of which differentiate as Ch neurons. Failure to generate Ch SOPs in *atonal* mutants results in individuals that are deaf and exhibit uncoordinated locomotion [19–21].

2.1: The regulation of *atonal* in mechanosensory cell development

atonal transcription is spatially and temporally regulated in two distinct phases of expression. In the first phase, *atonal* is activated by combinations of regionally expressed patterning factors and signals to give the PNCs at locations corresponding to the ultimate positions of Ch organs. In the second phase, expression becomes restricted to the SOPs – it is upregulated in these cells and downregulated in the remaining cells of each PNC during lateral inhibition. This entails positive autoregulation in the SOPs and Notch inhibition in the remaining cells, initiated by the SOPs themselves. In addition to the inhibitory signal, the SOPs also send out an EGFR pathway recruitment signal that causes upregulation of *atonal* in adjacent PNC cells [22–24]. The balance between Notch inhibition and EGFR induction determines the proportion of SOPs deriving from a PNC, and is at least part of the reason why Ch neurons can exist in large cohesive arrays, such as Johnston's Organ.

The *cis* regulation of *atonal* is achieved through extensive batteries of up- and downstream enhancer elements. In general, separate elements are required for activation of *atonal* in different locations, and also for the different temporal phases of expression [25]. In particular, it appears that the 3' region contains enhancer elements to initiate PNC expression whereas enhancers in the 5' region control upregulation in the SOPs. The 5' region contains separable autoregulatory enhancers for *atonal* upregulation in different regions (leg, eye, antenna, etc). Only one of these 5' enhancers has been characterised in any detail. This enhancer responds directly to the EGFR recruitment signal outlined above, in both the embryo and the leg imaginal disc. Within the enhancer are adjacent binding sites for Atonal and Pointed proteins (the ETS transcription factor activated by EGFR signaling) [26]. Thus this enhancer is activated in cells within the PNC (which are therefore expressing *atonal* at a low level) that receive EGFR signaling from previously selected SOPs. In other words, for this enhancer *atonal* autoregulation is contingent on EGFR signaling.

Another component of *atonal* autoregulation is the bifunctional Zn-finger protein, Senseless. This is a DNA-binding transcriptional repressor in the PNCs, but becomes a coactivator

when bound to *atonal* or other proneural proteins [27, 28]. *senseless* is also a target gene of *atonal*. Thus, *atonal* activates *senseless* expression, which then enhances *atonal* protein activity, thereby forming a positive feedback loop that helps to overcome Notch inhibition in SOPs.

Initial activation of *atonal* expression is directed by the 3' flanking region. The expectation was that this would contain different regulatory elements to initiate *atonal* expression in each of its different domains (antennal, leg, eye), but instead it appeared to comprise a single enhancer element that responds to the confluence of *dpp* (BMP) and *wg* (Wnt) signaling at each of these locations, as well a temporal ecdysone signal to trigger expression at the appropriate time of development [13, 25]. An intriguing explanation proposed for this was that all *atonal*-dependent sensory organs share a common developmental programme, and that cellular diversification arises through the later action of region-specific transcription factors such as *Pax* and *Hox* genes [13]. This developmental model contributed to the evolutionary idea of an *atonal*-dependent proto-sensory organ as discussed above. However, subsequent studies have shown that this characterisation of *atonal*'s regulation is simplistic since the 3' region can be split into separate elements for different expression domains [29]. The Ch neuron elements have not been characterised, but one element for the eye has binding sites for *eyeless* (*Pax6*) and *sine oculis* (*Six* gene) proteins [29, 30]. Therefore, region-specific 'patterning' factors are likely to initiate *atonal* activation in combination with spatial and temporal signals. In the adult and larval eyes (and possibly also Ch neurons), *hedgehog* signaling is also required for *atonal* regulation [31, 32], but it is not yet known whether this regulation is direct. Intriguingly, the trithorax-group chromatin remodeling factors are required in the eye specifically for *atonal* expression [33].

2.2: How *atonal* regulates target genes

Like other proneural bHLH factors, *atonal* regulates known target genes as a transcriptional activator by binding to E box motifs as a heterodimer with an E protein, Daughterless [2]. Investigation of several known or suspected target genes suggested that *atonal* regulates targets via an extended variant of the generic E box (A A/T C A G/T G T G T/G) [34]. A more recent systematic computational identification of putative target genes (albeit focused on the eye) suggested a related target gene-associated motif of G/A A C A C/G C T G C/T. In each case, the motif differs from the E box associated with target genes of the *achaete-scute* family (binding site: G C A G C/G T G T/G), which goes part way to explaining how *atonal* and *achaete-scute* differ in function and why they cannot substitute for each other [35].

The function of *atonal* protein is clearly context-dependent (i.e. it specifies mechanoreceptors, olfactory receptors and photoreceptors in different locations) implying that some proportion of *atonal*'s target genes will differ according to developmental context. As yet, there is little direct evidence of how *atonal*'s activity may be modulated by context, but we may expect that other region-specific transcription factors will be involved, either through binding to adjacent sites in *atonal*-dependent enhancers (combinatorial control), or through binding to and altering *atonal*'s DNA binding specificity directly (specificity coactivators). Indeed, there is some evidence that different *atonal* binding sites can support quite distinct expression patterns in reporter gene studies [34].

2.3: The function of *atonal* in mechanosensory neurons

What can target genes tell us about *atonal*'s function in mechanosensory cells? Clearly it regulates SOP specification but what about subsequent development and differentiation? Whilst several studies have sought to find target genes of proneural proteins, so far few have been directed specifically at *atonal*. The most comprehensive attempt to identify direct target

genes has been the combined transcriptome and computational analysis of Hassan and colleagues [36]. Although based on *atonal*'s function in the eye, most of the validated targets were expressed both in the eye and in Ch neurons, suggesting once more that the *atonal*-dependent gene expression programme has many common features in the different sensory organs.

Further functional genomic approaches have aimed not specifically at identifying direct *atonal* targets, but at the more general question of what are the pathways activated in mechanosensory differentiation. A recent study identified the time course of transcriptome changes downstream of *atonal* function in embryonic Ch cells [37]. GFP-tagged Ch cells were isolated from embryos at 1-hour intervals of development and subjected to microarray analysis. The most striking finding was that the predominant programme activated in Ch cells is associated with constructing the sensory cilium: at later timepoints, some 40% of the top 100 differentially expressed genes were associated with ciliogenesis or cilia function. Moreover, all *Drosophila* homologues of known ciliogenesis genes are upregulated in Ch neuron transcriptome. This leads to the question of how this pathway is regulated by *atonal*. The transcriptome analysis suggested two key transcription factors are activated by *atonal*. One is Rfx, a winged helix factor that regulates many ciliogenic genes in a variety of organisms, including in *Drosophila* sensory neurons [38]. The second is Fd3F, a FOX transcription factor. It was subsequently found that Fd3F regulates genes for specialisation of the Ch neuron cilium, notably genes of the intraflagellar transport A (IFT-A) complex, which is required to delineate the distinct motile and non-motile zones of the cilium (Fig. 1A), as well as genes for axonemal dyneins and associated TRPV channels, which populate the motile zone [39]. The dyneins contribute to hearing as part of the sensory transduction machinery, most likely as adaptation motors mediating amplification of sound reception via ciliary oscillations [16]. Loss of these specializations in *fd3F* mutant flies results in dysfunctional Ch neurons with immotile cilia leading to deafness and uncoordination [39]. Thus, *atonal* activates two transcription factors that work together to switch on the genes for the construction a major aspect of the Ch neuron – its mechanosensory cilium. In addition to this regulatory relay, a proportion of ciliogenic genes were found to be expressed surprisingly soon after Ch cell specification, and it was shown that at least one of these differentiation genes is a direct target of *atonal* [37, 40].

It is striking that clear links were made between *atonal* and the mechanosensory apparatus, thereby raising the question of whether *Atoh1* conserves such links in hair cells. Certainly, in the zebrafish otic vesicle, the Fd3F relative, *Foxj1b*, is necessary for the kinocilium [41], which is required for correct development of the microvilli-based stereocilia and for otolith tethering. Although it is itself immotile, the kinocilium is thought to be derived from a motile cilium. As yet there is no information on the regulation of *Foxj1* genes in the vertebrate ear, nor on the role of Rfx genes.

A further approach has identified genes expressed in the Ch organs of adult antennae (therefore, auditory genes) by comparing gene expression in wild-type and *atonal* mutants [16]. Some 274 auditory organ genes were identified. Of 42 genes analysed further, 27 showed defective auditory reception, including some homologues associated with human deafness. As mentioned above, a most surprising finding was that the 'auditory genes' included olfactory ionotropic receptors and visual rhodopsins. It will be interesting to determine whether *atonal* triggers a common pathway to regulate these genes in the three *atonal*-dependent sensory cell types. Interestingly, visual signal transduction genes have been shown to be required for thermosensation [42], whilst larval Ch neurons have been demonstrated to be thermoreceptive, allowing larvae to discriminate between their favored temperature (17.5°C) and slightly lower temperatures (down to 14°C) [43]. If this extends to

adults, there is the possibility of a link between TRPV channels and visual signal transduction apparatus.

Together, the developmental and adult mechanosensory gene data provide an excellent resource for exploring the regulatory network downstream of *atonal* and for providing clues to hair cell pathways downstream of *Atoh1*.

3: The role of vertebrate *Atoh1* genes in the development of mechanosensory hair cells

The developmental module that uses *atonal* family bHLH genes to regulate the development of mechanosensory cells is thought to be evolutionarily ancient [11]. As discussed above, invertebrates such as *Drosophila* use this module to generate intrinsically mechanosensitive neurons, whereas some vertebrate sensory systems have split the function of the mechanosensitive neuron into a sensory receptor cell (such as a hair cell or Merkel cell) that makes synaptic connections with a sensory neuron that is no longer mechanosensitive. The vertebrate homologues of *atonal*, *Atoh1*, are expressed in many mechanosensitive progenitor cells and are necessary for the development of hair cells of the inner ear and lateral line [5–7], and touch-sensitive Merkel cells in the skin [8, 9]. In this section, we focus on the factors that regulate *Atoh1* expression in hair cell progenitors and discuss how *Atoh1* regulates hair cell differentiation and survival through its known targets.

3.1: Do vertebrate *Atoh1* homologues function as proneural genes?

The *Drosophila atonal* gene fulfills several criteria of a proneural gene – its expression precedes and coincides with the selection of sensory neuronal progenitors, its expression is regulated by Notch-mediated lateral inhibition and its function is both necessary and sufficient for the development of those progenitors [44]. The situation is more complicated in vertebrates, as it is less clear if *Atoh1* genes mark committed hair cell progenitors as opposed to multipotent progenitor cells capable of forming hair cells, supporting cells or neurons [45]. This is an important question since it impinges on *Atoh1*'s potential in hair cell replacement therapies (see Section 4, below). We deal first with zebrafish, which has two *atonal* homologues, *atoh1a* and *atoh1b*. *Atoh1b* is expressed broadly in the otic placode before the differentiation of neurons and hair cells and is rapidly refined to two patches presaging distinct sensory epithelia [46]. It is necessary for the development of tether cells, precocious hair cells that seed and localize the formation of otoliths [46, 47], but not for the development of the majority of later hair cells in the ear, nor of lateral line neuromasts [46]. In contrast, *atoh1a* is expressed later in the progenitors of the majority of zebrafish hair cells and lateral line neuromasts [48, 49], and is necessary for their development, but not the development of the precocious *atoh1b*-dependent tether cells [7, 46]. As might be expected from this division of labor between two closely-related *atonal* homologues, knockdown of both genes in zebrafish eliminates both early- and late-developing hair cells (Millimaki et al., 2007). Conversely, ectopic expression of *atoh1a*, either constitutively or transiently, causes expanded regions of hair cells in the zebrafish ear [46, 50]. These results, together with the broad and early expression of *atoh1b* in the otic placode and the negative regulation of zebrafish *atoh1* genes by Notch signaling (Millimaki et al., 2007) suggest that fish *atoh1* homologues may act as true proneural genes.

In mammals, *Atoh1* is clearly necessary for hair cell formation [5, 51, 52], and ectopic expression of *Atoh1* is sufficient to induce ectopic hair cells in at least some non-sensory parts of the inner ear [53–55]. However, it is less clear whether *Atoh1* is expressed only in committed hair cell progenitors or in progenitors capable of giving rise to several cell types. A number of methods have been used to identify the onset of *Atoh1* expression in the inner

ear, including in situ hybridization for *Atoh1* mRNA [56], antibody staining of Atoh1 protein [51], GFP-expressing reporter mice driven by the *Atoh1* autoregulatory enhancer [57] and reporter expression resulting from knocking the β -galactosidase gene into the *Atoh1* locus [58]. These methods give somewhat contradictory results [59], with β -galactosidase driven from the *Atoh1* locus being expressed in a broad domain of sensory precursor cells, whereas Atoh1 protein or GFP expression from *Atoh1*-GFP mice suggesting that *Atoh1* is expressed in nascent hair cells.

A partial resolution of these data has come from recently developed transgenic mice in which either an *Atoh1*-GFP fusion gene or Cre recombinase were knocked into the *Atoh1* locus [60, 61]. Crossing *Atoh1*-Cre knock-in mice with Cre reporter lines show labeling of supporting cells [61]. Although this can be interpreted as *Atoh1* marking a bipotential hair cell-supporting cell progenitor, two interesting but cautionary points should be considered. First, the number of supporting cells labeled by Cre-mediated recombination in the different sensory organs of the ear varied widely, from about 60 labeled cells per 100 hair cells in the organ of Corti to only 7 per 100 hair cells in the utricular macula. Second, the pattern of Cre-labeled supporting cells in each organ is essentially random, with no obvious anatomical position or supporting cell sub-type being preferentially labeled (Yang et al., 2010; L. Gan, personal communication). Recent work from our lab using *Atoh1*-GFP fusion knock-in mice also suggests that Atoh1 protein is not broadly expressed in the prosensory domain of the cochlea, but rather appears in a salt-and-pepper pattern that segregates into a row of inner hair cells as the cochlear duct elongates (Cai, Zoghbi and Groves, unpublished observations). One interpretation of these data is that the mammalian *Atoh1* gene is expressed in hair cell progenitors immediately before they commit to a hair cell fate, but that some of these *Atoh1*-expressing cells can be re-directed to a supporting cell fate, most likely by Notch-mediated lateral inhibition. The labeling of supporting cells in different sensory organs by *Atoh1*-Cre mice would therefore vary according to the speed and efficiency with which Notch-mediated fate decisions are fixed as hair cells and supporting cells differentiate. Since the arrangement of hair cells and supporting cells in the organ of Corti is far more precise and invariant than in other sensory organs, it is reasonable to suggest that the higher number of Cre-labeled supporting cells in the organ of Corti is a consequence of the greater fine-tuning of hair cell and supporting cell numbers that occurs during its development.

3.2: What are the signals that activate Atoh1?

Although Atoh1 occupies a pivotal role in hair cell differentiation, very little is known of the factors that directly regulate its expression in differentiating prosensory cells. Several well-characterized signaling pathways have been shown to affect hair cell development, such as Wnt, BMP, FGF and Shh signals [62–69]. Further insights into how *Atoh1* is regulated have been gleaned from study of its well-characterized autoregulatory enhancer located downstream of the coding region [70]. This enhancer has E-box and N-box binding sites [70], and it is known that Atoh1 can itself activate this enhancer through E-box binding, and that this autoregulation is necessary for the maintenance of *Atoh1* transcription in hair cells [56]. Members of the Hes and Hey gene family, which are able to bind to N-box sequences, can repress hair cell differentiation [71, 72] and negatively regulate *Atoh1* expression [73, 74], but it is not yet clear whether this is due to direct binding of Hes or Hey factors to the N-box within the *Atoh1* enhancer, or by other mechanisms, such as sequestration of Atoh1 binding partners. The *Atoh1* autoregulatory enhancer can bind transcription factors known to be downstream of several signaling pathways, such as Wnt (β -catenin and Cdx2; [75, 76] and BMP (Zic1 and Cdx2; [76, 77]). A number of other transcription factor binding sites have been identified in the *Atoh1* enhancer [75–80], in particular for Sox2 [81]. Sox2 can form a transcriptional complex with Six1 and Eya1 that is sufficient to activate *Atoh1* when

electroporated into the greater epithelial ridge of the mouse cochlea [81]. *Sox2* can also act by itself to activate *Atoh1* expression in the otocyst of chicken embryos [82], and *Six1* and *Eya1* can also induce *Atoh1* in the absence of *Sox2*, albeit at lower efficiency [81]. Since *Sox2*, *Six1* and *Eya1* are all expressed more broadly in the prosensory domain of the cochlear duct prior to the differentiation of hair cells, it is likely that additional signals are required to allow this transcriptional complex to activate *Atoh1* expression specifically in nascent hair cells. Interestingly, *Six1* and *Eya1* are also expressed earlier in the progenitor domain that generates the cochleo-vestibular ganglion, and these factors are able to co-operate with *Sox2* to activate *Neurog1* and produce sensory neurons when electroporated into inner ear tissue [83]. Electroporation of *Six1* and *Eya1* cDNAs into the greater epithelial ridge of the cochlea generates large numbers of Myosin 7a+ hair cells and very few neurons, whereas electroporation of the same factors together with components of the SWI/SNF complex generates large numbers of neurons and very few hair cells [83]. Thus, the *Six1/Eya1/Sox2* complex can activate two closely-related bHLH transcription factors to produce neurons (*Neurog1*) or hair cells (*Atoh1*) depending on the cellular context, a decision that is mediated in part by the SWI/SNF chromatin remodeling complex. Some cells in the greater epithelial ridge may be derived from early *Neurog1*-expressing cells in the otocyst [56], and might be especially susceptible to reprogramming by neurogenic factors. The co-option of a chromatin remodeling complex in the choice between neurons and hair cells may have been an early evolutionary change as the function of an invertebrate mechanosensitive neuron was divided between a mechanosensitive hair cell and a non-mechanosensitive neuron in vertebrates.

The induction of *Atoh1* and the production of hair cells occurs over an extended period of time in all inner ear sensory organs. In the vestibular system, differentiation begins in the center of each sensory patch, with extra hair cells being added peripherally over time. In the case of the mouse utricular macula, the first *Atoh1*-expressing cells can be observed at embryonic day 11, but the last hair cells are not added until 14 days after birth [56, 84, 85]. In the cochlea, *Atoh1* is expressed in a basal-apical gradient starting in the mid-basal region at E13.5 and terminating in the apex 5–6 days later [57]. This sequential propagation of differentiation can occur correctly even in pieces of cochlea that have been mechanically separated and maintained in organ culture [86]. The basal-apical gradient of differentiation can be perturbed in mice carrying mutations for either *NeuroD* or *Neurog1* [87, 88], where precocious *Atoh1* expression and differentiation of hair cells is seen as early as E14.5. Neither *NeuroD* nor *Neurog1* are expressed in the cochlea at detectable levels during this period of differentiation, suggesting they may regulate the timing *Atoh1* expression indirectly. Since both mouse mutants lack most of the spiral ganglion, it is possible that the ganglion normally releases signals that regulate *Atoh1* induction. Interestingly, *Shh* is expressed transiently in the spiral ganglion, disappearing in a basal-apical sequence from E13.5 onwards [89]. This observation, together with the finding that inhibition of *Shh* signaling can increase hair cell production in the cochlea [62] suggests that *Shh* may be one of the factors that regulates the timing of *Atoh1* expression in the correct basal-apical sequence in the cochlea. Since *Shh* is required at earlier stages in ear development [90], conditional deletion of *Shh* will be required to test this hypothesis.

3.3: How does *Atoh1* regulate hair cell differentiation and survival?

At present, we know very little about mechanisms by which *Atoh1* regulates hair cell differentiation. A recent study which identified direct targets of *Atoh1* in cerebellar granule cells suggested that it not only regulates downstream transcription factors, but also genes involved in many other cellular processes, such as cell division, chromosomal organization, metabolism, cell migration and cell adhesion [91]. In the context of hair cells, it is possible that *Atoh1* may simply regulate generic aspects of hair cell differentiation (for example,

regulating apical-basal polarity, components of the mechanotransduction apparatus or actin polymerization), and that other factors may co-operate with *Atoh1* or act in parallel to regulate genes specific to particular classes of hair cell – for example, inner versus outer cochlear hair cells, or type I versus type II vestibular hair cells. Moreover, since *Atoh1* is down-regulated in hair cells as they mature, it is not clear how many direct targets of *Atoh1* continue to be expressed in mature hair cells, nor how the expression of these genes is maintained. Recent evidence suggests that other transcriptional regulators can co-operate with *Atoh1* to determine hair cell identity. For example, mutation of the *Jxc1* transcription factor causes production of ectopic hair cells in the greater epithelial ridge that have the morphology of vestibular hair cells [92], and activation of *-catenin* in the chicken basilar papilla generates cells with a vestibular, rather than an auditory hair cell morphology [69].

Resolution of these questions requires identification of direct targets of *Atoh1* in hair cells. Unlike tissues such as the cerebellum, where *Atoh1*-expressing cells are present in large numbers and constitute a significant proportion of the total cell population, there are only a few thousand *Atoh1*-expressing hair cells in inner ear sensory organs, and they represent only a small minority of the total cell types. Thus, techniques such as chromatin immunoprecipitation and deep sequencing (ChIP-seq) are extremely difficult to apply to the inner ear. Nevertheless, it may be possible to exploit and apply recent data sets obtained from the cerebellum [91] to help identify *Atoh1* targets in the inner ear. For example, analysis of *Atoh1* targets in the cerebellum has identified a consensus specific *Atoh1* binding motif based on an extended E box variant that is reminiscent of, but not identical, to that identified for *Drosophila atonal* (G/A,C/A,CA,G/T,C/A,TG,G/T,C/T) [91]. This *Atoh1* E-box associated motif, or AtEAM, is present close to the coding regions of over 65% of genes with sequences bound by *Atoh1* in the cerebellum, and may be of use in predicting candidate *Atoh1* targets in hair cells. However, as in *Drosophila*, it is also possible that hair cell-specific targets may be regulated by a different E box variant. It is also clear that some of the processes regulated by *Atoh1* in cerebellar granule cells, such as proliferation and migration, do not occur in hair cells, and one might therefore expect significant differences in the repertoire of genes regulated by *Atoh1* in hair cells and granule neurons. Indeed, a recent comparison the ChIP-sequencing data from the cerebellum with gene expression data sets from cerebellar granule neurons, dorsal spinal interneurons and hair cells have identified only three genes – *Rab15*, *Selenoprotein M (Selm)* and *Atoh1* itself – that are candidates to be direct targets of *Atoh1* in all three cell types [93].

Atoh1 is closely related to *Neurog1*, another bHLH factor that is necessary for the development of sensory neurons, including those generated by the inner ear [88, 94]. Despite the similar sequence of both proteins, *Neurog1* is not able to fully substitute for *Atoh1*. Mice in which the coding sequence of *Atoh1* was replaced with *Neurog1* have a highly abnormal organ of Corti with very few hair cells [95]. The phenotype of these homozygous gene replacement mice is somewhat less severe than regular *Atoh1* null mice, suggesting that *Neurog1* is only able to activate a subset of *Atoh1* target genes. By analogy to the recently-discovered AtEAM E-box binding variant in *Atoh1* target genes, it is likely that *Neurog1* has its own conserved DNA binding motif that is similar to, but distinct from, the AtEAM motif, and that subtle differences in the binding affinities of these two factors have far-reaching consequences on the range of genes each factor can activate.

3.4: The function of *Atoh1* in mechanosensitive Merkel cells

Although most attention has focused on the molecular function of *Atoh1* in hair cell development, *Atoh1* is also necessary for the development of Merkel cells, an epidermally-derived cell population believed to play a role in the discrimination of light touch [96, 97]. Merkel cells associate with the neurites of SAI sensory afferents in the skin and bear large microvilli that interdigitate with epidermal cells. Merkel cells contain the actin-binding

protein espin, (which is also seen in hair cell stereocilia), express a number of ion channels previously implicated in mechanotransduction, such as *PKD1*, *PKD2* and *TRPC1*, and show calcium influx in response to osmotic stress *in vitro* [98]. Together, these features suggest that Merkel cells may be intrinsically mechanosensitive [99], although this has yet to be directly demonstrated in the intact animal. Inactivation of *Atoh1*, either throughout the animal or specifically in the epidermis, causes a failure of Merkel cell development and an associated loss of SAI responses to light touch [8, 9, 100]. Little is known about the direct transcriptional targets of *Atoh1* in Merkel cells, although it was recently shown that Merkel cells express *Rab15* and *Selm*, two *Atoh1* target genes also expressed in cerebellar granule neurons, spinal dP1/dI1 neurons and hair cells [93]. Although *Rab15* is known to be involved in endosome recycling [101] its potential role in hair cells is unclear. It is possible that it may regulate the expression of Notch receptors or their ligands as has been shown for *Rab11* in *Drosophila* [102]. *Selm* encodes Selenoprotein M, which may participate in antioxidant protection in neuronal cells [103]. Since hair cells are particularly susceptible to oxidative stress, it is possible that *Selm* participates in hair cell survival following stress. *Selm* mutant mice are phenotypically normal (Jane Johnson and Helen Lai, personal communication), although it is not known if these mice are more susceptible to noise or ototoxic damage to their inner ears.

4: The role of *Atoh1* in the regeneration of hair cells

Mammals are unable to regenerate their auditory hair cells after damage, and display only a very limited degree of regeneration in the vestibular system (reviewed in [104]). In contrast, non-mammalian vertebrates show robust hair cell regeneration after damage due to the proliferation and trans-differentiation of supporting cells. Non-mammalian vestibular sensory organs and the lateral line organs of teleosts also show a steady ongoing turnover of hair cells [105, 106]. Accordingly, small numbers of *Atoh1*-expressing cells can be detected in these organs under normal conditions [107, 108], and *Atoh1* is rapidly-re-activated in many supporting cells during regeneration in non-mammalian vertebrates [105, 107–109]. Regenerating hair cells quickly start to re-establish a normal proportion of hair cells and supporting cells through regulation of *Atoh1* by lateral inhibitory Notch signaling [108, 110]. Inhibition of the Notch signaling pathway can further increase the proportion of cells expressing *Atoh1* in the chicken basilar papilla [109], and also in the adult mouse utricle [111], albeit to a much smaller degree.

The necessity and sufficiency of *Atoh1* for hair cell development, and the similarities in the regulation of *Atoh1* during hair cell development and regeneration has prompted much interest in using *Atoh1* as a potential target of gene therapy to promote hair cell regeneration in humans [112, 113]. However, recent work has suggested that although *Atoh1* is sufficient to generate new hair cells, the ability of inner ear tissue to respond to *Atoh1* over-expression in this manner declines rapidly with age. We describe some of these experiments below and discuss possible reasons for the age-dependent loss of responsiveness to *Atoh1* activation

4.1: Is there an age-dependent limit on the ability of *Atoh1* to induce hair cell differentiation in mammals?

As described above, *Atoh1* is one of the first genes to be up-regulated in supporting cells following hair cell loss in birds and fish. There are no reports of *Atoh1* being expressed in the mammalian cochlea after hair cell loss, although a small amount of *Atoh1* transcription has been seen in the mammalian vestibular system after damage, both *in vivo* and *in vitro* [111, 114] – for example, an average of about 200 cells activate reporter gene expression from an *Atoh1* enhancer in the drug-damaged adult mouse utricle [111]. However, in both studies, only a very small fraction of *Atoh1*-expressing cells – typically less than 5% – go on to express *Atoh1* protein or develop as hair cells [111, 114]. Although this number can be

increased somewhat by inhibiting Notch signaling [111], these results suggest that the ability to activate transcription from the *Atoh1* locus and translation of *Atoh1* mRNA are severely attenuated in the adult mammal.

In an attempt to overcome at least some of these limitations in *Atoh1* expression in mammals, a number of studies have used adenoviral or transgenic expression of *Atoh1* to generate new hair cells. Although a few studies have shown that adenovirally-transduced *Atoh1* has the ability to restore hair cells in animals treated with ototoxic drugs [112, 113, 115], it is less clear whether this represents the generation of new hair cells or the repair of damaged, surviving hair cells, and further studies are needed to replicate and extend these findings. Recently, two studies used either Cre-inducible or tetracycline-regulated transgenic mice to activate *Atoh1* expression throughout the inner ear epithelium or specifically in sub-populations of supporting cells [55, 116]. In both cases, expression of *Atoh1* in supporting cells or in non-sensory cochlear epithelium was able to induce new hair cells, some of which showed impressive morphological and functional maturation [55, 116]. Nevertheless in both studies, activation of *Atoh1* at progressively older ages produced fewer and fewer hair cells, by two weeks of age – a time corresponding to the onset of hearing – transgenic expression of *Atoh1* yielded virtually no new hair cells [55, 116]. This failure was also seen even after killing the resident hair cells with ototoxic drugs [116].

Why do supporting cells in the mature mammalian ear fail to respond to the activation of *Atoh1*? First, the *Atoh1* protein itself may be post-translationally modified, for example by phosphorylation at its serine-rich C-terminal domain [59]. Second, it is known that inhibitory helix-loop-helix family members such as the Id proteins can block or attenuate bHLH gene activity by competing for E proteins, and that Id family members in the inner ear are known to antagonize *Atoh1* function during development [117]. It is therefore possible that sustained expression of Id family members or other inhibitory factors in the adult would block the activity of ectopically-expressed *Atoh1* protein. Third, it is possible that in addition to forming heterodimers with E proteins, such as E12 or E47, *Atoh1* also requires other transcription factors or co-activators to regulate its targets. Such co-operating factors might be down-regulated in supporting cells with age, with the consequence that ectopically-expressed *Atoh1* protein would not be able to activate its targets alone. Finally, it is possible that *Atoh1* is active and functional when over-expressed mature mammalian supporting cells, but that its direct transcriptional targets have undergone epigenetic modification, rendering them unavailable for transcription. Some form of epigenetic reprogramming would therefore be required to allow supporting cells to trans-differentiate in response to *Atoh1* expression. This might involve changes in DNA methylation, or in the post-translational modification of histones to replace inhibitory epigenetic marks with marks associated with active chromatin. Testing these hypotheses will require the identification of more direct targets of *Atoh1* in order to understand their regulation and epigenetic modification in supporting cells with age. Alternatively, *Atoh1* could be used to reprogram supporting cells directly in concert with other, as-yet unidentified factors, by analogy to the reprogramming of fibroblasts into differentiated cells of the nervous system, muscle or pancreas [118]. In conclusion, it is clear that *Atoh1* has great potential as a therapeutic agent, but much more information is required about its regulators, cofactors, and targets in order to capitalize on this potential. The study of *atonal* family genes continues to be an exciting and important area of research.

REFERENCES

1. Fritsch B, Beisel KW, Pauley S, Soukup G. Molecular evolution of the vertebrate mechanosensory cell and ear. *Int J Dev Biol*. 2007; 51:663–678. [PubMed: 17891725]

2. Jarman AP, Grau Y, Jan LY, Jan YN. *atonal* is a proneural gene that directs chordotonal organ formation in the *Drosophila* peripheral nervous system. *Cell*. 1993; 73:1307–1321. [PubMed: 8324823]
3. Jarman AP, Grell EH, Ackerman L, Jan LY, Jan YN. *atonal* is the proneural gene for *Drosophila* photoreceptors. *Nature*. 1994; 369:398–400. [PubMed: 8196767]
4. Seipel K, Yanze N, Schmid V. Developmental and evolutionary aspects of the basic helix-loop-helix transcription factors Atonal-like 1 and Achaete-scute homolog 2 in the jellyfish. *Dev Biol*. 2004; 269:331–345. [PubMed: 15110704]
5. Bermingham NA, Hassan BA, Price SD, Vollrath MA, Ben-Arie N, Eatock RA, et al. Math1: an essential gene for the generation of inner ear hair cells. *Science*. 1999; 284:1837–1841. [PubMed: 10364557]
6. Nechiporuk A, Raible DW. FGF-dependent mechanosensory organ patterning in zebrafish. *Science*. 2008; 320:1774–1777. [PubMed: 18583612]
7. Sarrazin AF, Villablanca EJ, Nunez VA, Sandoval PC, Ghysen A, Allende ML. Proneural gene requirement for hair cell differentiation in the zebrafish lateral line. *Dev Biol*. 2006; 295:534–545. [PubMed: 16678150]
8. Ben-Arie N, Hassan BA, Bermingham NA, Malicki DM, Armstrong D, Matzuk M, et al. Functional conservation of atonal and Math1 in the CNS and PNS. *Development*. 2000; 127:1039–1048. [PubMed: 10662643]
9. Maricich SM, Wellnitz SA, Nelson AM, Lesniak DR, Gerling GJ, Lumpkin EA, et al. Merkel cells are essential for light-touch responses. *Science*. 2009; 324:1580–1582. [PubMed: 19541997]
10. Jarman AP. Studies of mechanosensation in *Drosophila*. *Human Molecular Genetics*. 2002:11.
11. Fritzsch B, Eberl DF, Beisel KW. The role of bHLH genes in ear development and evolution: revisiting a 10-year-old hypothesis. *Cell Mol Life Sci*. 2010; 67:3089–3099. [PubMed: 20495996]
12. Arendt D, Tessmar-Raible K, Snyman H, Dorresteyn AW, Wittbrodt J. Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science*. 2004; 306:869–871. [PubMed: 15514158]
13. Niwa N, Hiromi Y, Okabe M. A conserved developmental program for sensory organ formation in *Drosophila melanogaster*. *Nature Genetics*. 2004; 36:293–297. [PubMed: 14981517]
14. zur Lage PI, Prentice DRA, Holohan EE, Jarman AP. The *Drosophila* proneural gene *amos* promotes olfactory sensillum formation and suppresses bristle formation. *Development*. 2003; 130:4683–4689. [PubMed: 12925594]
15. Zhao C, Emmons SW. A transcription factor controlling development of peripheral sense organs in *C. elegans*. *Nature*. 1995; 373:74–78. [PubMed: 7800042]
16. Senthilan PR, Piepenbrock D, Ovezmyradov G, Nadrowski B, Bechstedt S, Pauls S, et al. *Drosophila* auditory organ genes and genetic hearing defects. *Cell*. 2012; 150:1042–1054. [PubMed: 22939627]
17. Wang VY, Hassan BA, Bellen HJ, Zoghbi HY. *Drosophila atonal* fully rescues the phenotype of Math1 null mice: new functions evolve in new cellular contexts. *Curr Biol*. 2002; 12:1611–1616. [PubMed: 12372255]
18. Eberl DF. Feeling the vibes: chordotonal mechanisms in insect hearing. *Current Opinion in Neurobiology*. 1999; 9:389–393. [PubMed: 10448164]
19. Jarman AP, Sun Y, Jan LY, Jan YN. Role of the proneural gene, *atonal*, in formation of *Drosophila* chordotonal organs and photoreceptors. *Development*. 1995; 121:2019–2030. [PubMed: 7635049]
20. Eberl DF, Hardy RW, Kernan MJ. Genetically similar transduction mechanisms for touch and hearing in *Drosophila*. *J Neurosci*. 2000; 20:5981–5988. [PubMed: 10934246]
21. Caldwell JC, Miller MM, Wing S, Soll DR, Eberl DF. Dynamic analysis of larval locomotion in *Drosophila* chordotonal organ mutants. *PNAS*. 2003; 100:16053–16058. [PubMed: 14673076]
22. Okabe M, Okano H. Two-step induction of chordotonal organ precursors in *Drosophila* embryogenesis. *Development*. 1997; 124:1045–1053. [PubMed: 9056779]
23. zur Lage P, Jan YN, Jarman AP. Requirement for EGF receptor signalling in neural recruitment during formation of *Drosophila* chordotonal sense organ clusters. *Current Biology*. 1997; 7:166–175. [PubMed: 9395407]

24. zur Lage P, Jarman AP. Antagonism of EGFR and Notch signalling in the reiterative recruitment of *Drosophila* adult chordotonal sense organ precursors. *Development*. 1999; 126:3149–3157. [PubMed: 10375505]
25. Sun Y, Jan LY, Jan YN. Transcriptional regulation of *atonal* during development of the *Drosophila* peripheral nervous system. *Development*. 1998; 125:3731–3740. [PubMed: 9716538]
26. zur Lage PI, Powell LM, Prentice DR, McLaughlin P, Jarman AP. EGF receptor signaling triggers recruitment of *Drosophila* sense organ precursors by stimulating proneural gene autoregulation. *Dev Cell*. 2004; 7:687–696. [PubMed: 15525530]
27. Acar M, Jafar-Nejad H, Giagtzoglou N, Yallampalli S, David G, He Y, et al. Senseless physically interacts with proneural proteins and functions as a transcriptional co-activator. *Development*. 2006; 133:1979–1989. [PubMed: 16624856]
28. Powell LM, Deaton AM, Wear MA, Jarman AP. The specificity of Atonal and Scute bHLH factors: analysis of cognate E box binding sites and the influence of Senseless. *Genes to Cells*. 2008; 13:915–929. [PubMed: 18681894]
29. Zhang T, Ranade S, Cai CQ, Clouser C, Pignoni F. Direct control of neurogenesis by selector factors in the fly eye: regulation of *atonal* by *Ey* and *So*. *Development*. 2006; 133:4881–4889. [PubMed: 17108002]
30. Tanaka-Matakatsu M, Du W. Direct control of the proneural gene *atonal* by retinal determination factors during *Drosophila* eye development. *Dev Biol*. 2008; 313:787–801. [PubMed: 18083159]
31. Domínguez M. Dual role for Hedgehog in the regulation of the proneural gene *atonal* during ommatidia development. *Development*. 1999; 126:2345–2353. [PubMed: 10225994]
32. Suzuki T, Saigo K. Transcriptional regulation of *atonal* required for *Drosophila* larval eye development by concerted action of *eyes absent*, *sine oculis* and *hedgehog* signaling independent of fused kinase and *cubitus interruptus*. *Development*. 2000; 127:1531–1540. [PubMed: 10704398]
33. Melicharek D, Shah A, DiStefano G, Gangemi AJ, Orapallo A, Vrillas-Mortimer AD, et al. Identification of novel regulators of *atonal* expression in the developing *Drosophila* retina. *Genetics*. 2008; 180:2095–2110. [PubMed: 18832354]
34. Powell LM, zur Lage PI, Prentice DRA, Senthinathan B, Jarman AP. The proneural proteins Atonal and Scute regulate neural target genes through different E-box binding sites. *Mol Cell Biol*. 2004; 24:9517–9526. [PubMed: 15485919]
35. Maung SM, Jarman AP. Functional distinctness of closely related transcription factors: a comparison of the Atonal and Amos proneural factors. *Mech Dev*. 2007; 124:647–656. [PubMed: 17709231]
36. Aerts S, Quan XJ, Claeys A, Sanchez MN, Tate P, Yan J, et al. Robust target gene discovery through transcriptome perturbations and genome-wide enhancer predictions in *Drosophila* uncovers a regulatory basis for sensory specification. *PLoS Biology*. 2010; 8:1–18.
37. Cachero S, Simpson TI, zur Lage PI, Ma L, Newton FG, Holohan EE, et al. The gene regulatory cascade linking proneural specification with differentiation in *Drosophila* sensory neurons. *PLoS Biol*. 2011; 9:e1000568. [PubMed: 21283833]
38. Laurençon A, Dubruille R, Efimenko E, Grenier G, Bissett R, Cortier E, et al. Identification of novel regulatory factor X (RFX) target genes by comparative genomics in *Drosophila* species. *Genome Biology*. 2007; 8 R: 195.
39. Newton FG, zur Lage P, Karak S, Moore D, Göpfert MC, Jarman AP. The forkhead domain factor, Fd3F, cooperates with Rfx to regulate a program of sensory ciliary specialisation. *Dev Cell*. 2012; 22:1221–1233. [PubMed: 22698283]
40. Ma L, Jarman AP. Dilatory is a *Drosophila* protein related to AZI1 (CEP131) that is located at the ciliary base and required for cilium formation. *J Cell Sci*. 2011; 124:2622–2630. [PubMed: 21750193]
41. Yu X, Lau D, Ng CP, Roy S. Cilia-driven fluid flow as an epigenetic cue for otolith biomineralization on sensory hair cells of the inner ear. *Development*. 2011; 138:487–494. [PubMed: 21205793]
42. Shen WL, Kwon Y, Adegbola AA, Luo J, Chess A, Montell C. Function of rhodopsin in temperature discrimination in *Drosophila*. *Science*. 2011; 331:1333–1336. [PubMed: 21393546]

43. Kwon Y, Shen WL, Shim HS, Montell C. Fine thermotactic discrimination between the optimal and slightly cooler temperatures via a TRPV channel in chordotonal neurons. *J Neurosci.* 2010; 30:10465–10471. [PubMed: 20685989]
44. Hassan BA, Bellen HJ. Doing the MATH: is the mouse a good model for fly development? *Genes Dev.* 2000; 14:1852–1865. [PubMed: 10921900]
45. Kelley MW. Regulation of cell fate in the sensory epithelia of the inner ear. *Nat Rev Neurosci.* 2006; 7:837–849. [PubMed: 17053809]
46. Millimaki BB, Sweet EM, Dhasan MS, Riley BB. Zebrafish *atoh1* genes: classic proneural activity in the inner ear and regulation by Fgf and Notch. *Development.* 2007; 134:295–305. [PubMed: 17166920]
47. Riley BB, Zhu C, Janetopoulos C, Aufderheide KJ. A critical period of ear development controlled by distinct populations of ciliated cells in the zebrafish. *Dev Biol.* 1997; 191:191–201. [PubMed: 9398434]
48. Itoh M, Chitnis AB. Expression of proneural and neurogenic genes in the zebrafish lateral line primordium correlates with selection of hair cell fate in neuromasts. *Mech Dev.* 2001; 102:263–266. [PubMed: 11287207]
49. Whitfield TT, Riley BB, Chiang MY, Phillips B. Development of the zebrafish inner ear. *Dev Dyn.* 2002; 223:427–458. [PubMed: 11921334]
50. Sweet EM, Vemaraju S, Riley BB. Sox2 and Fgf interact with Atoh1 to promote sensory competence throughout the zebrafish inner ear. *Dev Biol.* 2011; 358:113–121. [PubMed: 21801718]
51. Chen P, Johnson JE, Zoghbi HY, Segil N. The role of Math1 in inner ear development: Uncoupling the establishment of the sensory primordium from hair cell fate determination. *Development.* 2002; 129:2495–2505. [PubMed: 11973280]
52. Pan N, Jahan I, Kersigo J, Kopecky B, Santi P, Johnson S, et al. Conditional deletion of Atoh1 using Pax2-Cre results in viable mice without differentiated cochlear hair cells that have lost most of the organ of Corti. *Hear Res.* 2011; 275:66–80. [PubMed: 21146598]
53. Gubbels SP, Woessner DW, Mitchell JC, Ricci AJ, Brigande JV. Functional auditory hair cells produced in the mammalian cochlea by in utero gene transfer. *Nature.* 2008; 455:537–541. [PubMed: 18754012]
54. Zheng JL, Gao WQ. Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears. *Nat Neurosci.* 2000; 3:580–586. [PubMed: 10816314]
55. Kelly MC, Chang Q, Pan A, Lin X, Chen P. Atoh1 directs the formation of sensory mosaics and induces cell proliferation in the postnatal Mammalian cochlea in vivo. *J Neurosci.* 2012; 32:6699–6710. [PubMed: 22573692]
56. Raft S, Koundakjian EJ, Quinones H, Jayasena CS, Goodrich LV, Johnson JE, et al. Cross-regulation of Ngn1 and Math1 coordinates the production of neurons and sensory hair cells during inner ear development. *Development.* 2007; 134:4405–4415. [PubMed: 18039969]
57. Lumpkin EA, Collisson T, Parab P, Omer-Abdalla A, Haeberle H, Chen P, et al. Math1-driven GFP expression in the developing nervous system of transgenic mice. *Gene Expr Patterns.* 2003; 3:389–395. [PubMed: 12915300]
58. Woods C, Montcouquiol M, Kelley MW. Math1 regulates development of the sensory epithelium in the mammalian cochlea. *Nat Neurosci.* 2004; 7:1310–1318. [PubMed: 15543141]
59. Mulvaney J, Dabdoub A. Atoh1, an Essential Transcription Factor in Neurogenesis and Intestinal and Inner Ear Development: Function, Regulation, and Context Dependency. *J Assoc Res Otolaryngol.* 2012
60. Flora A, Klisch TJ, Schuster G, Zoghbi HY. Deletion of Atoh1 disrupts Sonic Hedgehog signaling in the developing cerebellum and prevents medulloblastoma. *Science.* 2009; 326:1424–1427. [PubMed: 19965762]
61. Yang H, Xie X, Deng M, Chen X, Gan L. Generation and characterization of Atoh1-Cre knock-in mouse line. *Genesis.* 2010; 48:407–413. [PubMed: 20533400]
62. Driver EC, Pryor SP, Hill P, Turner J, Ruther U, Biesecker LG, et al. Hedgehog signaling regulates sensory cell formation and auditory function in mice and humans. *J Neurosci.* 2008; 28:7350–7358. [PubMed: 18632939]

63. Hayashi T, Ray CA, Bermingham-McDonogh O. Fgf20 is required for sensory epithelial specification in the developing cochlea. *J Neurosci.* 2008; 28:5991–5999. [PubMed: 18524904]
64. Huh SH, Jones J, Warchol ME, Ornitz DM. Differentiation of the lateral compartment of the cochlea requires a temporally restricted FGF20 signal. *PLoS Biol.* 2012; 10:e1001231. [PubMed: 22235191]
65. Kamaid A, Neves J, Giraldez F. Id gene regulation and function in the prosensory domains of the chicken inner ear: a link between Bmp signaling and Atoh1. *J Neurosci.* 2010; 30:11426–11434. [PubMed: 20739564]
66. Li H, Corrales CE, Wang Z, Zhao Y, Wang Y, Liu H, et al. BMP4 signaling is involved in the generation of inner ear sensory epithelia. *BMC Dev Biol.* 2005; 5:16. [PubMed: 16107213]
67. Pirvola U, Ylikoski J, Trokovic R, Hebert JM, McConnell SK, Partanen J. FGFR1 is required for the development of the auditory sensory epithelium. *Neuron.* 2002; 35:671–680. [PubMed: 12194867]
68. Pujades C, Kamaid A, Alsina B, Giraldez F. BMP-signaling regulates the generation of hair-cells. *Dev Biol.* 2006; 292:55–67. [PubMed: 16458882]
69. Stevens CB, Davies AL, Battista S, Lewis JH, Fekete DM. Forced activation of Wnt signaling alters morphogenesis and sensory organ identity in the chicken inner ear. *Dev Biol.* 2003; 261:149–164. [PubMed: 12941626]
70. Helms AW, Abney AL, Ben-Arie N, Zoghbi HY, Johnson JE. Autoregulation and multiple enhancers control Math1 expression in the developing nervous system. *Development.* 2000; 127:1185–1196. [PubMed: 10683172]
71. Doetzelhofer A, Basch ML, Ohshima T, Gessler M, Groves AK, Segil N. Hey2 regulation by FGF provides a Notch-independent mechanism for maintaining pillar cell fate in the organ of Corti. *Dev Cell.* 2009; 16:58–69. [PubMed: 19154718]
72. Zheng JL, Shou J, Guillemot F, Kageyama R, Gao WQ. Hes1 is a negative regulator of inner ear hair cell differentiation. *Development.* 2000; 127:4551–4560. [PubMed: 11023859]
73. Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, et al. Control of endodermal endocrine development by Hes-1. *Nat Genet.* 2000; 24:36–44. [PubMed: 10615124]
74. Kim TH, Shivdasani RA. Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression. *J Biol Chem.* 2011; 286:11427–11433. [PubMed: 21282114]
75. Shi F, Cheng YF, Wang XL, Edge AS. Beta-catenin up-regulates Atoh1 expression in neural progenitor cells by interaction with an Atoh1 3' enhancer. *J Biol Chem.* 2010; 285:392–400. [PubMed: 19864427]
76. Mutoh H, Sakamoto H, Hayakawa H, Arao Y, Satoh K, Nokubi M, et al. The intestine-specific homeobox gene Cdx2 induces expression of the basic helix-loop-helix transcription factor Math1. *Differentiation.* 2006; 74:313–321. [PubMed: 16831200]
77. Ebert PJ, Timmer JR, Nakada Y, Helms AW, Parab PB, Liu Y, et al. Zic1 represses Math1 expression via interactions with the Math1 enhancer and modulation of Math1 autoregulation. *Development.* 2003; 130:1949–1959. [PubMed: 12642498]
78. Akazawa C, Ishibashi M, Shimizu C, Nakanishi S, Kageyama R. A mammalian helix-loop-helix factor structurally related to the product of *Drosophila* proneural gene *atonal* is a positive transcriptional regulator expressed in the developing nervous system. *J Biol Chem.* 1995; 270:8730–8738. [PubMed: 7721778]
79. Briggs KJ, Eberhart CG, Watkins DN. Just say no to ATOH: how HIC1 methylation might predispose medulloblastoma to lineage addiction. *Cancer Res.* 2008; 68:8654–8656. [PubMed: 18974104]
80. D'Angelo A, Bluteau O, Garcia-Gonzalez MA, Gresh L, Doyen A, Garbay S, et al. Hepatocyte nuclear factor 1alpha and beta control terminal differentiation and cell fate commitment in the gut epithelium. *Development.* 2010; 137:1573–1582. [PubMed: 20388655]
81. Ahmed M, Wong EY, Sun J, Xu J, Wang F, Xu PX. Eya1-Six1 interaction is sufficient to induce hair cell fate in the cochlea by activating Atoh1 expression in cooperation with Sox2. *Dev Cell.* 2012; 22:377–390. [PubMed: 22340499]

82. Neves J, Uchikawa M, Bigas A, Giraldez F. The prosensory function of Sox2 in the chicken inner ear relies on the direct regulation of Atoh1. *PLoS One*. 2012; 7:e30871. [PubMed: 22292066]
83. Ahmed M, Xu J, Xu PX. EYA1 and SIX1 drive the neuronal developmental program in cooperation with the SWI/SNF chromatin-remodeling complex and SOX2 in the mammalian inner ear. *Development*. 2012; 139:1965–1977. [PubMed: 22513373]
84. Burns JC, On D, Baker W, Collado MS, Corwin JT. Over Half the Hair Cells in the Mouse Utricle First Appear After Birth, with Significant Numbers Originating from Early Postnatal Mitotic Production in Peripheral and Striolar Growth Zones. *J Assoc Res Otolaryngol*. 2012
85. Kirkegaard M, Nyengaard JR. Stereological study of postnatal development in the mouse utricular macula. *J Comp Neurol*. 2005; 492:132–144. [PubMed: 16196029]
86. Montcouquiol M, Kelley MW. Planar and vertical signals control cellular differentiation and patterning in the mammalian cochlea. *J Neurosci*. 2003; 23:9469–9478. [PubMed: 14561877]
87. Jahan I, Pan N, Kersigo J, Fritzsche B. Neurod1 suppresses hair cell differentiation in ear ganglia and regulates hair cell subtype development in the cochlea. *PLoS One*. 2010; 5:e11661. [PubMed: 20661473]
88. Matei V, Pauley S, Kaing S, Rowitch D, Beisel KW, Morris K, et al. Smaller inner ear sensory epithelia in Neurog 1 null mice are related to earlier hair cell cycle exit. *Dev Dyn*. 2005; 234:633–650. [PubMed: 16145671]
89. Liu Z, Owen T, Zhang L, Zuo J. Dynamic expression pattern of Sonic hedgehog in developing cochlear spiral ganglion neurons. *Dev Dyn*. 2010; 239:1674–1683. [PubMed: 20503364]
90. Riccomagno MM, Martinu L, Mulheisen M, Wu DK, Epstein DJ. Specification of the mammalian cochlea is dependent on Sonic hedgehog. *Genes Dev*. 2002; 16:2365–2378. [PubMed: 12231626]
91. Klisch TJ, Xi Y, Flora A, Wang L, Li W, Zoghbi HY. In vivo Atoh1 targetome reveals how a proneural transcription factor regulates cerebellar development. *Proc Natl Acad Sci U S A*. 2011; 108:3288–3293. [PubMed: 21300888]
92. Chen Z, Montcouquiol M, Calderon R, Jenkins NA, Copeland NG, Kelley MW, et al. Jxc1/Sobp, encoding a nuclear zinc finger protein, is critical for cochlear growth, cell fate, and patterning of the organ of corti. *J Neurosci*. 2008; 28:6633–6641. [PubMed: 18579736]
93. Lai HC, Klisch TJ, Roberts R, Zoghbi HY, Johnson JE. In vivo neuronal subtype-specific targets of Atoh1 (Math1) in dorsal spinal cord. *J Neurosci*. 2011; 31:10859–10871. [PubMed: 21795538]
94. Ma Q, Chen Z, del Barco Barrantes I, de la Pompa JL, Anderson DJ. neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. *Neuron*. 1998; 20:469–482. [PubMed: 9539122]
95. Jahan I, Pan N, Kersigo J, Calisto LE, Morris KA, Kopecky B, et al. Expression of Neurog1 instead of Atoh1 can partially rescue organ of Corti cell survival. *PLoS One*. 2012; 7:e30853. [PubMed: 22292060]
96. Lumpkin EA, Marshall KL, Nelson AM. The cell biology of touch. *J Cell Biol*. 2010; 191:237–248. [PubMed: 20956378]
97. Morrison KM, Miesegaes GR, Lumpkin EA, Maricich SM. Mammalian Merkel cells are descended from the epidermal lineage. *Dev Biol*. 2009; 336:76–83. [PubMed: 19782676]
98. Haeberle H, Bryan LA, Vadakkan TJ, Dickinson ME, Lumpkin EA. Swelling-activated Ca²⁺ channels trigger Ca²⁺ signals in Merkel cells. *PLoS One*. 2008; 3:e1750. [PubMed: 18454189]
99. Haeberle H, Lumpkin EA. Merkel Cells in Somatosensation. *Chemosens Percept*. 2008; 1:110–118. [PubMed: 19834574]
100. Van Keymeulen A, Mascré G, Youseff KK, Harel I, Michaux C, De Geest N, et al. Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. *J Cell Biol*. 2009; 187:91–100. [PubMed: 19786578]
101. Elferink LA, Strick DJ. Functional properties of rab15 effector protein in endocytic recycling. *Methods Enzymol*. 2005; 403:732–743. [PubMed: 16473635]
102. Emery G, Hutterer A, Berdnik D, Mayer B, Wirtz-Peitz F, Gaitan MG, et al. Asymmetric Rab 11 endosomes regulate delta recycling and specify cell fate in the Drosophila nervous system. *Cell*. 2005; 122:763–773. [PubMed: 16137758]

103. Reeves MA, Bellinger FP, Berry MJ. The neuroprotective functions of selenoprotein M and its role in cytosolic calcium regulation. *Antioxid Redox Signal*. 2010; 12:809–818. [PubMed: 19769485]
104. Groves AK. The challenge of hair cell regeneration. *Exp Biol Med (Maywood)*. 2010; 235:434–446. [PubMed: 20407075]
105. Ma EY, Raible DW. Signaling pathways regulating zebrafish lateral line development. *Curr Biol*. 2009; 19:R381–R386. [PubMed: 19439264]
106. Roberson DF, Weisleder P, Bohrer PS, Rubel EW. Ongoing production of sensory cells in the vestibular epithelium of the chick. *Hear Res*. 1992; 57:166–174. [PubMed: 1733910]
107. Cafaro J, Lee GS, Stone JS. Atoh1 expression defines activated progenitors and differentiating hair cells during avian hair cell regeneration. *Dev Dyn*. 2007; 236:156–170. [PubMed: 17096404]
108. Ma EY, Rubel EW, Raible DW. Notch signaling regulates the extent of hair cell regeneration in the zebrafish lateral line. *J Neurosci*. 2008; 28:2261–2273. [PubMed: 18305259]
109. Lewis RM, Hume CR, Stone JS. Atoh1 expression and function during auditory hair cell regeneration in post-hatch chickens. *Hear Res*. 2012; 289:74–85. [PubMed: 22543087]
110. Stone JS, Rubel EW. Delta1 expression during avian hair cell regeneration. *Development*. 1999; 126:961–973. [PubMed: 9927597]
111. Lin V, Golub JS, Nguyen TB, Hume CR, Oesterle EC, Stone JS. Inhibition of Notch activity promotes nonmitotic regeneration of hair cells in the adult mouse utricles. *J Neurosci*. 2011; 31:15329–15339. [PubMed: 22031879]
112. Baker K, Brough DE, Staecker H. Repair of the vestibular system via adenovector delivery of Atoh1: a potential treatment for balance disorders. *Adv Otorhinolaryngol*. 2009; 66:52–63. [PubMed: 19494572]
113. Husseman J, Raphael Y. Gene therapy in the inner ear using adenovirus vectors. *Adv Otorhinolaryngol*. 2009; 66:37–51. [PubMed: 19494571]
114. Kawamoto K, Izumikawa M, Beyer LA, Atkin GM, Raphael Y. Spontaneous hair cell regeneration in the mouse utricle following gentamicin ototoxicity. *Hear Res*. 2009; 247:17–26. [PubMed: 18809482]
115. Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, et al. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. *Nat Med*. 2005; 11:271–276. [PubMed: 15711559]
116. Liu Z, Dearman JA, Cox BC, Walters BJ, Zhang L, Ayrault O, et al. Age-dependent in vivo conversion of mouse cochlear pillar and deiters' cells to immature hair cells by atoh1 ectopic expression. *J Neurosci*. 2012; 32:6600–6610. [PubMed: 22573682]
117. Jones JM, Montcouquiol M, Dabdoub A, Woods C, Kelley MW. Inhibitors of differentiation and DNA binding (Ids) regulate Math1 and hair cell formation during the development of the organ of Corti. *J Neurosci*. 2006; 26:550–558. [PubMed: 16407553]
118. Sancho-Martinez I, Baek SH, Izpisua Belmonte JC. Lineage conversion methodologies meet the reprogramming toolbox. *Nat Cell Biol*. 2012; 14:892–899. [PubMed: 22945254]

Chordotonal neurons

Figure 1.

Mechanosensory cells that require *atonal* family genes. (A) The *Drosophila* Ch neuron. A Ch organ consists of 1–2 neurons and several support cells. One of the latter forms the characteristic scolopale ‘basket’ that houses the distal part of the Ch neuron dendrites. The monopolar dendrites terminate in a specialized mechanosensory cilium with functionally distinct zones. (B) The vertebrate hair cell. The mechanosensory apparatus consists of microvilli-based stereocilia. The cells also bear a true cilium (kinocilium). In cochlear hair cells the kinocilium may be required developmentally to organize the stereocilia although it later degenerates. In vestibular hair cells the kinocilium is retained, but its role in mechanosensation is not clear. Hair cells contact sensory neurons of the spiral ganglion. The sensory neurons require the bHLH factor, Neurog1, rather than Atoh1.

Figure 2.

A diagram of the mouse *Atoh1* locus, showing the position of its 3' autoregulatory enhancer. Numbers above elements on the diagram refer to the position on mouse chromosome 6, according to the current build of the mouse genome (mm10 / GRCm38). The *Atoh1* autoregulatory enhancer consists of two conserved elements, A and B, with features of these enhancers numbered in parentheses relative to the start of enhancer A. Transcription factor binding sites that have been experimentally verified are shown in color on relevant regions of the enhancer sequence, with the remaining unverified but predicted sites shown in black.