

The Genetics of Hearing and Balance in Zebrafish

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Abstract

The zebrafish is an excellent model system for studying the molecular basis of inner ear development and function. The eggs develop *ex utero* and the ear is transparent for the first few weeks of life. Forward genetic screens and antisense technology have helped to elucidate the signaling pathways and molecules required for inner ear development and function. This review addresses the most recent advances in our understanding of how the ear forms and discusses the molecules in hair cells that are essential for sensing sound and movement in the zebrafish.

Contents

THE INNER EAR RECEPTOR: THE SENSORY HAIR CELL . . .	10
ANATOMY AND FUNCTION OF THE ZEBRAFISH EAR	10
DEVELOPMENT OF THE ZEBRAFISH EAR	13
Genes Implicated in Otic Induction	13
Genes Implicated in Morphogenesis of the Otic Vesicle	15
Genes Implicated in Specification of Hair Cells	16
HAIR-CELL FUNCTION IN ZEBRAFISH	16
Genes Implicated in Mechanotransduction	16
Genes Affecting Integrity of Hair Bundles	18
Genes Affecting Hair-Cell Synaptic Transmission	18
Genes Affecting Hair-Cell Survival	18

THE INNER EAR RECEPTOR: THE SENSORY HAIR CELL

The inner ear detects motion and sound. The sensory patches dedicated to this task are termed maculae, cristae, or the organ of Corti. Maculae contain receptors coupled to dense crystal structures known as otoconia or otoliths. Cristae are situated in the semicircular canals. The organ of Corti is a specialization for hearing found only in higher vertebrates. The sensory receptors of the inner ear, the hair cells, are exquisitely sensitive to the mechanical stimuli of movement and sound (20). Their highly specialized apical surfaces consist of unusual processes, termed stereocilia. Each stereocilium is densely packed with actin filaments in a paracrystalline array. The hair-like appearance of the bundle of apical stereocilia gave rise to the name “hair cells.” Within each hair bundle, stereocilia are arranged with remarkable precision

Crista: sensory epithelium of hair cells and supporting cells inside the semicircular canals

Macula: sensory epithelium associated with otoliths

Otolith: calcium carbonate stone situated above the macula

in rows, with increasing height toward one end of the bundle. Next to the tallest stereocilia in vestibular or lower vertebrate hair cells is a true cilium known as the kinocilium. Its role appears to couple the bundle of stereocilia to the overlying extracellular matrix or the otolithic membrane. In mammalian auditory hair cells, kinocilia appear during development but are reabsorbed in mature hair cells. In the cochlea, the tallest stereocilia of the outer hair cells are instead attached to the tectorial membrane, a gelatinous membrane very similar to the otolithic or cupula membranes present in maculae or cristae. All hair cells are bathed in a special fluid, the endolymph, a high potassium fluid unique to the ear. When hair cells are mechanically stimulated, stereocilia pivot about their bases and move together as a unit because of numerous extracellular linkages. Movement allows cations to flow into the hair bundles, resulting in depolarization.

It has been estimated that a movement of three angstroms is sufficient to excite hair cells. Unfortunately, this sensitivity may underlie the vulnerability to mutations. Deafness is one of the most common inherited diseases (31). In addition, age-related hearing loss occurs at a high frequency (11). There are many things that can go wrong in the ear (see Hereditary Hearing Loss Homepage at <http://webhost.ua.ac.be/hhh/>). The endolymph can be thrown out of balance by mutations affecting ion transport and homeostasis. Hair bundles can be malformed or degenerate over time. The calcium carbonate biominerals, the otoconia or otoliths, may be absent or dislodged from the sensory epithelia. Using genetics as a tool, we are beginning to grasp the molecular basis of how the ear develops and how these pathologies may arise.

ANATOMY AND FUNCTION OF THE ZEBRAFISH EAR

Orientation with respect to gravity and the environment is a key concern for both aquatic and terrestrial animals. In vertebrates, the

anatomical features of the vestibular labyrinth are highly conserved in terms of structure and function. These features include three semi-circular canals and two or more macular organs (**Figure 1**). At the base of each canal is a rounded mound of sensory epithelium called the crista. Within each crista, the long kinocilia of the hair cells are embedded in a gelatinous membrane called the cupula that

spans the canal. Movements of the head cause the fluid inside the canals to impinge upon the cupula or gelatinous membrane, deflecting the embedded kinocilia and hair bundles. Signals are subsequently sent from the excited hair cells to the brain, providing information about the position of the head and angular acceleration. All vertebrates possess two macular organs, the saccule and utricle.

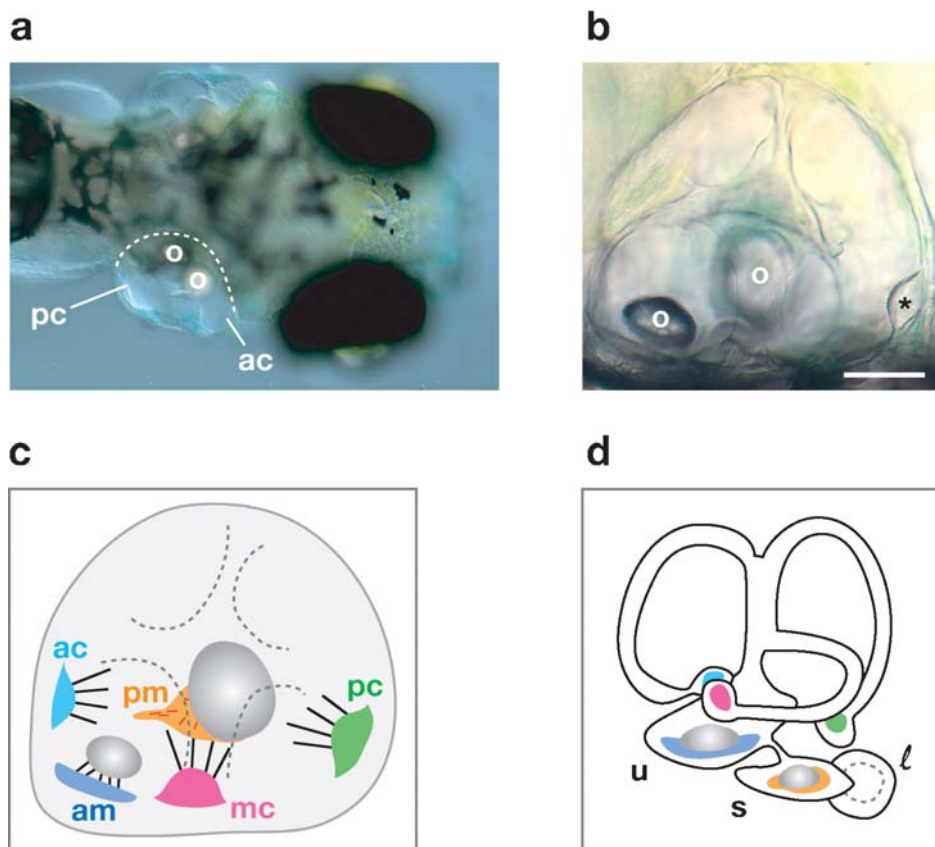


Figure 1

Larval and adult inner ear of the zebrafish. (A) Dorsal view of a live, 5 day-old zebrafish larvae. The plane of focus includes the anterior and posterior canals (*solid lines*). The dotted line indicates the medial wall of the developing otocyst. Both maculae are located relatively deep within the otocyst (out of focus otoliths). (B) Lateral view of the larval inner ear. The focal plane includes the anterior otolith and the neuroepithelium within the posterior canal (*asterisk*). The epithelial columns that join in the middle are also visible. (C) Diagrammatic representation of the larval ear. All five sensory patches are indicated in color. Dotted lines indicate the epithelial structures around which the semicircular canals form. (D) Diagram of the adult zebrafish ear. A third macular organ, the lagena (*dotted area*), forms later during the juvenile stage. Colors correspond to those in panel C. Scale bar indicates 100 μm in A and 60 μm in B. ac, anterior crista; am, anterior macula; l, lagena; mc, medial crista; o, otolith; pc, posterior crista; pm, posterior macula; s, saccule; u, utricle.

Lateral line organ: system of superficial and canal hair cells that detects water movements

Neuromast: group of hair cells at the surface of the skin

These pouch-like organs contain a bed of hair cells coupled to calcium carbonate crystals. In fish, the crystals coalesce to form a single large polycrystalline mass called an otolith (**Figure 1**). Forces impinging upon the otoliths cause them to move, which in turn deflects the underlying hair bundles. Macular organs are important for sensing linear acceleration and gravity. In lower vertebrates such as fish and frogs, they also are used in hearing (33). Fish use their saccular organs to detect frequencies between 10–4000 Hz.

Some fish are classified as hearing specialists based on the presence of a series of bones known as Weberian ossicles that connect the swim bladder to the saccule. Sound can set the air-filled swim bladder into motion and this motion is transmitted to the sensory epithelium via the ossicles, in effect amplifying the sound. The zebrafish, along with many related species within its clade, are hearing specialists. Additional endorgans include the macular organ called the lagena and the macula neglecta that develop after the larval stage. Their function is primarily auditory (33, 50).

In addition to the ear, fish (and frogs) possess another organ that employs sensory hair cells, the lateral line organ. This organ allows the detection of low-frequency stimuli such as water movements and is important in schooling, prey detection, and other behaviors. It is called the lateral line because the groupings of hair cells known as neuromasts (**Figure 2**) are located in a series extending along the trunk. Although an attractive hypothesis because of the anatomical simplicity of lateral line organ, still under debate is whether the lateral line organ was the first to evolve and then later give rise to the ear (34).

The organization and morphology of the inner ear neuroepithelium in fish resembles

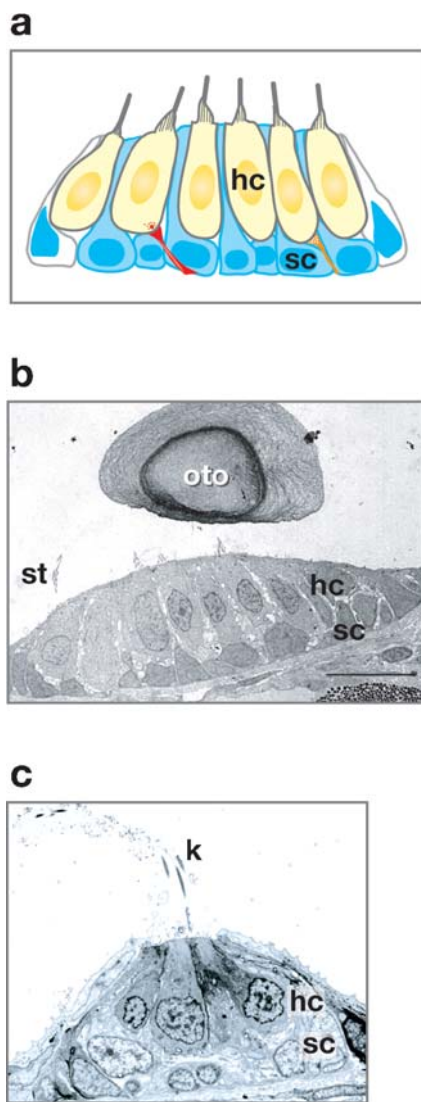


Figure 2

Organization of the sensory epithelium.

(A) Diagram of cross section of inner ear neuroepithelium (anterior macula). The oval-shaped hair cells (yellow) are present in the upper two thirds of the epithelium. The lower supporting cells (blue) are situated between hair cells. The epithelium is bathed in a high potassium solution, the endolymph. The outer cells (white) are presumably dark cells that regulate the potential of the endolymph. Also depicted are an example of an afferent synapse (red) and an efferent synapse (orange).

Transmission electron micrographs of an anterior macula (B) and a neuromast (C) at 120 hpf. The cylindrical oval-shaped sensory hair cells lie above the dark nuclei in the soma of the supporting cells. Scale bar in B indicates 7 μm in B and 3.5 μm in C. hc, hair cells; k, kinocilia; oto, otolith; sc, supporting cells; st, stereocilia.

that found in higher vertebrates (**Figure 2**). At the surface of the neuroepithelium, hair bundles project into the inner ear lumen or in the lateral line, directly into the aqueous environment. Each hair cell is surrounded by 5 to 6 supporting cells whose apical surfaces are covered with microvilli. The oval-shaped cell bodies of the hair cells are situated above the more cuboidal-like nuclei of the supporting cells. The eighth cranial nerve fibers create multiple synapses along the basolateral membrane of the hair cells. The specialized calyx surrounding Type I vestibular hair cells in higher vertebrates is not present in fish, though long protrusions or extensions of the basal surface into individual nerve fibers have been detected (5). At afferent synapses, one or more synaptic ribbons or dense bodies are located next to the basolateral membrane of the hair cell (**Figure 3**). These electron-dense structures are surrounded by synaptic vesicles and are thought to play a role in synaptic transmission. The same organization characterizes the mounded clusters of hair cells or neuromasts that comprise the superficial lateral line organ (**Figure 2**). Similar to hair cells in the semicircular canals, the kinocilia of lateral line hair cells are long and embedded in a gelatinous cupula.

DEVELOPMENT OF THE ZEBRAFISH EAR

The development of the zebrafish ear is similar to that observed in other vertebrates (35, 50). The one striking exception is the mechanism by which the lumen of the otocyst forms. In zebrafish, the otic placode forms a hollow space within the cluster of precursor cells. In chicks and mice, the otic placode invaginates, creating a cup that eventually closes to form a lumen. Other differences include the way in which the semicircular canals form and the lack of extensive cell death in remodeling during morphogenesis. Despite these differences, studies of develop-

ment of the zebrafish ear have increased our knowledge about the general mechanisms employed. Overall, the development of the ear proceeds quite rapidly in zebrafish. Within 16 hours post fertilization (hpf), a thickening of the ectoderm, the otic placode, becomes visible (13). Soon after cavitation (18 hpf), the first hair cells appear at opposite poles of the ventral portion of the lumen. These two sensory patches will give rise to the anterior and posterior maculae. When the first hair cells appear, otoliths attach to the kinocilia around 20 hpf (35). During this period neuroblasts delaminate from the otic vesicle to form the first-order neurons of the auditory/vestibular (VIIIth) nerve (13). At approximately 45 hpf, epithelial columns sprout from the otocyst wall and grow into the lumen. About 10 h later, these projections meet and fuse. Eventually they will form the central parts or hubs around which the semicircular canals develop (**Figure 1**). The sensory patches or cristae within the semicircular canals form about a day later than the anterior and posterior maculae. By 96 hpf, the larvae are free swimming and have fully functional ears (**Figure 1**).

The following sections focus on otic induction, semicircular canal development, and hair-cell specification. For more information on related developmental topics such as lateral line or otolith development, please see related reviews by Ghysen & Dambly-Chaudiere (2004) and Söllner & Nicolson (2004).

Genes Implicated in Otic Induction

An in-depth review on this topic can be found in an excellent review by Whitfield et al. (see related reviews). The purpose of this section is to summarize recent progress in understanding otic induction. The accessibility of the zebrafish inner ear coupled with forward genetics and antisense technology have allowed a big step forward in defining signaling events that give rise to placode development. Both early and late events have been described

Otic vesicle: developing inner ear fluid-filled, sac-like structure lined with inner ear progenitor cells

hpf: hours post fertilization



Figure 3

Fine structure of zebrafish inner ear hair cells (120 hpf). (A) A sensory hair cell of the anterior macula. Scale bar, 2 μm . (B) Transverse section of a hair cell bundle. The increase in height of the closely arranged stereocilia can be seen (only a third of the stereocilia are present in this section). Scale bar, 1 μm . (C) Two synaptic bodies surrounded by secretory vesicles at the basolateral end of the cell. (D) An efferent synapse filled with vesicles. (E) Very fine extracellular linkages at the tips of two stereocilia. The darkened areas of insertion plaques at the upper insertion site and the caps of the stereocilia are readily visible. (F) A single tip link interconnecting neighboring stereocilia. Scale bars, 0.5 μm in C-E, and 0.125 μm in F.

at the molecular level, leading the field of development in terms of our knowledge of otic induction.

Very early transplantation studies have suggested that the source of inductive cues for placode development was hindbrain tissue (47). Fibroblast growth factors (Fgfs) have been candidates for signaling as they are expressed in the region adjacent to the otic placode. More definitive evidence, however, was lacking until recent studies by several laboratories showing that *fgf3* and *fgf8* are required for otic induction (25, 28, 32). Recessive loss-of-function mutations in the *acerebellar/fgf8* gene cause the development of small ears, as does the removal of *fgf8* activity. If *fgf3* is knocked down using antisense oligonucleotides or morpholinos in the mutant *acerebellar* background, then the otic placode is completely absent.

Targets of the Fgf3 and Fgf8 pathway have also been identified by examining embryos harboring the deficiency *Df*^{b380} that lacks *sox9a*, *dlx3b*, and *dlx4b* (26). The absence of otic placodes in *Df*^{b380} mutants can be rescued by injection of *sox9a*, *dlx3b*, and *dlx4b* mRNA, and is mimicked by combined injection of morpholinos against all three genes. Only expression of *sox9a* is dependent on Fgf3 and Fgf8 signaling. The paired box transcription factors, Pax2 and Pax8, are also downstream targets of Fgf signaling (16). Onset of *pax8* expression in the preotic region occurs before the duplicates *pax2a* and *pax2b* are expressed. Loss of *pax2a* or *pax8* alone results in abnormally small ears. As with concomitant loss of *fgf3* and *fgf8* activity, loss of both *pax2a* and *pax8* causes otic induction to fail. The *bearsay* gene encodes a forkhead transcription factor, Foxi1, and mutations affect otic placode development (45). Analysis of both *bearsay* and *Df*^{b380} mutants reveals that Foxi1 influences the levels of *pax8* expression (16, 45). In addition, *Dlx3b* was shown to regulate expression of the later acting *pax2a* gene (16). Removal of both *foxi1* and *dlx3b* blocks the development of the otic placode.

Genes Implicated in Morphogenesis of the Otic Vesicle

The structure of the embryonic otocyst is very simple in comparison to the mature adult ear. To detect movement of the head in various dimensions, the semicircular canals are positioned in three different planes. How this remarkable arrangement is achieved at the molecular level is only now beginning to be understood. We do, however, have some hints at what is required to establish the initial outgrowth of the epithelial columns that are destined to shape and position the semicircular canals. Mutagenesis screens have uncovered a class of mutants with jaw defects that fail to fuse the inner ear epithelial columns near the center of the lumen (29). The canal rudiments initially form, but then fail to grow and project away from the wall of the otocyst. *jekyll* is one such mutant. The gene was cloned and found to encode an enzyme, Uridine 5'-diphosphate glucose dehydrogenase (Ugdh), that produces one of the subunit building blocks of hyaluronic acid (HA) (49). Another study found that the zebrafish orthologue of a human deafness gene, *dfna5*, was also required for proper canal and jaw development (4). If *dfna5* activity was knocked down, then expression of *ugdh* was reduced in the ear and the pharyngeal arches of the jaw. It is not clear what the function of *Dfna5* is because it shares no homology to other proteins, nor has it been localized; however, some evidence suggests that it may act at the transcriptional level (12), presumably upstream of *ugdh*. In both cases, removal of *ugdh* or *dfna5* results in loss of HA production. What does HA synthesis have to do with outgrowth of epithelial columns in the ear? It is a mystery at this point, though an interesting idea has been put forth proposing that HA acts as a propellant while it is secreted from the cells that extend forward into the lumen (14).

Disorganized epithelial columns are also a prominent feature of *dog-eared/eya1* mutants (24). Most of the otic vesicles in these mutants are abnormally small and the cristae fail

Fgf: fibroblast growth factor

Ugdh: Uridine 5'-diphosphate glucose dehydrogenase

HA: hyaluronic acid

to develop due to apoptosis of sensory neural precursors. In addition, cell death occurs within the migrating lateral line primordia, leading to loss of neuromasts at the end of the larval trunk. Larvae carrying *chameleon*^{tf18b} and *slow muscle omitted*^{b641}/*smoothened* mutations also have disorganized canals and abnormal ear morphology (15). The abnormalities are due to loss of inductive signals to the posterior region of the otic vesicle, resulting in a duplication of anterior structures. Overexpression of *patched1* mRNA phenocopies the mutants, demonstrating a role for Hedgehog signaling from underlying midline structures in patterning of the otic vesicle.

Genes Implicated in Specification of Hair Cells

Studies mainly from the mouse reveal that lateral inhibition plays a major role in determining the fate of the neuroepithelial cells (see related reviews, Bryant et al., 2002). A recent advance in zebrafish suggests that ubiquitination by the Mind bomb/ubiquitin ligase controls endocytosis of the Delta ligand in presumptive hair cells (21). Surprisingly, endocytosis of Delta leads to enhanced signaling by the Delta-Notch pathway.

Other recent studies have implicated glial cells in preventing premature development of lateral line hair cells (10, 27). Sox10 is required for specification of glial cells, and in the *colourless/sox10* mutant, hair cells develop precociously. Although the signaling events from glial cells to the lateral line primordial cells have yet to be described, this discovery reveals a novel role for glial cells in development. It will be interesting to determine whether such a mechanism is conserved in the development of other organs or in other vertebrates.

HAIR-CELL FUNCTION IN ZEBRAFISH

The overall morphology of the inner ear and lateral line hair cells closely resembles that seen in vestibular hair cells in other verte-

brates (**Figures 2 and 3**). To date, a number of genes required for hair-cell function in the zebrafish have been associated with auditory defects in mice and humans, thus revealing the conservation of function of these genes (**Table 1**). The phenotype of zebrafish mutants with balance and hearing defects is characterized by a swimming behavior in circular motions, hence the name “circler” mutants (30). In some cases, a complete lack or partial response to acoustic stimuli suggests that the animals are deaf. This can be easily tested by examining the acoustic startle reflex, which is very robust and consistently evoked at the free-swimming larval stage (>80 hpf).

Genes Implicated in Mechanotransduction

Mechanotransduction is the conversion of mechanical energy into electrical signals. At the hair bundle, the mechanical energy of sound or movement is converted to electrical impulses that propagate along the basolateral membrane of the hair cell body. These signals are then transmitted via first-order neurons to the brain. Transmission electron microscopic data of zebrafish hair bundles reveals that they possess what is thought to be a transduction apparatus at the tips of stereocilia (**Figure 3**) (44). The morphological correlates include an obliquely oriented tip link or fine extracellular filament of approximately 150 nm in length, and electron densities at either end of the tip link where mechanosensitive channels are presumed to be present. In addition, sensitivity of mechanotransduction to aminoglycosidic antibiotics or the calcium chelator EGTA has been demonstrated at the physiological level (30, 39). Over the past two decades, data from electrophysiological and biophysical studies of bullfrog hair cells suggest that the tip link pulls open channels when hair bundles are deflected in the excitatory direction (19). The search for the components required for mechanotransduction has been an ongoing effort. In recent studies using

Table 1 Genes required for inner ear development and function in zebrafish

Mutant/Gene	Expression pattern	Phenotype	References
Developmental			
<i>fgf3</i>	Hindbrain	Small malformed ear	(25, 26, 28, 32)
<i>acerebellar/fgf8</i>	Hindbrain	Small malformed ear	(25, 26, 28, 32)
<i>chameleon^{tf18b}</i>	Unknown	Anteriorized ear	(15)
<i>colourless/sox10</i>	Neural crest	Small malformed ear	(16)
<i>dfna5</i>	Inner ear	Defective canals	(4)
<i>Df^{b380} (sox9a, dlx3b-4b)</i>	Preotic region	Ear absent	(16)
<i>dog-eared/eya1</i>	Otic anlage	Fewer hair cells	(24)
<i>bearsay/foxi1</i>	Otic anlage	Small malformed ear	(45)
<i>jekyll/ugdb1</i>	Jaw, inner ear	Defective canals	(49)
<i>mind bomb/tubeE3-like</i>	n.d.	Extra hair cells	(21)
<i>no isthmus/pax2a</i>	Preotic region	Extra hair cells	(16)
<i>pax8</i>	Preotic region	Small malformed ear	(16)
<i>slow muscle omitted^{b641}/smo</i>	Otic vesicle, Hindbrain	Anteriorized ear	(15)
Hair-cell function/survival			
<i>gemini/cav1.3</i>	Hair cells, brain, retina	Hair-cell defect synaptic transmission affected?	(41)
<i>mariner/myoVIIa</i>	Hair cells	Hair-bundle defect	(7)
<i>orbiter/pcdb15a</i>	Hair cells, brain	Hair-bundle defect	(38)
<i>ru848/choroideremia</i>	Ubiquitous	Hair-cell death	(46)
<i>satellite/myo6b</i>	Hair cells	Hair-bundle defect abnormal membrane fusion	(22, 39)
<i>sputnik/cdb23</i>	Hair cells, brain, retina	Hair-bundle defect mechanotransduction affected	(44)
<i>trpn1</i>	Hair cells, neural crest, retina, brain, gills	Mechanotransduction affected	(42)

n.d. not done.

either forward genetics or antisense technology, two key players of the transduction apparatus have been identified in zebrafish, the tip link and transduction channel.

A candidate for the tip link was discovered by analysis of *sputnik/cadherin23* mutants (44). In homozygous larvae, the tip link is absent. In addition, an antibody against Cadherin 23 labeled the tips of inner ear hair bundles and produced some punctate labeling of the bundle. EM gold labeling was not performed in zebrafish; however, in murine and bullfrog hair bundles, labeling appears to be restricted to the tip links and the kinocilial links, a presumably related structural connec-

tion (43). The collective data argue for a role for Cadherin 23 in transduction, suggesting that its unusually long extracellular domain (27 cadherin repeats) is the fine tip link filament spanning between neighboring stereocilia.

A candidate gene approach to identify the transduction channel arose from studies of mechanosensory bristle mutants in *Drosophila* (23). One particular mutant, *nompC*, had an interesting defect in transduction currents, and the gene encodes a novel transient receptor potential (TRP) channel (48). The zebrafish orthologue, *trpn1*, is expressed in inner ear hair cells and knock-down of its activity results

TRP: transient receptor potential

in deafness and loss of receptor potentials in lateral line hair cells (42).

Although *Trpn1* is a promising candidate for the transduction channel in zebrafish inner ear hair cells, the subcellular localization of the channel is not known. In addition, a genetic mutation in *trpn1* has not been reported. The reason may be that saturation screens for circler mutants have not been performed, or mutations in *trpn1* may result in early embryonic lethality. Transient expression of *trpn1* in presumptive migrating neural crest cells lends some support to this idea.

Genes Affecting Integrity of Hair Bundles

Besides the above-mentioned tip links and kinocilial links, the hair bundle is interconnected by horizontal links and ankle links. Stabilization of the bundle is also provided by the dense actin meshwork known as the cuticular plate, located just below the apical surface. The actin filaments of the stereocilia converge at the base of each stereocilium and then insert their long rootlets into the cuticular plate. The structural integrity of the links and cuticular plate is crucial for mechanotransduction by the hair bundle.

Recent studies of *satellite/myosin VI* or *ru920/myosin VI* mutants suggest that Myosin VI is required for the integrity of the cuticular plate and prevents fusion of stereocilia (22, 37). A similar phenotype was detected in Snell's waltzer/myosin VI mice mutants (3, 40), suggesting that the role of myosin VI in bundle integrity is highly conserved.

Along with *cadherin23* and *myosin VIIA* (7, 44), more severe mutations in *protocadherin 15* cause splaying of bundles (38). Although Protocadherin 15 has been localized to the hair bundles in other vertebrates (1), it is not known where it is located within the zebrafish stereocilia. Based on its long extracellular domain, one might also expect it to be one of the linkages interconnecting stereocilia.

Genes Affecting Hair-Cell Synaptic Transmission

As sensory receptors, hair cells have the remarkable capability of producing prolonged, tonic synaptic transmission (8). How this is accomplished at the molecular level is not well understood. An unusual structure, the synaptic ribbon, tethers synaptic vesicles next to the synaptic cleft. Many hundreds of vesicles are located near the ribbon body at the basolateral membrane. Given its putative role in promoting vesicle fusion, it was not surprising to find that mutations in the L-type calcium channel, Cav1.3, caused deafness in mutant *gemini* larvae (41). Indeed, the Cav1.3 channel was found to localize in ring-like structures near the basolateral membrane of zebrafish lateral line hair cells. What is lacking in the zebrafish is the means to directly measure synaptic transmission or to perform patch clamp analysis of hair cell currents. However, transgenic lines carrying genetic calcium indicators such as chameleon (2, 9, 18) will be an important tool for studying transmission or perhaps even transduction in zebrafish hair cells. Such experiments can be done in live, undissected larvae, providing real advantages over studies that rely on explants or isolated cells. As the collection of zebrafish auditory/vestibular mutants includes genes that appear to be acting downstream of mechanotransduction (T. Nicolson, unpublished observations), it is likely that more proteins involved in synaptic transmission will be found.

Genes Affecting Hair-Cell Survival

Many forms of human hearing loss are due to hair-cell degeneration, especially among the elderly (11). Genes that are required for hair-cell survival are therefore of great interest; however, there are few that have been reported thus far. Mutations in the *dog-eared/eya1* gene result in the reduction of hair cells, but this appears to be mainly a failure in differentiation or death of progenitors. In *dog-eared* mutants, cell death occurs within

the developing otocyst and lateral line primordia, but does not appear to be restricted to hair cells (24). Mutations in the *choroidermia* gene also cause hair cell death during an early developmental stage such that otic vesicles and neuromasts in *ru848/choroidermia* fish have very few hair cells remaining at 120 hpf (46). Additional effects include abnormal pigmentation and disorganization of the retinal pigment epithelium and photoreceptors in the

eye. The *choroidermia* gene is named after an X-linked disorder that causes photoreceptor degeneration in humans and encodes a Rab GTPase escort protein (6, 36). The effect on hair cell survival in *choroidermia* mutants remains to be explored. Efforts to understand the molecular basis of hair cell survival and regeneration are under way (17) and may lead to the discovery of important players in this process.

SUMMARY POINTS

1. FGF signaling from the hindbrain to the overlying ectoderm plays an important role in otic induction.
2. After induction, modeling of the ear is accomplished by diverse mechanisms such as Hedgehog signaling and the production of hyaluronic acid that leads to outgrowth of epithelial columns that form the semicircular canals.
3. Hair cells are specified by the Delta-Notch signaling pathway that is enhanced through endocytosis of the Delta ligand by the ubiquitin ligase, Mind bomb.
4. Mechanotransduction in zebrafish hair cells requires Cadherin 23 and Trpn1, two candidates for components of the transduction apparatus: the tip link and transduction channel, respectively.
5. The transducing organelle of the hair cell, the hair bundle, is dependent upon unconventional myosins and novel cadherins for integrity and function as seen in higher vertebrates such as mice and humans.

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Contents

John Maynard Smith <i>Richard E. Michod</i>	1
The Genetics of Hearing and Balance in Zebrafish <i>Teresa Nicolson</i>	9
Immunoglobulin Gene Diversification <i>Nancy Maizels</i>	23
Complexity in Regulation of Tryptophan Biosynthesis in <i>Bacillus subtilis</i> <i>Paul Gollnick, Paul Babitzke, Alfred Antson, and Charles Yanofsky</i>	47
Cell-Cycle Control of Gene Expression in Budding and Fission Yeast <i>Jürg Bähler</i>	69
Comparative Developmental Genetics and the Evolution of Arthropod Body Plans <i>David R. Angelini and Thomas C. Kaufman</i>	95
Concerted and Birth-and-Death Evolution of Multigene Families <i>Masatoshi Nei and Alejandro P. Rooney</i>	121
<i>Drosophila</i> as a Model for Human Neurodegenerative Disease <i>Julide Bilen and Nancy M. Bonini</i>	153
Molecular Mechanisms of Germline Stem Cell Regulation <i>Marco D. Wong, Zbigang Jin, and Ting Xie</i>	173
Molecular Signatures of Natural Selection <i>Rasmus Nielsen</i>	197
T-Box Genes in Vertebrate Development <i>L.A. Naiche, Zachary Harrelson, Robert G. Kelly, and Virginia E. Papaioannou</i>	219
Connecting Mammalian Genome with Phenome by ENU Mouse Mutagenesis: Gene Combinations Specifying the Immune System <i>Peter Papathanasiou and Christopher C. Goodnow</i>	241
Evolutionary Genetics of Reproductive Behavior in <i>Drosophila</i> : Connecting the Dots <i>Patrick M. O'Grady and Therese Anne Markow</i>	263

Sex Determination in the Teleost Medaka, <i>Oryzias latipes</i> <i>Masura Matsuda</i>	293
Orthologs, Paralogs, and Evolutionary Genomics <i>Eugene V. Koonin</i>	309
The Moss <i>Physcomitrella patens</i> <i>David Cove</i>	339
A Mitochondrial Paradigm of Metabolic and Degenerative Diseases, Aging, and Cancer: A Dawn for Evolutionary Medicine <i>Douglas C. Wallace</i>	359
Switches in Bacteriophage Lambda Development <i>Amos B. Oppenheim, Oren Kobiler, Joel Stavans, Donald L. Court,</i> <i>and Sankar Adhya</i>	409
Nonhomologous End Joining in Yeast <i>James M. Daley, Phillip L. Palmbo, Dongliang Wu, and Thomas E. Wilson</i>	431
Plasmid Segregation Mechanisms <i>Gitte Ebersbach and Kenn Gerdes</i>	453
Use of the Zebrafish System to Study Primitive and Definitive Hematopoiesis <i>Jill L.O. de Jong and Leonard I. Zon</i>	481
Mitochondrial Morphology and Dynamics in Yeast and Multicellular Eukaryotes <i>Koji Okamoto and Janet M. Shaw</i>	503
RNA-Guided DNA Deletion in Tetrahymena: An RNAi-Based Mechanism for Programmed Genome Rearrangements <i>Meng-Chao Yao and Ju-Lan Chao</i>	537
Molecular Genetics of Axis Formation in Zebrafish <i>Alexander F. Schier and William S. Talbot</i>	561
Chromatin Remodeling in Dosage Compensation <i>John C. Lucchesi, William G. Kelly, and Barbara Panning</i>	615

INDEXES

Subject Index	653
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