Neural Circuit Development in the Mammalian Cochlea

The organ of Corti, the sensory epithelium of the mammalian auditory system, uses afferent and efferent synapses for encoding auditory signals and top-down modulation of cochlear function. During development, the final precisely ordered sensorineural circuit is established following excessive formation of afferent and efferent synapses and subsequent refinement. Here, we review the development of innervation of the mouse organ of Corti and its regulation.

It is generally assumed that synaptic activity is dispensable for establishment of synaptic networks (111, 129) but important for their refinement (e.g., Refs. 136, 138) in vertebrates. However, deeper investigation of this issue is hampered by the complexity of the central nervous system. For this reason, systems with less complex and well-ordered synaptic connectivity like the organ of Corti, the hearing organ of mammals, may serve as valuable systems to study circuit establishment and refinement. There, sensory information is transmitted from hair cells onto spiral ganglion neurons (SGNs) at specialized afferent synapses; the ribbon synapses and modulatory top-down control is exerted by neurons of the superior olive-forming efferent synapses on hair cells and postsynaptic boutons.

Here, we review 1) structure and features of the afferent and efferent innervation of the organ of Corti, 2) developmental changes of hair cells and their innervation, and 3) factors regulating this development. We discuss how, in the organ of Corti, both establishment and refinement of the afferent innervation of the inner hair cells (IHCs) are independent of presynaptic activity (firing of Ca$^{2+}$ action potentials and neurotransmitter release), whereas the refinement of the efferent synaptic innervation at the IHC (elimination of the axosomatic efferent synapses) requires the occurrence of normal Ca$^{2+}$ signaling inside the IHC. We focus on findings in mice, where the potential for genetic manipulation meets with the typical anatomy of the mammalian organ of Corti, enabling insights into the mechanisms that regulate development of innervation. This way, we deal with a single time axis of development within the limits of what is possible, given that the data were derived from different strains and of colonies bred in different laboratories. Where no corresponding data are available for mice, data from other rodents (rat, hamster) will be considered. Excellent reviews are available on the development of afferent (18, 29, 137) and efferent (115, 117) systems of the inner ear, of axonal guidance cues (23, 134), as well as on the developing auditory system in general (2, 103).

Innervation of the Organ of Corti

The organ of Corti is a suitable model for analyzing circuit formation and refinement. During development, it acquires a well-ordered afferent and efferent innervation: a single row of IHCs and three rows of outer hair cells (OHCs) form specific and well-defined afferent and efferent synapses with spiral ganglion and olivary neurons, respectively. A simplified model of the mature innervation of the organ of Corti is shown in FIGURE 1A. The nerve fibers innervating the organ of Corti form multiple radial and a few spiral bundles. Radial bundles include all types of neurites (SGN peripheral axons as well as axons of olivocochlear neurons), whereas the inner spiral bundle solely consists of lateral olivocochlear fibers. The outer spiral bundles comprise the peripheral processes of type II SGNs. However, the synaptic network within the organ of Corti may be still more complex than shown in FIGURE 1A involving reciprocal synaptic transmission (121, 124).

IHCs, the genuine sensory cells of the organ of Corti, receive most (95%) of the afferent innervation. In contrast to an average central neuron, forming $10^4$ to $10^5$ synapses (e.g., Ref. 60), each mature IHC only forms synapses with 6–20 type I SGNs (80). The number of synaptic contacts depends on the tonotopic position of the IHC within the organ of Corti and is maximal in the middle cochlear region (10–24 kHz), which transmits sounds with the highest sensitivity (19, 25, 58, 80). In contrast to the IHCs, each OHC contacts two to three afferent fibers of type II SGNs (5). Each type I SGN is thought to contact only one IHC (63), whereas each type II SGN innervates 3–10 OHCs (5).
Efferent innervation of the mature organ of Corti is constituted by non-myelinated lateral olivocochlear fibers forming axodendritic synapses with type I SGNs in close proximity to IHCs and by myelinated medial olivocochlear fibers directly innervating OHCs. The number of efferent synapses in the mouse organ of Corti was estimated by Maisson and coworkers (69). They showed that the density of efferent boutons contacting OHCs depends on the tonotopic position in the cochlea, similar to the number of the IHC afferent synapses, and rises from one contact per OHC in the very apical or basal part of the cochlea to three boutons per OHC in the 10-kHz region. At the same time, the authors found a rather uniform density of efferent innervation in the IHC region along the cochlear spiral. In contrast to other species [cat (64), guinea pig (17)], no radial gradient of efferent OHC innervation was observed.

According to their morphology, synapses in the organ of Corti can be divided into two types: “conventional” efferent synapses (FIGURE 1B) and efferent ribbon synapses (FIGURE 1C). Efferent synapses onto hair cells (medial olivocochlear axosomatic synapses with OHCs in the mature organ of Corti and with IHCs mostly during development) and afferent postsynaptic boutons (lateral olivocochlear axodendritic synapses) have a conventional presynaptic terminal filled with small clear-core and round synaptic vesicles and contain the “typical” complement of synaptic proteins [e.g., SNAREs (90, 110), complexins 1,2 (122, 127), synaptotagmin 1 (100, 110), and Bassoon and Piccolo (52)]. Their pharmacology is complex and best established for synapses formed by the medial olivocochlear bundle, which use acetylcholine as the main neurotransmitter (20, 21, 37). The postsynaptic membrane of these synapses is characterized by the “subsynaptic cistern” (FIGURE 1B), which by Ca\(^{2+}\)-induced Ca\(^{2+}\) release via ryanodine receptors boosts postsynaptic Ca\(^{2+}\) increase initiated by synaptic Ca\(^{2+}\) influx through \(\alpha 9/\alpha 10\) acetylcholine-receptors (e.g., Refs. 32, 66, 91). The ensuing localized Ca\(^{2+}\) increase activates small conductance K\(^{+}\) channels that, via hyperpolarization, inhibit the hair cell.

The hallmark feature of ribbon synapses, also found in cells of the vestibular organ, retina, pineal gland, lateral line, and electroreceptors, is a presynaptic electron-dense structure, whose shape varies from “ribbon-like” to spherical (then called dense body), dependent on species and organ (reviews in Refs. 78, 84). The precise function of the synaptic ribbon is still unknown. Current hypotheses state that it enables a large, readily releasable vesicle pool required for synchronous signaling.
and/or is involved in replenishing the readily releasable pool (27, 31, 67, 118, 140). The molecular anatomy and physiology of the IHC ribbon synapses (reviewed in Ref. 89) deviates from that of conventional synapses. On the one hand, the synapse uses specialized synaptic proteins such as RIBEYE (52), otoferlin (100, 102), and CaV1.3 L-type Ca2+ channels. On the other hand, several molecules with important roles in neurotransmission at other synapses like synapsins and synaptophysins (110), synaptotagmin 1 and 2 (6, 100, 110), complexins (122, 127), and neuronal SNAREs seem to be missing (Ref. 90, but see Ref. 127).

Early Development of the Circuitry

Innervation of the emerging mouse cochlea begins well before the organ of Corti is differentiated. However, only a few nerve fibers reach the otocyst or nascent cochlea at that time. The main events relevant to this process are summarized in FIGURE 2. The developmental time course varies within the organ of Corti: development proceeds in longitudinal or radial gradients. To a first approximation, the development of the innervation occurs in progressive basal-to-apical (bottom-to-top) and modiolar-to-strial (inside-to-outside) mode. SGNs exit terminal mitoses in a basal-to-apical gradient at around embryonic day (E) 9.5–13.5, whereas hair cells become postmitotic at E11.5–14.5 in an apical-to-basal gradient (77). Postmitotic SGNs immediately start sending their processes to the differentiating prosensory epithelium so that the fibers can be first visualized at E10.5 (22). The initial phase of afferent fiber outgrowth (up to E12.5 in mouse embryos) is directed by the otocyst [chicken (40)] but is neurotrophin independent (123). The mouse otocyst is innervated by afferent fibers as early as E11.5 (98) and efferent (28) fibers at E12. However, at E12.5, peripheral projections of the SGNs are branched and tangled and change their appearance to single unbranched fibers in the base of the cochlea only at E15.5 (55) as a result of the first phase of fiber pruning. The development of innervation proceeds in parallel with the differentiation of the prosensory epithelium, which presumably starts already in the otic cup at E9 (83) (see Ref. 48 for review). At E14.5, Atoh1 (one of the key transcription factors necessary for hair cell differentiation) is expressed in the midbasal turn of the organ of Corti (14). Also at E14.5, physiological differentiation of IHCs becomes evident by the appearance of a small potassium current (70). Half a day later, the development of stereocilia begins in the IHCs of the midbasal turn (65) and, probably with a short delay like in other mammals, in the OHCs (for reviews, see Refs. 49, 85, 96). At E15.5, IHCs localized in the base of the cochlea can be recognized by enhanced expression of the hair cell marker proteins myosin VIIa and Atoh1 (15, 135). At the same time and in the same region of the organ of Corti, OHCs only begin to express myosin VIIa in a modiolar-to-strial gradient (15). The lag in the differentiation of the OHCs persists also after birth (1). According to the morphological analysis of Nishida and coworkers (88), the delay in maturation of the apical hair cells is ~2–4 days.

Afferent Synaptogenesis in the Organ of Corti

At present, it is not exactly known when synaptogenesis in the mouse organ of Corti begins. First evoked exocytic membrane capacitance changes were recorded as early as E16.5 (45). However, neurotransmitter release in neurons is known to
Postnatal Maturation of IHCs and Their Afferent Innervation

IHCs and their innervation undergo a complex process of postnatal maturation that has recently been studied using functional and morphological approaches. After a brief general introduction, we will focus on changes related to afferent and efferent neurotransmission. Transduction likely becomes functional already during the late embryonic phase in vestibular hair cells (38) and around birth and early postnatally in cochlear outer hair cells (62, 131). The IHC repertoire of voltage-gated channels changes drastically during postnatal development, supporting the main IHC functions before and after the onset of hearing: generation of action potentials that drive presensory afferent signaling and faithful transmission of sensory information to the spiral ganglion. Action potentials of immature hair cells (FIGURE 4, H, I, AND K), although not yet directly demonstrated by in vivo experiments, are indicated to commence spontaneously by a large body of evidence and to be important for maturation of IHCs and their innervation (see below) and proper maintenance and refinement of central auditory projections. Action potentials are mediated mostly by Ca\(^{2+}\) channels (11, 56, 72) and are efficient triggers of exocytosis (8, 11, 33, 46). In parallel to the postnatal increase of number of afferent synapses (FIGURE 3), immature IHCs upregulate their Ca\(_{\text{v}1.3}\) Ca\(^{2+}\) channels, such that current density at the end of the first postnatal week exceeds the mature levels several fold (FIGURE 4A) (8, 11, 43, 45, 113, 139). Potassium channels expressed before the onset of hearing include voltage-gated delayed rectifier and Ca\(^{2+}\)-activated potassium channels of small conductance (SK2; FIGURES 4, J AND K, AND 5A), which are activated by Ca\(^{2+}\) influx through \(\alpha9/\alpha10\) ACh receptor or voltage-gated Ca\(^{2+}\) channels (11, 32, 42, 56, 71, 74). Recently, careful in vitro experimentation using near physiological conditions (42) and minimal perturbation of IHCs has addressed the underlying of spike generation (42, 125, 126). Action potentials of immature hair cells are likely driven by purinergic ionotropic receptors (but see Ref. 42) and the standing transduction current. Spiking activity is regulated primarily by inhibitory synaptic transmission via efferent synapses of olivocochlear neurons (32, 42) and ATP released by supporting cells (42, 125, 126). Spike patterns, the prevalence of spontaneous spikes, and their regulation have been studied (12, 42, 125), revealing tonotopic differences and complex control.
The appearance of additional potassium conductances around the onset of hearing has early on been considered as a mechanism disabling action potential spiking (FIGURE 5B) (56, 107, 109). Most importantly, Ca\(^{2+}\)- and voltage-activated K\(^+\) channels of large conductance (BK) enable a large hyperpolarizing conductance that secures graded and non-regenerative voltage responses of mature IHCs in response to transduction. In hair cells, this current shows surprisingly hyperpolarized activation and little Ca\(^{2+}\) dependence (11, 56, 71, 93). Even more negative activation is found for K\(_\text{v}7.4\) (KCNO\_4) defect in human deafness DFNA2 (51, 57), which is developmentally upregulated in IHCs (FIGURE 5B) (92) and OHCs (73) and contributes to setting their resting membrane potential (51, 73, 92).

Presynaptic structure and function of IHCs undergo major development beyond the described changes in Ca\(^{2+}\) channel expression. In immature IHCs, multiple round ribbons per synapse (FIGURE 3), sometimes not surrounded by synaptic vesicles (119, 120), face extended postsynaptic contacts of branched type I SGNs that engulf the IHCs in a calyceal manner (FIGURE 4D). Morphological and functional maturation of afferent synapses continues until ~2-3 wk of age.

During the first 3 days after birth, IHCs show little Ca\(^{2+}\) current and exocytosis. Exocytosis seems to rely on a different set of synaptic proteins than later in development and in the mature organ of Corti (6). Interestingly, these cells express the Ca\(^{2+}\) binding protein synaptotagmin 2 (6, 100), and their exocytosis seems unaffected when the protein otoferlin (6), essential for later presynaptic function (102), is absent. The number of synapses peaks at around the end of the first postnatal week, when exocytosis of IHCs also reaches a maximum (FIGURE 4A) (8, 11, 43, 87). The efficiency for incoming Ca\(^{2+}\) to trigger exocytosis increases during postnatal maturation, such that the smaller Ca\(^{2+}\) influx of mature IHCs causes comparable amounts of exocytosis for brief stimuli (FIGURE 4A) (8, 45, 113, 43). Since the first description, the quest of whether this reflects tighter spatial coupling or developmental changes of the synaptic proteins has been addressed by several studies (43–45, 113, 139) but remains open. It seems likely that Ca\(^{2+}\) channel distribution becomes more confined during postnatal maturation, which is one possible reason for the homogenous submembrane Ca\(^{2+}\) increase reported for immature IHCs (50) that contrasts the spot-like of Ca\(^{2+}\) influx in mature IHCs (26). First, immunohistochemical observations of Ca\(^{2+}\) channel distributions in immature IHCs suggest that Ca\(_{\text{a},1.3}\) Ca\(^{2+}\) channel clusters exist also in separation from the ribbon-type active zones (139), whereas the vast majority of Ca\(^{2+}\) channel clusters colocalizes with synaptic ribbons in mature IHCs (10, 26, 27, 86). Further morphological and functional imaging studies are required for a detailed characterization of the developmental changes of IHC Ca\(^{2+}\) signaling. Although Ca\(_{\text{a},1.3}\) is the dominant Ca\(^{2+}\) channel of immature and mature IHCs (11, 95), the properties of Ca\(^{2+}\) influx change (35) beyond the different channel number and distribution during development. These changes may reflect expression of different splice variants and/or changed protein interactions (36). It should be interesting to study whether differential changes in the number and functional properties of Ca\(^{2+}\) channels at the individual maturing synapse underlie the heterogeneity of synaptic Ca\(^{2+}\) signaling (26). If so, these changes could contribute to the developmentally emerging heterogeneity of auditory nerve fiber responses (133). Mature synapses typically display a single ellipsoid ribbon and a spatially confined active zone holding ~10–20 membrane-proximal synaptic vesicles (27, 94) and probably on average ~80 Ca\(_{\text{a},1.3}\) Ca\(^{2+}\) channels. Unlike the calyceal arrangement of glutamate receptor clusters in the mature organ of Corti (113), AMPA receptors form well separated and confined ring-like clusters closely matching the number of presynaptic active zones (52, 80).

Interestingly, during the early stages of development (up to P5–P6), the OHCs form as many ribbons per cell as IHCs but lose most of them before the onset of hearing (FIGURE 3) (119). Accordingly, substantial Ca\(^{2+}\) influx and exocytosis were observed during postnatal development (7, 54, 81). This suggests that initially both types of hair cells receive comparable numbers of afferent fibers, most of which are lost by OHCs when the animal matures (114). This phenomenon, synaptic pruning, is paralleled by neurite retraction (41) and likely also by apoptosis of extra-numerous SGNs, as shown for the normal development of the rat (105). The pruning of peripheral projections of type
I SGNs extending to the OHCs takes place around P3–P6 (FIGURE 3) (41). Importantly, this phenomenon does not seem to be the major determinant of SGN fate, since the type I and II SGNs can be distinguished by means of differential labeling as early as E18 (41). Taking the differential expression of the marker proteins to reflect fate decision, the later selective pruning as well as the final connections chosen by SGNs seem to result from different properties of type I and II neurons (for a more detailed review on differentiation of type I and II SGNs, see Ref. 2). Pruning of branched peripheral projections of type I SGNs contacting IHCs continues up to the onset of hearing, leading to a ~50% decrease of ribbon synapse number in mice between P7 and P8 and P14 and P15 (FIGURE 4, B AND D) (113).

Establishment and Refinement of Efferent Innervation

The formation of the efferent circuitry will only be briefly discussed here since it was previously reviewed in detail (115, 117). Efferent fibers begin to invade the sensory epithelium as early as E14.5 (82). At birth, axosomatic and axodendritic efferent synapses can be observed only around IHCs, whereas OHCs are devoid of efferent innervation.

FIGURE 4. Dependence of IHC maturation and developmental refinement of their innervation on thyroid hormone

A: developmental changes in exocytosis (top) and Ca2+ current density (bottom) in IHCs of normal (NMRI, white; Pax8+/−, orange) and hypothyroid (Pax8−/−, green) as well as Pax8−/− mice rescued by thyroid hormone injections (violet). B: afferent ribbon synapses in a hearing heterozygous (Pax8+/−) phenotypically normal mouse, note well confined postsynapses (labeled by GluR2/3 glutamate receptors, green) juxtaposed to synaptic ribbons (stained for RIBEYE, red). Ribbon synapses in a deaf athyroid Pax8−/− mouse (C) note more diffuse staining similar to immature pattern in younger wild-type (D) or mutant (E) animals (labeled as in B). F and G: representative electron micrographs of IHC ribbon synapses from P15 Pax8−/− (F) and P15 Pax8+/− (G) mice. H and I: fraction of IHCs that show spontaneous (H) or electrically evoked (I) action potential firing of Pax8−/− and Pax8+/− mice at different postnatal days. J: representative projections of confocal sections obtained from organs of Corti of P15 Pax8−/− (top) and P15 Pax8+/− (bottom) mice after synaptophysin (red) and SK2 (green) immunostaining: heterogeneous expression of efferent markers/maintenance of efferent synapses in wild-type IHCs at P15. K: a membrane potential recording from a P15 Pax8−/− IHC displaying spontaneous action potentials and small biphasic potentials (arrowheads; example enlarged in inset) characteristic for cholinergic postsynaptic potentials. Scale bars: B–D, 2 μm; E, 500 nm. H and I have been taken from Ref. 12 and the other panels from Ref. 113 and have been used here with permission.
FIGURE 5. Mutational analysis of the postnatal development IHCs and their innervation

Schematic representation of basolateral IHC properties (A) sketches the immature IHC morphological and functional properties found in wild-type IHCs before the onset of hearing and in 2-wk-old Pax8^−/− IHCs: immature afferent synapses, large amplitude of Ca^{2+} influx, efferent axosomatic synapses, and lack of BK and Kv7.4 channels. B: mature basolateral makeup of IHCs after the onset of hearing for wild-type mice and mouse mutants with impaired transmitter release. C: summarizes the IHC properties found in IHCs of 2- to 4-wk-old mouse mutants with defects of Ca^{2+} influx, disabling firing of Ca^{2+} action potentials: normal maturation of afferent synapses but maintained efferent IHC innervation and lack of BK potassium channels. D: illustrates the IHC properties of mouse mutants lacking TMC1 or myosin VI function: impaired afferent maturation, immature basolateral ion channel makeup. Efferent innervation is not yet investigated. For simplicity, we only drew one afferent and efferent synapse each (at the same size) and did not depict the branching of afferent fibers seen in immature organs of Corti. Structures (ion channels and synapses) are not drawn to scale.
At P7 the first axodendritic and at P9 the first axosomatic efferent synapses are found around OHCs (FIGURE 3) (114). The direct efferent innervation of IHCs declines around the onset of hearing (114) and functionally vanishes until postnatal week 4 (FIGURE 5B; Ref. 11). The transient axodendritic synapses around OHCs disappear until P20 (114). Data in the rat show that the development of the efferent innervation proceeds with modiolar to strial and basal to apical gradients (16). Interestingly, from birth and until the end of postnatal week 3, at least part of the efferent synapse population surrounding the IHCs can originate from medial olivocochlear neurons, since it was shown that their anterogradely labeled axons terminated under IHCs at this developmental stage in rat (16) and hamster (116), and IHCs respond to acetylcholine in the neonatal rat (32). However, both medial and lateral olivocochlear neurons express enzymes required for acetylcholine biosynthesis, and their axons are still differentiating after arrival under IHCs (4). Thus the origin of the transient efferent synapses surrounding hair cells in immature animals still has not been unequivocally identified. As described above for afferent fibers, pruning probably takes place also during formation of the efferent circuit since plenty of synapses form and later disappear and, moreover, the elimination is accompanied by death of efferent neurons in the brain stem (16).

Regulation of the Circuit Formation and Refinement

Reviewing recent literature, we can address important questions concerning the regulation of circuit development in the organ of Corti. 1) Is afferent synaptogenesis dependent on synaptic activity? 2) Is afferent synaptic activity important for the refinement of organ of Corti circuitry? 3) Which factors are required for circuit development in the organ of Corti?

It was tempting to speculate that synaptic activity is a driving force for the establishment and/or refinement of the circuitry, since it becomes functional before, or nearly simultaneously with, the appearance of the very first synapses (8, 45). Also, synaptic activity was shown to promote synaptogenesis in vitro (76) and circuit refinement in the auditory system (61) (for review, see Ref. 47) as well as in other systems in vivo (108). However, recent studies on knockout mice show that the afferent IHC circuitry develops (i.e., is established and refined) even in the absence of Ca\textsuperscript{2+} action potentials (FIGURE 5C) (87) and IHC neurotransmitter release (FIGURE 5B) (106, 112). This, to our knowledge, is the first example of activity-independent circuit development in vertebrates. The knockout mice used in these studies are deaf due to the absence of either the pore-forming calcium channel subunit Ca\textsubscript{v1.3}, providing calcium ions for synaptic vesicle exocytosis (11, 95) or the vesicular glutamate transporter (Vglut3, loading synaptic vesicles with neurotransmitter). Interestingly, the same knockout animals give insights into the control of efferent IHC circuitry refinement: The loss of Vglut3 does not affect the developmental elimination of axosomatic efferent synapses on IHCs (112), whereas in Ca\textsubscript{v1.3}\textsuperscript{-/-} IHCs this innervation was retained until at least postnatal week 4 (FIGURE 5C) (11, 87). Therefore, it is likely that the elimination of the axosomatic efferent synapses of IHCs is promoted by Ca\textsuperscript{2+} action potential firing, consistent with Ca\textsuperscript{2+} signals being well known regulators of gene expression in neurons (24).

Another factor driving maturation of the circuitry of the organ of Corti is thyroid hormone. It was shown that different models of hypothyroidism cause severe retardation of maturation of the afferent and efferent innervation of IHCs (113) and OHCs (104, 128) as well as failure to eliminate extranumerous SGNs (104). Although the formation of afferent synapses proceeds apparently unaltered, IHCs maintain their immature phenotype (FIGURE 5A): high number (FIGURE 4C) of immature (FIGURE 4G) afferent synapses, large Ca\textsubscript{v1.3} Ca\textsuperscript{2+} currents (FIGURE 4A), and Ca\textsuperscript{2+} action potential firing (FIGURE 4, H, I, AND K) (12, 113) as well as the low Ca\textsuperscript{2+} efficiency of exocytosis (FIGURE 4A) (113). Interestingly, otoferlin immunofluorescence was strongly reduced in a pharmacological model of hypothyroidism, despite the presence of robust exocytic membrane capacitance changes (12). This indicates a clear distinction from the physiological immaturity state of control IHCs during the end of the first postnatal week, where a similar large Ca\textsuperscript{2+} current is observed but exocytosis is otoferlin-dependent (102). It should be interesting to see whether the IHCs of these hypothyroid rats use a different program of synaptic proteins, as has been suggested for early postnatal IHCs of wild-type mice (6, 100). In keeping with retarded maturation, the normally observed upregulation during postnatal week 2 of the large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (BK) (11, 56) is delayed (12, 107, 113), and only some BK channel immunolabeling was found in the late second postnatal month (12). Moreover, IHCs keep their efferent axosomatic synapses with acetylcholine receptors and small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels characteristic for immature IHCs (FIGURES 4, J AND K, AND 5A). Further insights into the regulation can be expected from mouse mutants for transmembrane cochlear-expressed gene 1 (TMC1) and myosin VI (FIGURE 5D). TMC 1 is a transmembrane protein of hair cells
important for hair cell maturation and survival (75) and hearing (9, 59, 75, 130). IHCs of TMCI mouse loss-of-function mutants maintain immature levels of Ca\(^{2+}\) current and exocytosis and seem not to acquire BK and K\(_{\text{Ca}}\) channels (75). Myosin VI is an unconventional myosin required for hair cell maturation (39, 101) and hearing (3, 79). Genetic inactivation impairs the maturation of afferent synapses and the developmental acquisition of BK and K\(_{\text{Ca}}\) channels (39, 101). It should be interesting to test in future studies whether TMCI and myosin VI are regulated by thyroid hormone and how they affect maturation.

Conclusions

In conclusion, the innervation of the organ of Corti provides a valuable model for analyzing formation and refinement of neuronal circuitry: 1) extranumerous synapses are formed; 2) appropriate connections mature; and 3) inappropriate connections are eliminated. Furthermore, the studies on this model show that both establishment and refinement of circuitry can be independent of synaptic activity and demonstrate that thyroid hormone coordinates the process of refinement.

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