Seminars in Cell & Developmental Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear

Kaidi D. Zhang*, Thomas M. Coate

Department of Biology, Georgetown University, Washington, DC, USA

ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 12 August 2016
Accepted 25 September 2016
Available online xxx

Keywords:
Spiral ganglion
Cochlea
Inner ear
Hair cell
Afferent innervation

ABSTRACT

In hearing, mechanically sensitive hair cells (HCs) in the cochlea release glutamate onto spiral ganglion neurons (SGNs) to relay auditory information to the central nervous system (CNS). There are two main SGN subtypes, which differ in morphology, number, synaptic targets, innervation patterns and firing properties. About 90–95% of SGNs are the type I SGNs, which make a single bouton connection with inner hair cells (IHCs) and have been well described in the canonical auditory pathway for sound detection. However, less attention has been given to the type II SGNs, which exclusively innervate outer hair cells (OHCs). In this review, we emphasize recent advances in the molecular mechanisms that control how type II SGNs develop and form connections with OHCs, and exciting new insights into the function of type II SGNs.

Contents

1. Introduction

The auditory sensory epithelium in mammals, the organ of Corti, is composed of a spectacularly arranged set of specialized epithelial cells called HCs and supporting cells (SCs). As illustrated in Fig. 1, the organ of Corti is comprised of one row of IHCs and three (occasionally four) rows of OHCs that are embedded in a series of diverse supporting cells, all of which serve distinct roles in hearing function [1]. IHCs encode acoustic information by transducing

Abbreviations: CNS, central nervous system; DPOAE, distortion product otoacoustic emissions; E, embryonic day; EPSC, excitatory postsynaptic currents; EPSP, excitatory postsynaptic potentials; GER, greater epithelial ridge; HC, hair cell; IHC, inner hair cell; OHC, outer hair cell; P, postnatal day; SC, supporting cell; SGN, spiral ganglion neuron.

* Corresponding author at: Regents Hall Room 411, Georgetown University, 37th and O St. NW, Washington, DC, 20007, USA.
E-mail address: kz115@georgetown.edu (K.D. Zhang).

http://dx.doi.org/10.1016/j.semcdb.2016.09.017
1084-9521/

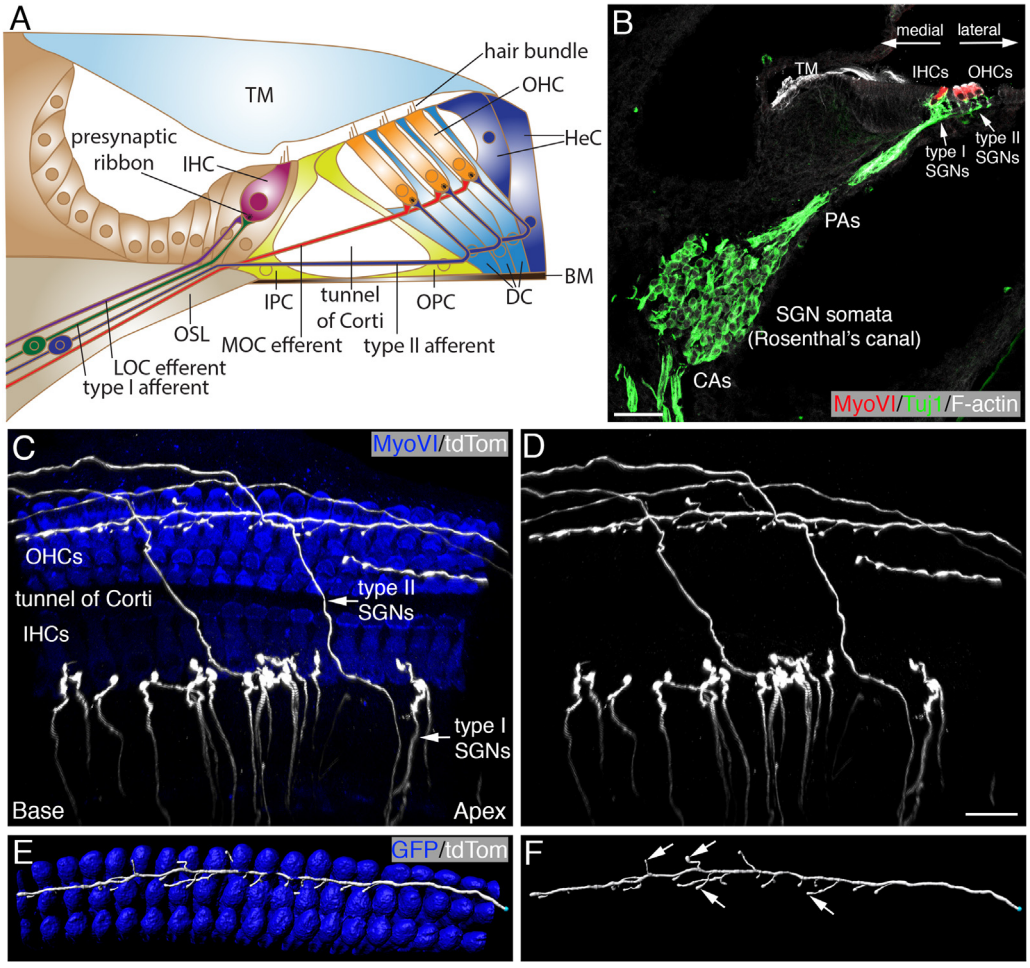

Fig. 1. Innervation of the organ of Corti. (A) A cartoon of the mature organ of Corti. There is one row of IHCs and three rows of OHCs separated by the tunnel of Corti. The organ of Corti is innervated by two types of afferents (type I and type II) and two types of efferents (MOC and LOC). OSL: osseous spiral lamina. LOC: lateral olivocochlear neuron. MOC: medial olivocochlear neuron. IPC: inner pillar cell. OPC: outer pillar cell. DC: Deiters' cell. BM: basilar membrane. HeC: Hensen's cell. TM: tectorial membrane. (B) A cross-sectional image of a P0 mouse cochlea at the base. The SGNs are labeled with an antibody against Tuj1 (green), a class III β-tubulin, and hair cells are labeled with an antibody against Myosin VI (red). Phallodin labeling of F-Actin (white) marks hair cell bundles, SGNs and TM. The majority of SGNs are type I SGNs innervating IHCs, while the rest SGNs are type II SGNs making connections with OHCs. PAs: peripheral axons. CAs: central axons. Scale bar: 50 μm. (C and D) A whole-mount image of a P8 cochlea from a mouse carrying *Neurog1-Cre*^{ERT2}, *R26R-tdTomato* and *Atoh1-GFP* [42,96]. Sparse numbers of labeled SGNs are detected using an anti-dsRED antibody that binds to tdTomato (white). Hair cells are labeled with Myosin VI (blue). Each type I SGN has an unbranched peripheral axon contacting a single IHC. Type II SGN processes pass through the tunnel of Corti, turn towards the base and form *en passant* contacts with OHCs. (E and F) 3D reconstruction of a type II SGN process (white) and OHCs (blue) in B. OHCs are reconstructed with GFP expressed by *Atoh1-GFP* (psuedocolored blue). Arrows point to a few examples of *en passant* contacts between the type II SGN and OHCs. Scale bar in C–F: 15 μm.

mechanical stimuli into electrochemical signals, which are relayed by type I SGNs into the brainstem and ascending auditory pathways [2]. OHCs, on the other hand, mediate a process known as "cochlear amplification," in which voltage driven somatic elongation and contraction and active hair bundle motility promote sound amplification and frequency selectivity [3–7].

In terms of nerve supply, the organ of Corti receives innervation for both afferent input and efferent feedback. Afferent innervations arise from SGN somata (Fig. 1B) located in Rosenthal's canal in the cochlea. SGNs are bipolar or pseudounipolar neurons with peripheral axons (Fig. 1B; "PA") terminating at HCs and central axons (Fig. 1B; "CA") projecting into the cochlear nuclei within the brainstem. SGN peripheral axons cross the osseous spiral lamina before passing through the habenula perforata to enter the organ of Corti [8]. We will discuss the two types of SGNs, type I and type II, in detail in the following sections. Although it will not be elaborated upon here, the SGNs along the tonotopic axis show clear distinctions in terms of physiological firing properties and the expression of synaptic proteins and channels [9,10]. Thus, beyond the known type I and type II populations, there must be additional SGN subtypes (or gradients of types) yet to be fully characterized. There are also two classes of cochlear efferent innervations (Fig. 1A), both of which provide inhibitory and excitatory feedback [11]. Unmyelinated lateral olivocochlear efferent neurons form "axodendritic" synapses with type I SGNs underneath IHCs, and myelinated medial olivocochlear efferent neurons form axosomatic synapses with OHCs [12,13]. The cochlear efferent system ultimately plays an important role in many auditory functions, including protection from damaging noise and sound discrimination in noisy backgrounds. Cochlear efferent modulation of the auditory system has been reviewed recently [13–17].

Hearing loss is one of the most common health issues in the United States affecting at least 15% of adults [18] and often involves a loss of hair cell or spiral ganglion neuron function. The current common treatment of hearing loss includes hearing aids for patients with functional HCs and cochlear implants for patients with profound or complete hearing HC loss and mostly intact SGNs. In a cochlear implant, an electrode array substitutes for IHCs in transmitting electrical impulses to the auditory nerve. In both cases, functional SGNs are indispensable in sending information from

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017

either HCs or the electrode array to the CNS. Therefore, it is necessary to understand the development and patterning of SGNs, so perhaps the neural circuitry in the ear can be maintained or regenerated after impairment. The development of type I SGNs has been discussed in a few recent reviews [10,19–24] and in a recently published book "The Primary Auditory Neurons of the Mammalian Cochlea" [25]. In this review, we touch on some well-known and recently discovered elements of type I SGNs, but most of our attention is devoted toward some recent and exciting findings related to type II SGNs.

1.1. Neuroanatomical features of type I SGNs

Type I SGNs represent 90–95% of the total SGN population and are thus responsible for the vast majority of hearing input. Each type I SGN extends one unbranched peripheral process, or "radial fiber," which forms a single ribbon synapse with one IHC (Fig. 1). Each IHC is innervated by a total of 6 to 20 type I SGNs in the mature mouse cochlea [26]. Each type I SGN also extends a long central projection that shows a remarkable stereotyped branching pattern, which is dependent on the receptor guanylyl cyclase Npr2 [27]. One branch extends into the anteroventral cochlear nucleus and the second branch crosses the posteroventral cochlear nucleus to terminate at the dorsal cochlear nucleus [8]. The branch directed toward the antereoventral cochlear nucleus forms a large synapse with globular bushy cells called "the endbulb of Held," a structure that is highly conserved from birds to mammals [8,28]. One fascinating neuroanatomical aspect of type I SGNs (which is similar to other sensory neurons) is that their axons are insulated by both of the major classes of myelinating glia: Schwann cells ensheathe the SGN peripheral and central axons in the inner ear and oligodendrocytes ensheathe the central axons after their passage through the internal auditory meatus [29]. There are some anatomical differences among mammalian species, which was nicely discussed in Nayagam *et al.*, 2011 [8].

Classic work by Liberman in cats demonstrated that type I SGNs can be further subdivided into two types based on where they terminate on IHCs. SGNs projecting onto the medial side of IHCs are reported to have low spontaneous discharge rates, high thresholds and relatively thin peripheral fibers in comparison with SGNs terminating on the lateral side of IHCs [30,31]. Interestingly, studies in mouse models have suggested that, despite these morphological and physiological differences, ribbon sizes do not differ between the lateral and medial side of the IHCs [26]. Overall, relatively little is known about how different subtypes of type I SGNs vary in function.

1.2. Neuroanatomical features of type II SGNs

The type II SGNs are a fascinating population of neurons that constitutes only 5–10% of the total SGN population. During development, their peripheral processes cross the tunnel of Corti to the OHC region, then turn toward the cochlear base and make *en passant* synaptic connections with anywhere from 5 to 30 OHCs (in mouse). The number of type II-OHC contacts in different species is summarized in Weisz *et al.*, 2012 [32]. The portion of the type II SGNs that extends basally in the OHC region has been classically referred to as "outer spiral fibers" (Fig. 1) and each OHC is innervated by 2–5 type II SGNs [33,34]. Interestingly, because of their turning and synapse formation with more basal OHCs, type II SGNs have higher characteristic frequencies than IHCs that neighbor them in Rosenthal's canal. Peripheral processes of type II SGNs are unmyelinated and thin, but can extend hundreds of micrometers along the cochlear spiral [8]. Morphologically, type II SGNs actually resemble pain-sensing sensory fibers from the dorsal root ganglion [8,35], which is especially interesting given new findings on their function in auditory nociception (see Section 4.2 here). Central processes of type II SGNs project into the cochlear nucleus together with type I SGNs and extend into both the anteroventral and dorsal cochlear nuclei and also in the granule cell region [36–38]. Interestingly, whereas type II SGNs have emerged in mammals during evolution, they appear to be absent in the avian basilar papilla [39], suggesting they are a mammalian auditory system specialization.

2. Establishment of cochlear innervation patterns with focus on type II SGNs

Specification of neuronal fate in the otocyst is dependent on transcriptional regulation by Sox2, Neurogenin 1 and others. These events along with other early events, such as SGN migration, lamination and early process outgrowth have been reviewed recently [10,19]. Here, we touch briefly on type II SGN origins, then focus on the mechanisms that control how peripheral processes of type I and type II SGNs explore the organ of Corti and synapse with their HC targets.

2.1. Origins, timing and specification of type II SGN – questions mostly unanswered

In the mouse cochlea, the exploration of SGN processes within the sensory domain starts at the base at embryonic day 14.5 (E14.5) and progresses towards the apex, similar to the timing of HC and SC differentiation [40,41]. There is then a prolonged phase of type I and type II SGN axon growth, guidance, synapse formation and pruning that occurs up until the onset of hearing around postnatal day 10 (P10). The number of presynaptic ribbons in OHCs decreases dramatically from P3 to P12 [33]. Where do type II SGNs come from and when do they arise? It is tempting to think type II SGNs may arise from an origin such as neural crest, given their morphological and functional similarities to pain-sensing C-fibers (Section 4.2). But, fate-mapping studies have showed type I and II SGNs arise from a common pool of neural precursors derived from the otocyst [42]. These studies also demonstrated that type II SGNs are visible by E16.5 so it is reasonable to predict their differentiation occurs around the time of sensory domain innervation (E14.5) or possibly earlier. Auditory neurons become visibly separated from vestibular neurons as early as E10.5 [43] and are molecularly distinct from them as early as E12.5 [44]. So, it is somewhere in this window when we would expect type II SGNs to become specified, but the precise time point has not been determined. Another, and perhaps more important mystery is what factor(s) differentiate type II SGNs away from the type I fate. As described below, the type II SGNs show some distinctions in expression patterns and early developmental behaviors, but any sort of transcriptional cascade associated with their delineation remains unknown. Recently, it was found that the transcriptomes from single cells from cochlear and vestibular epithelial tissues could be characterized [45] and similar technology will be needed to identify factors that determine type II SGN identity.

2.2. Mechanisms implicated in the establishment of type I vs. type II innervation patterns

The developmental mechanisms controlling selective HC innervation in the cochlea have been a topic of study by others and us over the past few decades. Echteler discovered originally (in the neonatal gerbil) that SGN peripheral axons innervate both IHCs and OHCs simultaneously and then undergo periods of pruning and refinement during the first postnatal week, which generates the adult innervation patterns [46]. In addition, increased activity of apoptosis before hearing onset seems to play a role in decreasing

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017

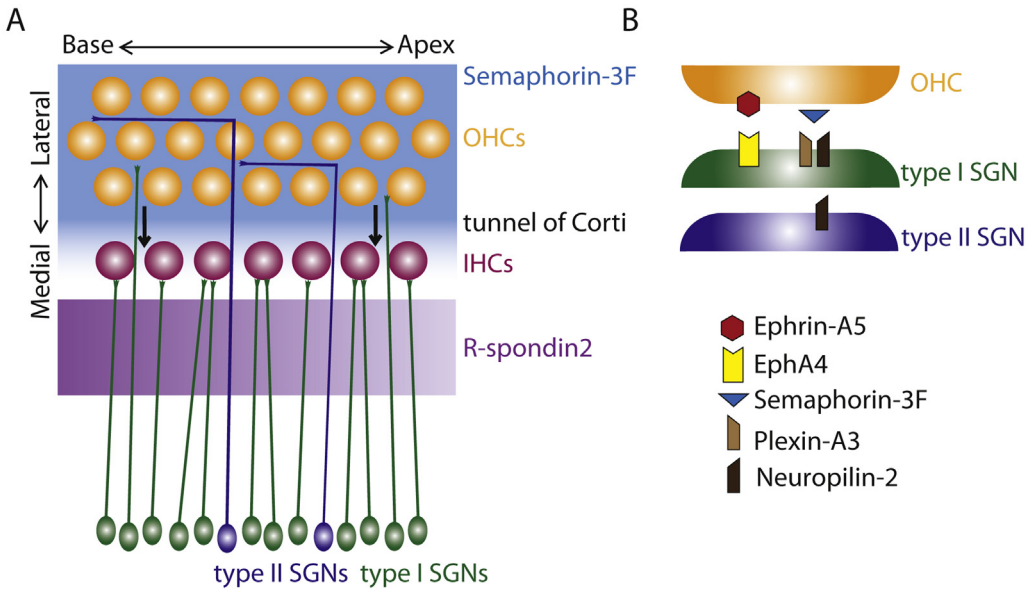

Fig. 2. A model of development of cochlear afferent innervation. (A) During late embryonic stages, SGN peripheral processes explore the organ of Corti and make connections with HCs. Some type I SGNs (green) enter the OHC (orange) region and retract (black arrows) during this stage. In the end of the exploration, they will form ribbon synapses with IHCs (magenta). Type II SGNs (blue) cross the tunnel of Corti, enter the OHC region and turn toward the base. Later, they will form *en passant* synapses with multiple OHCs. Semaphorin-3F (light blue) is secreted by OHCs and SCs in the lateral domain and is hypothesized to form a gradient across the organ of Corti. There is also a gradient of R-spondin2 (purple) along the cochlea with high level at the base. Central axons of SGNs are not shown in the cartoon. (B) Ephrin-A5 (red), present in the OHC membrane, can cause type I SGNs with EphA4 receptors (yellow) to retract from the OHC region. Semaphorin-3F (blue) secreted by OHCs and SCs leads to retraction of type I SGNs with both Plexin-A3 (light brown) and Neuropilin-2 (dark brown), but does not affect type II SGNs with only Neuropilin-2 co-receptor.

type II SGN numbers and refining SGN neurites [47,48]. In mouse, it was also shown that type I SGNs and OHCs form transient ribbon synapses between P0 and P3, most of which disperse around P6 and are drastically reduced by P12 [33]. This suggested that postnatal synapse elimination might play an important role in establishing the mature cochlear innervation pattern. However, SGN labeling in mouse using *Neurog1*^{CreERT2} determined that the "mature" innervation pattern, where only a small minority of SGN fibers was present in the OHC region, was apparent by P0 [42]. Recently, we followed up on this work [40] using the same Cre line and demonstrated that type I retraction from the OHC region (see black arrows in Fig. 2) mainly occurs between E15.5 and E18.5, which decreases the percentage of SGN fibers in the OHC region to 5–10% by P0. Time-lapse experiments using semi-intact E16.5 cochleae provided additional evidence for an active retraction process, while apoptotic cell death was negligible compared to total SGN numbers. Furthermore, Druckenbrod and colleagues found that, at P0, few branches of type I SGNs contact OHCs, with more off-target branches contacting pillar cells or were positioned close to IHCs but ending more toward the apical surface [49]. Type I SGNs were morphologically identified by their clear radial peripheral processes. Their time-lapse studies at E16 and E18 suggest that retraction of off-target branches plays a dominant role in refinement of SGN processes. Overall, these data demonstrate that SGNs have established their innervation patterns as type I or type II near the time of birth in mice.

From a development and signaling standpoint, it is now clear that type II SGNs are programmed differently compared to their type I counterparts. Recent 3D reconstructions of confocal z-stacks show that type I and type II processes differ along the scala media-scala tympani axis: type I processes locate closer to the scala media, whereas branches of type II processes are positioned closer to the scala tympani [49]. These new findings confirm previous observations by Berglund and Ryugo and others [33,50]. During development, type II branches that are located closer to the scala media are more likely to retract from OHCs [49]. The model of branch retraction of SGN peripheral processes raises the question: what are the underlying mechanisms? One possible mechanism is the presence of cell-surface or secreted guidance repellents that either keep type I SGN processes away from the OHC region or keep type II SGN processes away from the IHC region. In support of the idea that the OHC region represents an inhibitory zone for type I SGN processes, Defourney and colleagues showed that the repulsive ligand Ephrin-A5 is expressed in OHCs between E18 and P4 [51], whereas its receptor EphA4 was shown possibly to be present on the cell surface of type I SGNs. Loss of *Efna5* or *Epha4* led to a "radial shift" of type I SGN processes into the OHC region by P14, suggesting that Ephrin-A5 normally repels type I SGNs that express EphA4, preventing them from entering the OHC region [51] (Fig. 2). To highlight the complexity of Eph/Ephrin signaling in the ear, at a younger age (E16.5–E18.5), EphA4 protein is exclusively expressed in otic mesenchyme and binds with Ephrin-B2 on SGN peripheral processes to promote SGN fasciculation [52]. We reported recently a second and conceptually similar mechanism involving Semaphorin-3F, which is expressed in the lateral domain of the organ of Corti including OHCs, Dieters' cells, outer pillar cells and Hensen's cells [40]. Semaphorin-3F is secreted and diffusible. Therefore, we predict that it acts in a gradient across the organ of Corti where it becomes progressively less concentrated in the IHC region (Fig. 2). Cochleae lacking *Sema3f* or its receptor *Nrp2* (expressed by SGNs) also had increased number of SGN processes in the OHC region at P0-P1, suggesting Semaphorin-3F/Neuropilin-2-mediated repulsion normally restricts type I SGNs to the IHC region [40].

But, what is it about type II SGNs that allows them to bypass these repulsive cues present in the OHC region? Type II SGNs appear to lack EphA4 altogether, which prevents them from being repelled by Ephrin-A5 [51] (Fig. 2). Interestingly, Neuropilin-2 is expressed by both type I and type II SGNs, raising the question of why type II SGNs are not responsive to Semaphorin-3F. Plexin-A3, a required

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017

co-receptor for Nrp2, was found to be strongly expressed in type I SGN processes, but only faintly detectable in type II SGNs [40]. Thus, type II SGNs may not be sensitive to Semaphorin-3F because they lack the ability to mediate its repulsive signal. Since both Ephrin-A5 and Semaphorin-3F prevent type I SGN neurites from existing in the OHC region, it will be important to determine if the two factors act redundantly. What keeps type II SGNs from innervating IHCs is an entirely open question and no clues have emerged at this point. One important message from these studies, however, is that type I and II SGNs do have some different "molecular signatures," which supports the idea that they are specified differently during development.

In addition to epithelial surface cues, it is clear that SC and HC morphogenesis is also required for normal innervation patterns. *Fgfr3* knockout mice show a higher number of type II-like fibers, which accompanies an absence of inner pillar cells, and increased numbers and differentiation defects in both OHCs and Deiters' cells [53]. This phenotype suggests that FGF signaling may normally promote the expression of repulsive ligands laterally in the OHC region. In addition, the ablation of pillar and Deiters' cells by induced expression of diphtheria toxin fragment A at P0-P1 results in fewer and disorganized type II fibers in the OHC region by P8 (and occurs before OHC loss) [54]. This result highlights the importance of SCs in that they are likely a source of either trophic or structural support for type II SGNs. But, it is possible that type II SGN loss occurs secondarily to defects in OHC metabolism or viability that results from the loss of SCs. In either case, it is clear that SCs are paramount for the maintenance of both OHCs and type II SGN connections.

2.3. Mechanisms underlying turning of type II SGNs toward the base

One fascinating question about type II SGNs is the set of pathfinding mechanisms that controls the coordinated turning of their peripheral axons toward the base (Fig. 2). The cochlear duct elongates, for the most part, from base to apex [55]. If peripheral processes of type II SGNs simply followed this elongation, they would turn toward the apex. Therefore, the turning of type II SGNs toward the base should be a directed process. One secreted signaling molecule, R-spondin2 (Rspo2), has been shown necessary for the establishment of this feature of cochlear innervation [56]. *Rspo2* is expressed in the greater epithelial ridge (GER; medial to IHCs) with a base-to-apex gradient (high at the base) at E17.5 (Fig. 2). Loss of *Rspo2* results in disorganized type II SGNs and an improperly formed organ of Corti at the cochlear apex. However, *Rspo2* null cochleae at the base show a normal organ of Corti and some type II fibers turning toward the apex, which suggests Rspo2 may act directly on type II SGNs and that the innervation defects do not occur secondarily as a result of primary defects in epithelial patterning. Rspo2 is an Lgr receptor ligand that augments canonical Wnt signaling [57], but it is unknown which Lgr receptors are expressed by SGNs. Interestingly, while we do not know whether Wnt signaling is directly involved in mammalian cochlear innervation, studies done in chick show that treating statoacoustic ganglion neurons with Wnt ligands or inhibitors has no effect on axon outgrowth [58]. It has also been reported that SGNs express the homeobox protein Prox1 during neurite extension around E13.5-P0 and that the conditional loss of *Prox1* in SGNs disrupts type II innervation patterns [59]. In this case, the peripheral processes of type II SGNs turn randomly toward the apex or the base, or aberrantly bifurcate. Future studies should investigate possible transcriptional targets of Prox1 that may encode cell-surface receptors or other guidance cues that control growth cone attraction toward the base, or repulsion away from the apex.

3. Distinguishing features of synapses connecting OHCs and type II SGNs

Both IHCs and OHCs have presynaptic ribbons, which are electron-dense bodies surrounded by vesicles that facilitate glutamate release from HCs to postsynaptic SGNs (Figs. 1, 3). Whereas the IHC ribbon is well studied [20,23,28,60–62], less is known about synapses between OHCs and type II SGNs. In terms of ribbon numbers, the difference by adulthood is stark: IHCs have about 20 CtBP2-positive ribbons, and OHCs have an average of only 3 [33]. OHCs appear to start out with abundant ribbon bodies, but through pruning mediated by thyroid hormone signaling and likely other events [63], the number of ribbons in OHCs reduces by about 80% from P6 to P10 [64]. Transmission electron microscopy studies of P7-P9 rat cochleae revealed that the dense body volume of ribbons is similar between IHCs and OHCs, although there are significant differences in ribbon shape, with IHCs having shorter and wider ribbons [32]. In addition, it appears that OHC/type II synapses show substantially fewer vesicles overall near the ribbon body compared to their IHC/type I counterparts [32]. For example, experiments in the P3 gerbil cochlea suggested that immature OHCs have 135–143 vesicles in their readily releasable pool with about 8 vesicles per ribbon, in comparison with 400–500 readily releasable vesicles per IHC and 11–14 vesicles per IHC ribbon [65]. These morphological data support the conventional wisdom that, on a per-cell basis, OHCs play a more limited role in transducing acoustic signals compared to IHCs. In addition to these morphological variations, the composition of glutamate receptors may also be different between postsynaptic type I and type II afferents [33,66]. Kainate-type glutamate receptors GluK2 and GluK5 are expressed in both type I and type II SGNs [66]. AMPA-type glutamate receptors are present in type I afferents throughout life, but were previously shown to be downregulated in type II afferents just prior to hearing [33]. This implied that perhaps type II SGNs primarily used kainate receptors as opposed to AMPA receptors to transmit OHC input. However, more recent histological and physiological findings [34] showed that AMPA receptors are present at the OHC-type II synapse in the adult rat cochlea. Pharmacology experiments done in young rats suggested that glutamatergic excitation in type II SGNs is mainly mediated by AMPA receptors, and that a role for kainate receptors is unclear. Notably, about half of the postsynaptic densities in type II afferents were negative for GluA2 immunoreactivity and lacked a presynaptic ribbon. The authors suggested that these "ribbonless" contacts may be involved in cochlear long-term plasticity by converting into ribbon synapses after OHC damage.

4. Newly emerging concepts in the function of type II SGNs

Historically, determining the physiological properties of type II SGNs has been difficult because of their scarcity compared to type I SGNs, and because it is difficult to record from their thin, unmyelinated fibers [67–69]. Some of the initial indications of differences of type II SGNs came from whole-cell patch clamp recordings of their cell bodies in cochlear slices, which showed a significant difference in membrane properties compared to type Is [70]. In addition, recording of dissociated neurons *in vitro* revealed that type II SGNs have lower action potential thresholds and slow kinetics compared to type Is [71]. Together, these findings suggest that type II SGNs may not be involved in frequency coding. In the following sections, we discuss new insights into functional roles of type II SGNs.

4.1. Activation of type II SGNs by glutamate released from OHCs

Although there were notable differences compared to IHC firing properties, Weisz and colleagues found that type II SGNs can

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017

indeed be activated by OHC depolarization and glutamate release [32,72,73]. An important conclusion from their work, and one that may be related to more recent findings on pain sensation, is that the activation of a type II afferent results only after its entire presynaptic OHC pool is depolarized by very strong input. Using a preparation in which peripheral axons of type II SGNs were recorded from an excised cochlea, the authors found that excitatory postsynaptic currents (EPSCs) could be blocked by glutamate receptor and voltage-gated $Ca_V1.3$ channel antagonists, indicating that type II SGNs are excited by glutamate released from OHCs [72]. The evoked EPSCs and spontaneous EPSCs had similar amplitudes, suggesting each EPSC is evoked by one single vesicle released from one OHC. This contrasts sharply with an immature IHC, which releases about 50 vesicles during one action potential and is thus significantly more effective [20,74]. Follow up computational modeling revealed that excitatory postsynaptic potentials (EPSPs) evoked simultaneously by neurotransmitter release from six OHCs are necessary to reach the threshold (25 mV) to evoke an action potential [73]. Considering the low vesicle release probability of OHCs, one type II afferent may actually need to connect with a pool of 24 OHCs that are all stimulated simultaneously [32]. However, since the OHCs in the experiments were activated by increased extracellular potassium concentration or mechanical stimulation, it is unclear whether sounds can actually be loud enough to depolarize OHCs and activate type II SGNs without causing tissue damage. One additional question is whether type II afferents in the adult cochlea are able to respond to OHC depolarization and neurotransmitter release, as all the findings mentioned here were from rat cochleae before P20.

What is the function of type II SGN activation *in vivo*? A recent study by Froud *et al.* proposes an interesting model that type II SGNs activate the medial olivocochlear efferent system to suppress cochlear amplification [75]. In this study, the authors used *Prph* (encoding Peripherin, a type III intermediate filament protein) null mice, which apparently lack all type II SGNs, but maintain normal auditory brainstem response thresholds indicating normal type I function. Using distortion product otoacoustic emission (DPOAE)-based measurements, the authors found that *Prph* null mice showed diminished contralateral and ipsilateral olivocochlear suppression, suggesting defects in efferent reflex and suppression of cochlear amplification. However, in contrast with the previously stated hypothesis that type II SGNs are only activated by very loud sounds, the sound stimuli used in the DPOAE tests in this report were only 82 dB. Further, the response latencies of medial olivocochlear neurons are very short (5 ms) [76], which conflicts with reports of slow impulse conduction in unmyelinated type II fibers [67–69]. In addition, since Peripherin is expressed in type I SGNs during development [77] and possibly also in cochlear efferents, the *Prph* null mice may have subtle defects in type I SGN synaptogenesis and auditory transmission that may not have been detectable by auditory brainstem response thresholds. Although this study suggests a fascinating model, future investigations will certainly be needed to clarify some of these issues.

4.2. Auditory nociception

There is now growing convincing evidence that type II SGNs can be activated by extracellular ATP after mechanical trauma and noise exposure. Immunostaining studies show that the ATP-gated ion channel $P2X_2$ is present in the synaptic regions of type I and type II SGNs beneath IHCs and OHCs [78,79], suggesting that SGNs may respond to extracellular ATP. Application of ATP to dissected cochleae not only depolarizes OHCs and indirectly evokes EPSCs in type II afferents, but also evokes an inward current to depolarize type II afferents [72,80]. Upon mechanical trauma, ATP is released by damaged HCs, generating intracellular calcium increase

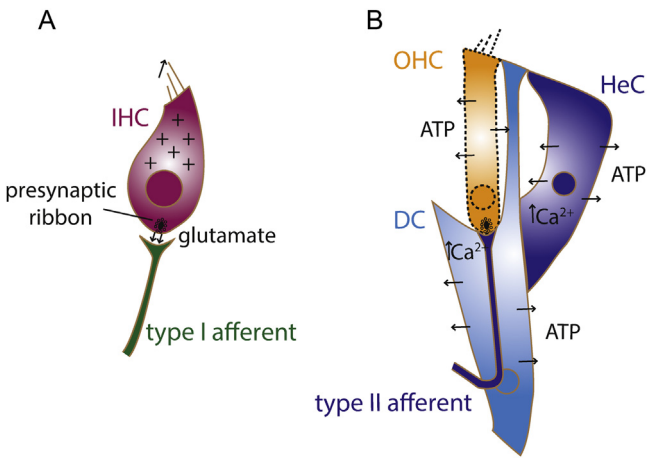

Fig. 3. Activation of type I and type II SGNs. (A) IHCs are depolarized when their hair bundles are displaced in response to sound stimuli. Presynaptic vesicles around ribbons release glutamate to activate postsynaptic type I SGNs. (B) ATP released by damaged OHCs leads to a propagation of Ca^{2+} wave among both HCs and SCs (only DC and HeC are shown here). Increased intracellular Ca^{2+} triggers ATP release from SCs. Extracellular ATP activates type II SGNs. Presynaptic ribbons are also present in the OHCs. DC: Deiters' cell. HeC: Hensen's cell.

and ATP release among surrounding HCs and SCs (Fig. 3B) [81–84], which may directly activate type II SGNs. It has been shown previously that, after loud noise exposure, cochlear stria vascularis cells release ATP into the endolymph, leading to a significant increase in ATP concentration in scala media [85]. Elevated ATP in endolymph could theoretically trigger OHC depolarization and indirectly activate type II afferents. One noteworthy observation is that there is an increase in both mRNA and protein levels of $P2X_2$ receptor in organ of Corti and SGNs after noise exposure [86].

Two recent studies provide direct evidence that type II SGNs are involved in a response termed "auditory nociception" (Fig. 3B) [80,87]. In one study, Flores *et al.* made use of *Vglut3* (vesicular glutamate transporter 3) null mice, in which IHCs fail to release glutamate and thus cannot activate type I SGNs [87]. Interestingly, noxious noise exposure destroyed most OHCs and activated cochlear nucleus neurons even in the absence of *Vglut3* (when type I SGNs are not functional). From their results, the activation of cochlear nuclei was not observed in degenerated cochleae with massive HC loss and did not seem to be mediated by somatosensory or vestibular afferents, which strongly supported the possibility that type II SGNs mediate a response to noxious noise and OHC damage. In support of this idea, the activation of the granule cell region of the cochlear nucleus (normally innervated by type II SGNs [36–38]) was not affected by the loss of *Vglut3* [87]. This strongly supports the idea that type II SGNs, but not type I SGNs, can serve a role in pain sensation within the auditory system. A second study by Liu *et al.* revealed that the function of type II SGNs in auditory nociception is mediated by extracellular ATP mainly released by SCs after HC damage [80]. In this study, the authors mechanically ablated HCs and detected slow activation of type II SGNs, which was dependent on both ATP-gated P2X receptors and G-protein-coupled P2Y receptors. The application of a connexin hemichannel antagonist prevented ATP release from SCs and led to shortened type II activation. What's especially interesting about this is how type II afferents and somatic C fiber nociceptors (whose cell bodies are housed in dorsal root ganglion) share common characteristics [20,80,87]. C fibers are also small in diameter, unmyelinated, activated by ATP released from damaged tissue [88], and inhibited by KCNQ activator retigabine that also can suppress type II afferents [89]. Thus, upon cochlear tissue damage, type II SGN afferents likely function very similarly to pain-sensing neurons of the mammalian somatosensory system. Although it is pure speculation, perhaps

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017

over evolutionary time it became advantageous for type II SGNs to extend toward the base to make preferential contact with the more basal OHCs, which are most vulnerable to noise damage and aging [90–93]. Possible auditory nociception connectivity patterns in the CNS need further investigation, as it is uncertain what kind of pain noxious noise exposure generates (earache or headache) [87], and to what extent there may be connectivity with somatosensory centers in the CNS. No matter what kind of pain is generated, auditory nociception may help mammals avoid painful noise and thus protect the cochlea, given the limited capacity for HCs to regenerate [94].

5. Summary and perspectives for future research

Overall, current work has begun to unveil a few of the mysteries related to type II SGNs in the mammalian cochlea. As described in Section 2, we have made considerable strides in understanding the mechanisms controlling the selective innervation of different HC regions by type I and type II SGNs. Perhaps not surprisingly, two of the most well-known axon guidance families (ephrins and semaphorins) are involved in this process. In addition, from genetic labeling studies, we know that type I and II SGNs may share a common progenitor during development [42]. But, the unique transcriptional networks that determine type I *vs*. type II fates still need to be discovered. A greater understanding of these specification events would certainly benefit any efforts to regenerate SGNs in a damaged cochlea. To further these investigations, it will be imperative to develop specific markers that distinguish type I *vs*. type II SGNs during development – tools that presently do not exist. It is known that postnatal type II SGNs can be labeled by anti-peripherin immunostaining, but peripherin is expressed by both type I and type II SGNs before birth [77,95]. From a developmental standpoint, there are also several other questions that remain, like what differences in extracellular cues facilitate the myelination of type I SGNs, but not type IIs? Also, how are SGN guidance events coupled to HC and SC maturation events (as noted in Section 2.1)? From the standpoint of function, and as described in Section 4, we now have convincing evidence that type II SGNs are involved in auditory nociception and may serve a protective function in noxious sound environments. But, what is not entirely clear is the function of the glutamatergic ribbon synapses that connect type II SGNs and OHCs. Answers to these questions, as well as others, should be achievable in the coming decade and will certainly enhance our overall understanding of auditory development and function.

Acknowledgements

We are grateful to N.A. Spita for the original image in Fig. 1C. We thank P.A. Fuchs (John Hopkins University) and C.J. Weisz (NIH) for critical reading of this manuscript. Funding: NIHDC13107 to T.M.C.

References

[1] P.A. Fuchs, Oxford Handbook of Auditory Science: The Ear, Oxford University Press, 2010.
[2] T.G. Nienhuys, G.M. Clark, Frequency discrimination following the selective destruction of cochlear inner and outer hair cells, Science 199 (4335) (1978) 1356–1357.
[3] A.J. Hudspeth, Integrating the active process of hair cells with cochlear function, Nat. Rev. Neurosci. 15 (9) (2014) 600–614.
[4] J. Ashmore, Cochlear outer hair cell motility, Physiol. Rev. 88 (1) (2008) 173–210.
[5] A.J. Hudspeth, Making an effort to listen: mechanical amplification in the ear, Neuron 59 (4) (2008) 530–545.
[6] P. Dallos, Cochlear amplification, outer hair cells and prestin, Curr. Opin. Neurobiol. 18 (4) (2008) 370–376.
[7] R. Fettiplace, C.M. Hackney, The sensory and motor roles of auditory hair cells, Nat. Rev. Neurosci. 7 (1) (2006) 19–29.
[8] B.A. Nayagam, M.A. Muniak, D.K. Ryugo, The spiral ganglion: connecting the peripheral and central auditory systems, Hear. Res. 278 (1–2) (2011) 2–20.
[9] S.H. Green, E. Bailey, Q. Wang, R.L. Davis, The Trk A, B, C's of neurotrophins in the cochlea, Anat. Rec. (Hoboken) 295 (11) (2012) 1877–1895.
[10] T.M. Coate, M.W. Kelley, Making connections in the inner ear: recent insights into the development of spiral ganglion neurons and their connectivity with sensory hair cells, Semin. Cell Dev. Biol. 24 (5) (2013) 460–469.
[11] J.A. Groff, M.C. Liberman, Modulation of cochlear afferent response by the lateral olivocochlear system: activation via electrical stimulation of the inferior colliculus, J. Neurophysiol. 90 (5) (2003) 3178–3200.
[12] D.D. Simmons, Development of the inner ear efferent system across vertebrate species, J. Neurobiol. 53 (2) (2002) 228–250.
[13] R.R. Ciuman, The efferent system or olivocochlear function bundle – fine regulator and protector of hearing perception, Int. J. Biomed. Sci. 6 (4) (2010) 276–288.
[14] U.J. Sienknecht, C. Köppl, B. Fritzsch, Evolution and development of hair cell polarity and efferent function in the inner ear, Brain Behav. Evol. 83 (2) (2014) 150–161.
[15] R.D. Rabbitt, W.E. Brownell, Efferent modulation of hair cell function, Curr. Opin. Otolaryngol. Head Neck Surg. 19 (5) (2011) 376–381.
[16] E. Wersinger, P.A. Fuchs, Modulation of hair cell efferents, Hear. Res. 279 (1–2) (2011) 1–12.
[17] D. Ryugo, R. Fay, A. Popper, Auditory and Vestibular Efferents, 1 ed., Springer-Verlag, New York, 2011.
[18] D.L. Blackwell, J.W. Lucas, T.C. Clarke, Summary health statistics for U.S. adults: national health interview survey, 2012, Vital Health Stat. 10 (260) (2014) 1–161.
[19] L. Delacroix, B. Malgrange, Cochlear afferent innervation development, Hear. Res. 330 (Pt. B) (2015) 157–169.
[20] P.A. Fuchs, E. Glowatzki, Synaptic studies inform the functional diversity of cochlear afferents, Hear. Res. 330 (Pt. A) (2015) 18–25.
[21] A.V. Bulankina, T. Moser, Neural circuit development in the mammalian cochlea, Physiology (Bethesda) 27 (2) (2012) 100–112.
[22] A.C. Meyer, T. Moser, Structure and function of cochlear afferent innervation, Curr. Opin. Otolaryngol. Head Neck Surg. 18 (5) (2010) 441–446.
[23] C. Wichmann, T. Moser, Relating structure and function of inner hair cell ribbon synapses, Cell Tissue Res. 361 (1) (2015) 95–114.
[24] D.O. Reijntjes, S.J. Pyott, The afferent signaling complex: regulation of type I spiral ganglion neuron responses in the auditory periphery, Hear. Res. 336 (2016) 1–16.
[25] A. Dabdoub, B. Fritzsch, A.N. Popper, R.R. Fay, The Primary Auditory Neurons of the Mammalian Cochlea, 1 ed., Springer, New York, 2016.
[26] A.C. Meyer, T. Frank, D. Khimich, G. Hoch, D. Riedel, N.M. Chapochnikov, Y.M. Yarin, B. Harke, S.W. Hell, A. Egner, T. Moser, Tuning of synapse number, structure and function in the cochlea, Nat. Neurosci. 12 (4) (2009) 444–453.
[27] C.C. Lu, X.J. Cao, S. Wright, L. Ma, D. Oertel, L.V. Goodrich, Mutation of Npr2 leads to blurred tonotopic organization of central auditory circuits in mice, PLoS Genet. 10 (12) (2014) e1004823.
[28] W.M. Yu, L.V. Goodrich, Morphological and physiological development of auditory synapses, Hear. Res. 311 (2014) 3–16.
[29] E.J. Jeon, N. Xu, L. Xu, M.R. Hansen, Influence of central glia on spiral ganglion neuron neurite growth, Neuroscience 177 (2011) 321–334.
[30] M.C. Liberman, Single-neuron labeling in the cat auditory nerve, Science 216 (4551) (1982) 1239–1241.
[31] M.C. Liberman, M.E. Oliver, Morphometry of intracellularly labeled neurons of the auditory nerve: correlations with functional properties, J. Comp. Neurol. 223 (2) (1984) 163–176.
[32] C.J. Weisz, M. Lehar, H. Hiel, E. Glowatzki, P.A. Fuchs, Synaptic transfer from outer hair cells to type II afferent fibers in the rat cochlea, J. Neurosci. 32 (28) (2012) 9528–9536.
[33] L.C. Huang, M. Barclay, K. Lee, S. Peter, G.D. Housley, P.R. Thorne, J.M. Montgomery, Synaptic profiles during neurite extension, refinement and retraction in the developing cochlea, Neural Dev. 7 (2012) 38.
[34] R. Martinez-Monedero, C. Liu, C. Weisz, P. Vyas, P.A. Fuchs, E. Glowatzki, GluA2-containing AMPA receptors distinguish ribbon-associated from ribbonless afferent contacts on rat cochlear hair cells, eNeuro 3 (2) (2016).
[35] L. Li, M. Rutlin, V.E. Abraira, C. Cassidy, L. Kus, S. Gong, M.P. Jankowski, W. Luo, N. Heintz, H.R. Koerber, C.J. Woodbury, D.D. Ginty, The functional organization of cutaneous low-threshold mechanosensory neurons, Cell 147 (7) (2011) 1615–1627.
[36] A.M. Berglund, M.C. Brown, Central trajectories of type II spiral ganglion cells from various cochlear regions in mice, Hear. Res. 75 (1–2) (1994) 121–130.
[37] M.C. Brown, A.M. Berglund, N.Y. Kiang, D.K. Ryugo, Central trajectories of type II spiral ganglion neurons, J. Comp. Neurol. 278 (4) (1988) 581–590.
[38] M.C. Brown, J.V. Ledwith, Projections of thin (type-II) and thick (type-I) auditory-nerve fibers into the cochlear nucleus of the mouse, Hear. Res. 49 (1–3) (1990) 105–118.
[39] D.K. Ryugo, T.N. Parks, Primary innervation of the avian and mammalian cochlear nucleus, Brain Res. Bull. 60 (5–6) (2003) 435–456.
[40] T.M. Coate, N.A. Spita, K.D. Zhang, K.T. Isgrig, M.W. Kelley, Neuropilin-2/semaphorin-3F-mediated repulsion promotes inner hair cell innervation by spiral ganglion neurons, Elife 4 (2015).
[41] A.K. Groves, K.D. Zhang, D.M. Fekete, The genetics of hair cell development and regeneration, Annu. Rev. Neurosci. 36 (2013) 361–381.

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017

[42] E.J. Koundakjian, J.L. Appler, L.V. Goodrich, Auditory neurons make stereotyped wiring decisions before maturation of their targets, J. Neurosci. 27 (51) (2007) 14078–14088.
[43] L.L. Sandell, N.E. Butler Tjaden, A.J. Barlow, P.A. Trainor, Cochleovestibular nerve development is integrated with migratory neural crest cells, Dev. Biol. 385 (2) (2014) 200–210.
[44] C.C. Lu, J.M. Appler, E.A. Houseman, L.V. Goodrich, Developmental profiling of spiral ganglion neurons reveals insights into auditory circuit assembly, J. Neurosci. 31 (30) (2011) 10903–10918.
[45] J.C. Burns, M.C. Kelly, M. Hoa, R.J. Morell, M.W. Kelley, Single-cell RNA-Seq resolves cellular complexity in sensory organs from the neonatal inner ear, Nat. Commun. 6 (2015) 8557.
[46] S.M. Echteler, Developmental segregation in the afferent projections to mammalian auditory hair cells, Proc. Natl. Acad. Sci. U. S. A. 89 (14) (1992) 6324–6327.
[47] S.M. Echteler, T. Magardino, M. Rontal, Spatiotemporal patterns of neuronal programmed cell death during postnatal development of the gerbil cochlea, Brain Res. Dev. Brain Res. 157 (2) (2005) 192–200.
[48] M. Barclay, A.F. Ryan, G.D. Housley, Type I vs type II spiral ganglion neurons exhibit differential survival and neuritogenesis during cochlear development, Neural Dev. 6 (2011) 33.
[49] N.R. Druckenbrod, L.V. Goodrich, Sequential retraction segregates SGN processes during target selection in the cochlea, J. Neurosci. 35 (49) (2015) 16221–16235.
[50] A.M. Berglund, D.K. Ryugo, Hair cell innervation by spiral ganglion neurons in the mouse, J. Comp. Neurol. 255 (4) (1987) 560–570.
[51] J. Defourny, A.L. Poirrier, F. Lallemend, S. Mateo Sánchez, J. Neef, P. Vanderhaeghen, E. Soriano, C. Peuckert, K. Kullander, B. Fritzsch, L. Nguyen, G. Moonen, T. Moser, B. Malgrange, Ephrin-A5/EphA4 signalling controls specific afferent targeting to cochlear hair cells, Nat. Commun. 4 (2013) 1438.
[52] T.M. Coate, S. Raft, X. Zhao, A.K. Ryan, E.B. Crenshaw 3rd, M.W. Kelley, Otic mesenchyme cells regulate spiral ganglion axon fasciculation through a Pou3f4/EphA4 signaling pathway, Neuron 73 (1) (2012) 49–63.
[53] C. Puligilla, F. Feng, K. Ishikawa, S. Bertuzzi, A. Dabdoub, A.J. Griffith, B. Fritzsch, M.W. Kelley, Disruption of fibroblast growth factor receptor 3 signaling results in defects in cellular differentiation, neuronal patterning, and hearing impairment, Dev. Dyn. 236 (7) (2007) 1905–1917.
[54] M.M. Mellado Lagarde, B.C. Cox, J. Fang, R. Taylor, A. Forge, J. Zuo, Selective ablation of pillar and deiters' cells severely affects cochlear postnatal development and hearing in mice, J. Neurosci. 33 (4) (2013) 1564–1576.
[55] J. Wang, S. Mark, X. Zhang, D. Qian, S.J. Yoo, K. Radde-Gallwitz, Y. Zhang, X. Lin, A. Collazo, A. Wynshaw-Boris, P. Chen, Regulation of polarized extension and planar cell polarity in the cochlea by the vertebrate PCP pathway, Nat. Genet. 37 (9) (2005) 980–985.
[56] J.F. Mulvaney, A. Yatteau, W.W. Sun, B. Jacques, K. Takubo, T. Suda, W. Yamada, A. Dabdoub, Secreted factor R-spondin 2 is involved in refinement of patterning of the mammalian cochlea, Dev. Dyn. 242 (2) (2013) 179–188.
[57] V. Munnamalai, D.M. Fekete, Wnt signaling during cochlear development, Semin. Cell Dev. Biol. 24 (5) (2013) 480–489.
[58] K.N. Fantetti, Y. Zou, D.M. Fekete, Wnts and Wnt inhibitors do not influence axon outgrowth from chicken statoacoustic ganglion neurons, Hear. Res. 278 (1–2) (2011) 86–95.
[59] B. Fritzsch, M. Dillard, A. Lavado, N.L. Harvey, I. Jahan, Canal cristae growth and fiber extension to the outer hair cells of the mouse ear require Prox1 activity, PLoS One 5 (2) (2010) e9377.
[60] S. Safieddine, A. El-Amraoui, C. Petit, The auditory hair cell ribbon synapse: from assembly to function, Annu. Rev. Neurosci. 35 (2012) 509–528.
[61] F. Schmitz, The making of synaptic ribbons: how they are built and what they do, Neuroscientist 15 (6) (2009) 611–624.
[62] P.A. Fuchs, E. Glowatzki, T. Moser, The afferent synapse of cochlear hair cells, Curr. Opin. Neurobiol. 13 (4) (2003) 452–458.
[63] S. Sundaresan, S. Balasubbu, M. Mustapha, Thyroid hormone is required for the pruning of afferent type II spiral ganglion neurons in the mouse cochlea, Neuroscience 312 (2016) 165–178.
[64] H.M. Sobkowicz, J.E. Rose, G.L. Scott, C.V. Levenick, Distribution of synaptic ribbons in the developing organ of Corti, J. Neurocytol. 15 (6) (1986) 693–714.
[65] S.L. Johnson, C. Franz, M. Knipper, W. Marcotti, Functional maturation of the exocytotic machinery at gerbil hair cell ribbon synapses, J. Physiol. 587 (Pt. 8) (2009) 1715–1726.
[66] T. Fujikawa, R.S. Petralia, T.S. Fitzgerald, Y.X. Wang, B. Millis, J.A. Morgado-Díaz, K. Kitamura, B. Kachar, Localization of kainate receptors in inner and outer hair cell synapses, Hear. Res. 314 (2014) 20–32.
[67] D. Robertson, Horseradish peroxidase injection of physiologically characterized afferent and efferent neurones in the guinea pig spiral ganglion, Hear. Res. 15 (2) (1984) 113–121.
[68] D. Robertson, P.M. Sellick, R. Patuzzi, The continuing search for outer hair cell afferents in the guinea pig spiral ganglion, Hear. Res. 136 (1–2) (1999) 151–158.
[69] M.C. Brown, Antidromic responses of single units from the spiral ganglion, J. Neurophysiol. 71 (5) (1994) 1835–1847.
[70] D.J. Jagger, G.D. Housley, Membrane properties of type II spiral ganglion neurones identified in a neonatal rat cochlear slice, J. Physiol. 552 (Pt. 2) (2003) 525–533.
[71] M.A. Reid, J. Flores-Otero, R.L. Davis, Firing patterns of type II spiral ganglion neurons in vitro, J. Neurosci. 24 (3) (2004) 733–742.
[72] C. Weisz, E. Glowatzki, P. Fuchs, The postsynaptic function of type II cochlear afferents, Nature 461 (7267) (2009) 1126–1129.
[73] C.J. Weisz, E. Glowatzki, P.A. Fuchs, Excitability of type II cochlear afferents, J. Neurosci. 34 (6) (2014) 2365–2373.
[74] E. Glowatzki, P.A. Fuchs, Transmitter release at the hair cell ribbon synapse, Nat. Neurosci. 5 (2) (2002) 147–154.
[75] K.E. Froud, A.C. Wong, J.M. Cederholm, M. Klugmann, S.L. Sandow, J.P. Julien, A.F. Ryan, G.D. Housley, Type II spiral ganglion afferent neurons drive medial olivocochlear reflex suppression of the cochlear amplifier, Nat. Commun. 6 (2015) 7115.
[76] M.C. Brown, R.K. de Venecia, J.J. Guinan, Responses of medial olivocochlear neurons. Specifying the central pathways of the medial olivocochlear reflex, Exp. Brain Res. 153 (4) (2003) 491–498.
[77] A. Hafidi, G. Després, R. Romand, Ontogenesis of type II spiral ganglion neurons during development: peripherin immunohistochemistry, Int. J. Dev. Neurosci. 11 (4) (1993) 507–512.
[78] L.E. Järlebark, G.D. Housley, P.R. Thorne, Immunohistochemical localization of adenosine 5'-triphosphate-gated ion channel P2X(2) receptor subunits in adult and developing rat cochlea, J. Comp. Neurol. 421 (3) (2000) 289–301.
[79] G.D. Housley, R. Kanjhan, N.P. Raybould, D. Greenwood, S.G. Salih, L. Järlebark, L.D. Burton, V.C. Setz, M.B. Cannell, C. Soeller, D.L. Christie, S. Usami, A. Matsubara, H. Yoshie, A.F. Ryan, P.R. Thorne, Expression of the P2X(2) receptor subunit of the ATP-gated ion channel in the cochlea: implications for sound transduction and auditory neurotransmission, J. Neurosci. 19 (19) (1999) 8377–8388.
[80] C. Liu, E. Glowatzki, P.A. Fuchs, Unmyelinated type II afferent neurons report cochlear damage, Proc. Natl. Acad. Sci. U. S. A. 112 (47) (2015) 14723–14727.
[81] J.E. Gale, V. Piazza, C.D. Ciubotaru, F. Mammano, A mechanism for sensing noise damage in the inner ear, Curr. Biol. 14 (6) (2004) 526–529.
[82] V. Piazza, C.D. Ciubotaru, J.E. Gale, F. Mammano, Purinergic signalling and intercellular Ca2+ wave propagation in the organ of Corti, Cell Calcium 41 (1) (2007) 77–86.
[83] M. Lahne, J.E. Gale, Damage-induced cell–cell communication in different cochlear cell types via two distinct ATP-dependent Ca waves, Purinergic Signal. 6 (2) (2010) 189–200.
[84] F. Anselmi, V.H. Hernandez, G. Crispino, A. Seydel, S. Ortolano, S.D. Roper, N. Kessaris, W. Richardson, G. Rickheit, M.A. Filippov, H. Monyer, F. Mammano, ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca2+ signals across the inner ear, Proc. Natl. Acad. Sci. U. S. A. 105 (48) (2008) 18770–18775.
[85] D.J. Muñoz, I.S. Kendrick, M. Rassam, P.R. Thorne, Vesicular storage of adenosine triphosphate in the guinea-pig cochlear lateral wall and concentrations of ATP in the endolymph during sound exposure and hypoxia, Acta Otolaryngol. 121 (1) (2001) 10–15.
[86] J.C. Wang, N.P. Raybould, L. Luo, A.F. Ryan, M.B. Cannell, P.R. Thorne, G.D. Housley, Noise induces up-regulation of P2X2 receptor subunit of ATP-gated ion channels in the rat cochlea, Neuroreport 14 (6) (2003) 817–823.
[87] E.N. Flores, A. Duggan, T. Madathany, A.K. Hogan, F.G. Márquez, G. Kumar, R.P. Seal, R.H. Edwards, M.C. Liberman, J. García-Añoveros, A non-canonical pathway from cochlea to brain signals tissue-damaging noise, Curr. Biol. 25 (5) (2015) 606–612.
[88] S.P. Cook, E.W. McCleskey, Cell damage excites nociceptors through release of cytosolic ATP, Pain 95 (1–2) (2002) 41–47.
[89] D.A. Brown, G.M. Passmore, Neural KCNQ (Kv7) channels, Br. J. Pharmacol. 156 (8) (2009) 1185–1195.
[90] G.P. Richardson, I.J. Russell, Cochlear cultures as a model system for studying aminoglycoside induced ototoxicity, Hear. Res. 53 (2) (1991) 293–311.
[91] A. Forge, G. Richardson, Freeze fracture analysis of apical membranes in cochlear cultures: differences between basal and apical-coil outer hair cells and effects of neomycin, J. Neurocytol. 22 (10) (1993) 854–867.
[92] T. Kamimura, C.A. Whitworth, L.P. Rybak, Effect of 4-methylthiobenzoic acid on cisplatin-induced ototoxicity in the rat, Hear. Res. 131 (1–2) (1999) 117–127.
[93] R.J. Salvi, A.N. Popper, R.R. Fay, Hair Cell Regeneration, Repair, and Protection, Springer, New York, 2008.
[94] E.W. Rubel, S.A. Furrer, J.S. Stone, A brief history of hair cell regeneration research and speculations on the future, Hear. Res. 297 (2013) 42–51.
[95] A. Hafidi, Peripherin-like immunoreactivity in type II spiral ganglion cell body and projections, Brain Res. 805 (1–2) (1998) 181–190.
[96] E.A. Lumpkin, T. Collisson, P. Parab, A. Omer-Abdalla, H. Haeberle, P. Chen, A. Doetzlhofer, P. White, A. Groves, N. Segil, J.E. Johnson, Math1-driven GFP expression in the developing nervous system of transgenic mice, Gene Expr. Patterns 3 (4) (2003) 389–395.

Please cite this article in press as: K.D. Zhang, T.M. Coate, Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.09.017