

Hearing Loss, Protection, and Regeneration in the Larval Zebrafish Lateral Line

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1 Introduction

The goal for this chapter is to introduce the zebrafish (*Danio rerio*) lateral line as a model system for hearing habilitation studies and to survey the literature on sensory hair cell loss, protection, and regeneration in the lateral line, specifically of larval zebrafish. The chapter opens with a brief history of zebrafish research and introduces some of the techniques that help make zebrafish a valuable species for these studies (Section 1). Subsequent sections discuss lateral line studies of genetic deafness (Section 2), drug-induced hair cell damage and protection (Sections 3 and 4), and hair cell regeneration (Section 5).

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1.1 *Zebrafish in the Lab*

Zebrafish (*Danio rerio*) are small, striped cyprinid fishes found in slow-moving streams and rice paddies in southern Asia (Engeszer et al., 2007). Although a hobby aquarist favorite for years, zebrafish have more recently found fame in studies of vertebrate development and neurobiology. Pioneering work was done by George Streisinger and his colleagues at the University of Oregon in the 1970s (see Grunwald & Eisen, 2002 for a historical review of the field). Although the main focus of this chapter is the use of the zebrafish lateral line as a model system for auditory research, the chapter begins with a brief overview of major technical advances in zebrafish research to familiarize readers with some of the techniques that are applied to the auditory work discussed later in the chapter.

Increasing popularity of zebrafish for laboratory experiments is attributable to several features. Adults are small (3–5 cm), hardy, and can be kept at relatively high densities in affordable, commercially available aquaculture systems. Given appropriate temperature, light cycle, and water conditions, adults will breed year-round in captivity, with a single pair capable of producing upwards of several hundred embryos per week from the time of reproductive maturity (around 3–4 months in the lab) until senescence at 18–24 months of age. The embryos are transparent and develop externally, making them an ideal organism in which to study vertebrate development *in vivo*. At optimal temperatures (24–28°C), development proceeds in a highly reproducible and stereotyped fashion, as described in detail by Kimmel et al. (1995).

1.2 *Techniques for Zebrafish Studies*

There are a number of techniques that facilitate the versatility of zebrafish larvae for developmental and applied biomedical studies. Unlike most vertebrate species, zebrafish are suited to high-throughput screens designed to identify genetic or pharmacological targets of interest. These screens can be subdivided into reverse or forward approaches. Reverse approaches start with a target, genetic or pharmacological, that holds promise based on available data. These studies are more directed and often limited to a specific category of genes or drugs that investigators are particularly interested in. In contrast to reverse-directed approaches, forward-screens are often referred to as unbiased because the researcher begins by isolating a phenotype of interest and then trace it back to the cause, genetic or pharmacological, without any “bias” as to the cause of the phenotype.

The first large-scale forward genetic screens in zebrafish were performed in the mid-1990s (Mullins et al., 1994). These screens identified mutations that alter vertebrate development, including development of the notochord, brain, inner ear, and lateral line (Schier et al., 1996; Stemple et al., 1996; Whitfield et al., 1996). Publication of the first zebrafish linkage map (Postlethwait et al., 1994), followed

by the sequencing of the zebrafish genome by the Sanger Institute, provided the tools to map and ultimately identify the genes underlying specific mutations of interest.

Complementing random mutagenesis methods are a suite of techniques for over- and underexpressing specific genes in zebrafish. Transgenic zebrafish lines (those that carry an inserted or “foreign” gene) can be generated using the *tol2* transposon, allowing for tissue-specific overexpression of a gene of interest or of a fluorescent reporter such as green fluorescent protein (GFP) (Kawakami et al., 2004; Burket et al., 2008; Suster et al., 2009). Injection of antisense morpholino oligonucleotides (morpholinos) at the one-cell stage is a powerful method of globally reducing gene expression in developing embryos, although the knock-down effect is efficient only during the first few days post-fertilization owing to a dilution of the morpholino during subsequent rounds of cell division (Nasevicius & Ekker, 2000). Morpholino knockdown of specific mRNA transcripts has helped reveal a suite of genes necessary for lateral line and inner ear development (Whitfield et al., 2002; Kerstetter et al., 2004). More recently, zinc-finger nuclease (ZFN) technology has been applied to zebrafish, allowing researchers to create targeted mutations that are stably inherited (Doyon et al., 2008; Meng et al., 2008; Foley et al., 2009).

Screening of compound libraries, sometimes also referred to as “chemical genetics,” is another major advance in zebrafish research, providing key insights into both fundamental biological processes and disease mechanisms. Larval zebrafish are exposed to a known drug, a small molecule, or other drug-like compound from a library of hundreds or thousands of molecules, and assayed for a specific phenotype (reviewed in Kaufman et al., 2009). Zebrafish small molecule screens have identified compounds that affect many processes including organ and tissue development, tissue regeneration, and behavior patterns (Peterson et al., 2000; Mathew et al., 2007; Rihel et al., 2010). One advantage of chemical genetics is that zebrafish can be exposed to small molecules for precisely controlled intervals, not usually available with traditional mutagenesis screens. However, a drawback to small molecule screens is that in many cases, the molecular targets of these compounds are unknown. Both traditional genetic and chemical genetic screens have been applied to studies of hair cell loss, protection, and regeneration in the zebrafish lateral line (Chiu et al., 2008; Owens et al., 2008; Namdaran et al., 2012).

1.3 Zebrafish Lateral Line

The larval zebrafish lateral line consists of a series of neuromasts on the head (anterior lateral line) and the body (posterior lateral line) (see the chapter by Webb in this volume and Fig. 1 for a comparison of the adult and larval neuromast distribution). Neuromasts are located in stereotyped positions on the developing animal (Metcalf et al., 1985; Raible & Kruse, 2000). The posterior lateral line develops from a migrating primordium that deposits neuromasts along the trailing edge of the

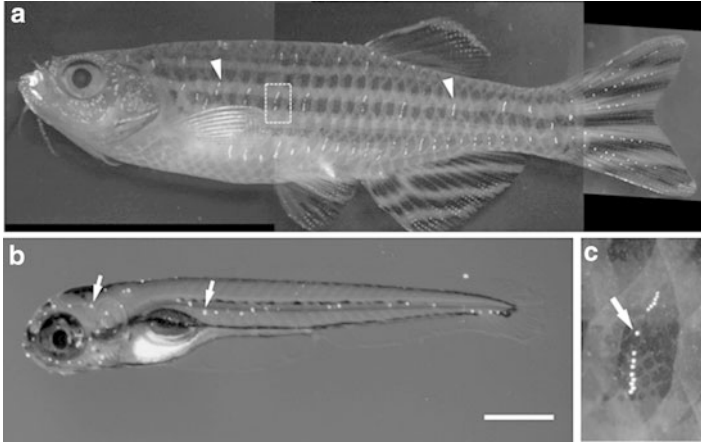


Fig. 1 Adult zebrafish (a) and 5 dpf larva (b), labeled with the vital dye DASPEI. In adults, neuromasts bud off and create stiches, the vertical lines of neuromasts (arrowheads). Example neuromasts are indicated by arrows and appear as white dots along the head and body of larva and as individual dots within stiches of adult (inset, c). Scale bar in b = 500 μ m. (The image in b is from *Zebrafish*, 2010. Reprinted with permission of Mary Ann Liebert, New Rochelle, NY)

migratory body (David et al., 2002; reviewed in Ghysen & Dambly-Chaudiere, 2004). Each neuromast contains about 10–20 sensory hair cells and associated supporting cells and receives afferent and efferent innervation from the lateral line ganglia (Raible & Kruse, 2000; Ghysen & Dambly-Chaudiere, 2004). In zebrafish, the lateral line is already partially functional by 4 days post-fertilization (dpf), allowing for studies in relatively young larvae. As in other species (see the chapter by McHenry & Liao), it is thought that the zebrafish lateral line contributes to a variety of behaviors that involve detection of near-field water movements including prey detection, predator avoidance, orientation in current, and schooling.

Just like mammalian inner ear hair cells, zebrafish lateral line hair cells are susceptible to ototoxic drugs such as aminoglycoside antibiotics and platinum-based chemotherapy compounds (Harris et al., 2003; Ou et al., 2007; Owens et al., 2007). Moreover, homologs of several human deafness genes cause loss of hair cell function in the zebrafish lateral line (Ernest et al., 2000; Seiler et al., 2004). These genetic similarities in hair cell development and maintenance, combined with the similar responses to known ototoxic compounds, serve as the foundation for using the zebrafish lateral line as a model system for biomedical studies of hair cell loss and protection. Additional advantages include the external location of the hair cells that facilitates drug delivery and imaging for studies of dynamic events and hair cell viability, and the small size and high fecundity of the adults. Remarkably, zebrafish, as well as other fishes and most nonmammalian vertebrates, can regenerate lost hair cells and restore sensory function, making them an appealing model for hearing restoration and regenerative medicine (Corwin & Cotanche, 1988; Ryals & Rubel, 1988; Ma et al., 2008).

1.4 Hearing and Balance Disorders: What Is Being Modeled?

Hearing loss caused by damage to or death of sensory hair cells is the most common worldwide sensorineural disorder. As of 2004 the World Health Organization estimated that more than 275 million people live with moderate to profound hearing impairment, defined as a hearing loss that interferes with daily activities (WHO 2012). This can have a devastating impact on an individual with hearing loss, his or her family, and on the economy. Affected children often have delayed language development and impaired learning, while adults report feelings of isolation and reduced quality of life (Dalton et al., 2003; Kral & O'Donoghue, 2010). In the United States alone, the economic burden was estimated at \$154–186 billion per year as of 2000, making hearing loss not only common but also exceedingly costly (Ruben, 2000).

Hearing loss can be caused by many factors including genetic mutations, workplace and recreational noise exposure, ototoxic drugs, and aging (see Keats et al., 2002 [SHAR Vol. 14]; Schacht et al., 2008 [SHAR Vol. 31]). Zebrafish provide a platform for study of individual factors and for analysis of epistatic interactions in hearing loss. Hair cells in the vestibular system can also be damaged by similar means, with equally devastating consequences (Zingler et al., 2007; Eppsteiner & Smith, 2011). Peripheral vestibular dysfunction causes dizziness and vertigo and is partially responsible for many of the falls experienced by the elderly (Barin & Dodson, 2011). Therefore, a better understanding of hair cell death, protection, and regeneration will facilitate habilitation of both auditory and vestibular function.

1.5 Tools of the Trade

1.5.1 Visualizing the Lateral Line

Several tools and techniques have been developed in recent years that facilitate studies of the lateral line in hair cell death, protection, and regeneration, particularly in the area of neuromast visualization. Figures 1 and 2 show representative examples of some of the labeling techniques available.

Vital dyes are arguably the easiest and most accessible way to label lateral line hair cells, with several dyes in widespread use. The mitochondrial potential dye DASPEI is a favorite, having been used to label the lateral line of many fish species, including both adult and larval zebrafish (Harris et al., 2003; Van Trump et al., 2010). Yo-Pro1 and related compounds are DNA-binding dyes that delineate hair cell nuclei, while FM1-43 is generally considered a marker for endocytosis and labels the hair cell cytoplasm (Meyers et al., 2003; Santos et al., 2006). All of these dyes are quickly taken up by hair cells when added to the embryo medium

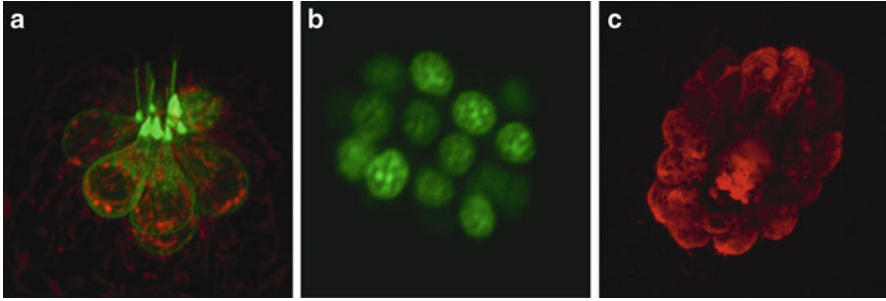


Fig. 2 Examples of different hair cell labeling techniques for lateral line visualization and quantification. **(a)** Hair cells in Brn3c:mGFP transgenic fish express GFP in the plasma membrane (green). This fish is also labeled with the vital dye MitoTracker Red to visualize mitochondria (red). **(b)** Hair cell nuclei labeled with the vital dye Yo-Pro1. Images in both **a** and **b** were collected in live, anesthetized larvae. In contrast, **(c)** shows an example of a post-fixation labeling technique, in this case labeling with an antibody to parvalbumin. (The image in **b** is from *Zebrafish*, 2010. Reprinted with permission of Mary Ann Liebert, New Rochelle, NY)

surrounding the fish. FM1-43 is additionally useful in that it is taken up in a transduction-dependent manner and is therefore considered a proxy for healthy hair cells (Meyers et al., 2003).

In addition to vital dye labeling, several groups have generated transgenic fish lines that express fluorescent proteins in hair cells, including Green Fluorescent Protein (GFP) driven by the Brn3c, parvalbumin3, or atp2b1a promoters (Parinov et al., 2004; Xiao et al., 2005; McDermott et al., 2010). These fish lines are useful for visualizing immature hair cells, as the promoters are often turned on early in hair cell development before some vital dyes can be internalized.

Finally, several classic post-fixation techniques are useful for visualizing zebrafish hair cells. Actin-rich stereocilia can be labeled with fluorescently tagged phalloidin (Williams & Holder, 2000; Harris et al., 2003), while antibodies to myosin VI, myosin VIIa, S100, and parvalbumin label hair cell bundles and soma (Germana et al., 2004; Lopez-Schier & Hudspeth, 2006; Coffin et al., 2007). Electron microscopy, although more labor-intensive than other techniques, also provides information on hair cell morphology and ultrastructure (Williams & Holder, 2000; Harris et al., 2003; Owens et al., 2007).

1.5.2 Assessing Lateral Line Function

Although most hearing habilitation studies in the zebrafish lateral line have relied heavily on imaging techniques, methods for electrophysiological and behavioral assessment of lateral line function have recently been developed. Work in Teresa Nicolson's laboratory has established protocols for recording hair cell microphonics and responses from individual lateral line afferents under both spontaneous and fluid jet-stimulated conditions in intact, immobilized larvae (Obholzer et al., 2008;

Trapani and Nicolson, 2011). These techniques have been useful in dissecting auditory pathways in zebrafish mutants to identify the physiological level of defects. Further behavioral assays include rheotaxis (orientation to water flow) (Suli et al., 2012) and flow-mediated startle responses (McHenry et al., 2009) for quantifying lateral line-mediated behavior. These techniques add to zebrafish lateral line research by facilitating comparisons of structure, function, and behavior under the same experimental conditions.

2 Genetic Deafness

There are many causes of deafness, but it is estimated that 60–70% of all deafness is the result of heterogeneous genetic factors (Raviv et al., 2010). There is a massive body of literature in this area, and an overview of clinical genetics is available in an earlier volume of the SHAR series, *Genetics of Auditory Disorders* (Keats et al., 2002). Although clinical genetics is an active area of research, there are difficulties inherent in human studies, such as geographical dispersion of family members, identification of families of appropriate size and genetic background, gaps in pedigrees, and obtaining approval to collect, transport, and process samples from patients that make the appeal of model organisms obvious. Model systems have been critical for identifying and understanding the function of genes linked to hearing and hearing loss, with mice in particular playing an important role. The following section examines the role of zebrafish in identifying novel deafness genes and elucidating the underlying causes of genetic deafness.

2.1 Mutagenesis Screens for Deafness Genes

The majority of genetic screens in zebrafish use the mutagen *N*-ethyl-*N*-nitrosurea (ENU) (Mullins et al., 1994; Solnica-Krezel et al., 1996). ENU introduces point mutations that can be later identified by traditional linkage mapping techniques. ENU mutagenesis screens have generated hundreds of mutant lines, many of which display morphological and/or functional defects in hearing and balance (reviewed in Whitfield, 2002; Nicolson, 2005).

Mutagenesis screens in zebrafish initially focused on isolating mutants exhibiting morphological defects visible under a dissecting microscope (Haffter et al., 1996; Malicki et al., 1996; Whitfield et al., 1996). The first major genetic screen for ear mutations produced 78 mutants with phenotypes such as very small or large ear size, abnormal position, size or number of otoliths, or malformation of internal structures such as the shape of the vestibular organs or changes in other aspects of development (Malicki et al., 1996; Whitfield et al., 1996). Secondary screening was performed to determine whether sensory patches in the auditory, vestibular, and lateral line systems developed normally in these ear-morphology mutants. Two of the mutants

displayed posterior lateral line abnormalities; *dog* mutants had fewer neuromasts than wild-type animals whereas *hypersensitive* mutants had nearly double the number of neuromasts (Whitfield et al., 1996).

Many of the mutants identified on the basis of morphological defects also displayed abnormal responses to noise and/or unusual swimming behaviors, as might be expected in animals with compromised function of the ear. Balance-defective swimming behaviors included swimming in circles, either horizontal or vertical, or swimming in a corkscrew path when stimulated. In the absence of stimulation, many of these mutants rested sideways or upside down on the bottom despite the presence of an inflated swim bladder, again suggesting compromised vestibular function. Behavioral phenotypes also revealed mutants that failed to respond to sound or vibrational stimuli, such as a tap on the dish, although they swam away from tactile stimulation (Malicki et al., 1996; Whitfield et al., 1996).

Genetic screening for motility in response to touch led to the identification of 15 additional mutants with balance-defective swim phenotypes in the absence of gross morphological defects of the ear (Granato et al., 1996). On further investigation, these “circler” mutants revealed more subtle defects such as decreased hair bundle integrity or defects in transduction (Nicolson et al., 1998).

Together, these morphological- and behavioral-genetic screens for ear-related abnormalities have isolated more than 90 mutant lines that have since been linked to at least 30 genes and the numbers continue to increase. The majority of genes isolated in these screens have been linked to mammalian orthologs, and more than a third are associated with human deafness syndromes including Usher syndrome and Waardenburg syndrome (reviewed in Whitfield, 2002; and Nicolson, 2005).

One such example of a zebrafish model of human deafness is the *mariner* mutant. *Mariner* was one of the mutants initially identified by its circler phenotype and further analysis revealed that *mariner* is also unresponsive to acoustic/vibrational stimuli (Granato et al., 1996; Nicolson et al., 1998). The mutation was later mapped to *myosin7a*, an unconventional myosin highly expressed in both zebrafish and mammalian hair cells (Ernest et al., 2000). A mouse model also exists for *myosin7a*. First described in 1929, *shaker* mice have progressive hearing loss and a tendency to walk in circles, a phenotype similar to the circular swimming pattern observed in *mariner* (Lord & Gates, 1929; Gibson et al., 1995). These models are also similar at the cellular level: lack of a robust electrophysiological response to stimuli and disorganized hair bundles are observed in *mariner* zebrafish (Nicolson et al., 1998; Ernest et al., 2000) and *shaker* mice (Richardson et al., 1997; Self et al., 1998). These models with mutations in *myosin7a* also contribute to the study of human deafness diseases. In patients, *MYOVI*A has been linked to Usher1B syndrome (Weil et al., 1995) and both recessive and dominant forms of nonsyndromic deafness (Liu et al., 1997a,b).

Similar cases, where genetic mutations in zebrafish mutants act as models for human deafness, and often in parallel with other mammalian models, exist for many genes. Additional zebrafish mutants exist in structural genes such as *myosin6b* and *cadherin23* and developmental genes including *eyal*, *sox10*, and *pax2*. These and additional zebrafish models of hearing disease are covered in more details in a wide

variety of reviews (Whitfield, 2002; Nicolson, 2005). The ability of zebrafish to phenocopy many of the major aspects of human hearing by single gene mutations provides strong support for the continued use of zebrafish as a model for the behavioral and molecular studies of deafness genes.

The majority of screens have been performed on larval zebrafish 5 dpf or younger. However, at least one screen was performed on adult zebrafish by testing their ability to respond to tone bursts at 400 Hz (Bang et al., 2001). In screening more than 6,500 adult animals roughly 1% had an abnormal response to the sound stimuli. The majority of the mutants isolated in this screen had defects limited to structures important for sound conduction, such as the swim bladder. Morphological defects were also detected in specialized hearing structures specific to Otophysan fishes, such as the Weberian ossicles. Although this particular screen did not identify any new genes important for hair cell development or function, it is the sole example of high-throughput, sound-based screening of adult zebrafish and introduced a technique that may have exciting future applications.

3 Ototoxicity

Certain therapeutic drugs can also cause sensorineural hearing loss. Aminoglycoside antibiotics (e.g., streptomycin, neomycin, gentamicin) and platinum-based chemotherapeutics (e.g., cisplatin) are the most well studied ototoxic drugs, both in terms of documented cases of deafness in human patients and in animal studies aimed at understanding mechanisms underlying ototoxic responses. Table 1 lists drugs known to cause hair cell loss in zebrafish.

3.1 *Effects of Known Ototoxins on the Zebrafish Lateral Line*

3.1.1 Aminoglycosides

Aminoglycoside ototoxicity was first discovered in the 1940s when reports of hearing loss cropped up in patients receiving streptomycin treatment (Schacht & Hawkins, 2006). The first lateral line experiment was performed in 1964, showing that streptomycin reduced lateral line microphonic potentials in the burbot *Lota lota*, a freshwater gadiform fish indigenous to parts of Europe and North America (Wersäll & Flock, 1964). Subsequent studies on the lateral line of other fish and amphibian species have confirmed the ototoxic effects of several aminoglycoside drugs (Kroese & van den Bercken, 1980, 1982; Kaus, 1987; Song et al., 1995). As a result, aminoglycoside treatment has become a standard tool for blocking sensory input in behavioral studies designed to investigate lateral line function (e.g., Montgomery et al., 1997; Coombs et al., 2001).

Table 1 Ototoxic drugs identified or confirmed in zebrafish lateral line

Drug name	Drug category/use	Reference
Neomycin	Aminoglycoside antibiotic	Williams & Holder, 2000; Harris et al., 2003
Gentamicin	Aminoglycoside antibiotic	Ton & Parng, 2005; Owens et al., 2009
Kanamycin	Aminoglycoside antibiotic	Owens et al., 2009
Cisplatin	Antineoplastic, DNA crosslinker	Ton & Parng, 2005; Ou et al., 2007
Chloramphenicol	Antibiotic	Chiu et al., 2008
Chlortetracycline HCl	Antibiotic	Chiu et al., 2008
Pentamidine isethionate	Antiprotozoal	Chiu et al., 2008
Spermadine	Ornithine decarboxylase inhibitor	Chiu et al., 2008
Tobramycin	Antibiotic	Chiu et al., 2008
Propantheline bromide	Anticholinergic	Chiu et al., 2008
Ethacrynic acid	Loop diuretic	Chiu et al., 2008
Pomiferin	Antioxidant	Chiu et al., 2008
Chlorophyllide	Antineoplastic	Chiu et al., 2008
Estradiol valerate	Estrogen	Chiu et al., 2008
Pentetrazole	CNS/respiratory/circulatory stimulant	Chiu et al., 2008
Guaiazulene	Antioxidant	Chiu et al., 2008
Rosolic acid	Diagnostic aid	Chiu et al., 2008
Vincamine	Vasodilator	Chiu et al., 2008
Demeclocycline HCl	Antibiotic	Chiu et al., 2008
Mefloquine	Antiprotozoal	Chiu et al., 2008
Candesartan	Angiotensin 1 receptor antagonist	Chiu et al., 2008
Simvastatin	HMGCoA reductase inhibitor, antihyperlipidemic	Chiu et al., 2008
Oxaliplatin	Antineoplastic, DNA cross linker	Hirose et al., 2011
Nitrogen mustard	Antineoplastic, alkylating agent	Hirose et al., 2011
Vincristine	Antineoplastic, microtubule inhibitor	Hirose et al., 2011
Vinorelbine	Antineoplastic, microtubule inhibitor	Hirose et al., 2011
Imatinib	Antineoplastic, receptor tyrosine kinase inhibitor	Hirose et al., 2011
Doxorubicin	Antineoplastic, topoisomerase poison	Hirose et al., 2011
Vinblastine	Antineoplastic, microtubule inhibitor	Ton & Parng, 2005; Hirose et al., 2011
Sunitinib	Antineoplastic, receptor tyrosine kinase inhibitor	Hirose et al., 2011
Raloxifene	Antineoplastic, estrogen receptor modulator	Hirose et al., 2011
Dactinomycin	Antineoplastic, transcription inhibitor	Hirose et al., 2011
Carmustine	Antineoplastic, alkylating agent	Hirose et al., 2011
Exemestane	Antineoplastic, steroidal aromatase inhibitor	Hirose et al., 2011
Quinine	Anti-malarial	Ton & Parng, 2005

At the beginning of the twenty-first century investigators began using the zebrafish lateral line as a model system to study hair cell toxicity due to therapeutic drugs. An initial study by Williams and Holder (2000) showed that neomycin robustly kills lateral line hair cells, and Harris et al. (2003) documented these effects more thoroughly by demonstrating that the response to neomycin is dose-dependent and similar in each neuromast. Hair cell susceptibility is dependent on both fish age and hair cell age, as hair cells in 4 dpf fish are significantly less sensitive to neomycin damage than those in 5 dpf or older animals (Murakami et al., 2003; Santos et al., 2006). These findings are consistent with mammalian studies that show a correlation between aminoglycoside sensitivity and hearing onset (Marot et al., 1980). Hair cells in adult zebrafish lateral line are also susceptible to aminoglycoside damage, suggesting that ototoxic responses of mature hair cells remain stable throughout the life of the animal (Van Trump et al., 2010).

More recent studies in the zebrafish lateral line seek to discover underlying cell death mechanism(s) responsible for aminoglycoside toxicity. Mitochondrial swelling, loss of mitochondrial membrane potential, and the necessity for mitochondrial-associated Bcl2 proteins all suggest that aminoglycosides activate mitochondrial-dependent cell death pathways (Owens et al., 2007; Coffin et al., 2013). Similar events have been demonstrated in rodent and chick inner ear, showing concordance between zebrafish and mammalian hair cell death responses (Hirose et al., 1999; Cunningham et al., 2004; Mangiardi et al., 2004; Matsui et al., 2004). Nuclear condensation and activation of classical caspase-dependent apoptosis may also occur, although the necessity of this class of proteases in hair cell death is debated (Williams & Holder 2000; Cunningham et al., 2002; Matsui et al., 2004; Jiang et al., 2006; Owens et al., 2007). Interestingly, recent research demonstrates that the time course of hair cell death differs dramatically for different aminoglycoside antibiotics, suggesting that these aminoglycosides may kill hair cells via different intracellular signaling cascades (Coffin et al., 2009; Owens et al., 2009). Further elucidation of hair cell death mechanisms is necessary to provide targeted therapy for patients who receive aminoglycoside treatment.

Although several studies agree that aminoglycosides cause dose-dependent hair cell death in the zebrafish lateral line, closer examination of these studies reveals interesting differences in concentration-dependent effects. Little hair cell death occurs with neomycin concentrations under 50 μM , and that at least 200 μM neomycin is required for virtually complete hair cell loss (Harris et al., 2003; Murakami et al., 2003; Owens et al., 2007). In contrast, concentrations as low as 10 μM were shown to cause significant hair cell loss by other groups, with complete hair cell death seen after treatment with just 16 μM neomycin (Williams & Holder, 2000; Ton & Parg, 2005). These dose-dependent differences are due to differences in ion composition in the embryo medium used by the different research groups (Coffin et al., 2009). Reducing the concentration of calcium or magnesium makes hair cells more sensitive to neomycin such that low concentrations become highly toxic (Kaus, 1992; Coffin et al., 2009). Divalent cations, specifically calcium, are thought to regulate the open probability of the hair cell transduction channel such that a greater fraction of channels are open at rest under low calcium conditions

(Corey & Hudspeth, 1983; Ricci & Fettiplace, 1998). Given that aminoglycoside uptake by hair cells is transduction-dependent and likely enters directly through the transduction channel itself (Steyger et al., 2003; Marcotti et al., 2005), the ability to shift the dose–response relationship of neomycin via modulation of divalent cation concentration is logical. Collectively, these findings underscore the need for careful documentation and appropriate controls so that lateral line ototoxicity data may be accurately compared between labs. They also lend additional support to recent evidence that post-treatment verification of hair cell loss is necessary when aminoglycosides are used as tools for blocking sensory input to the lateral line (Van Trump et al., 2010; Brown et al., 2011).

3.1.2 Cisplatin

Cisplatin is a highly toxic, platinum-based chemotherapeutic agent used in the treatment of a variety of solid tumors. Whereas aminoglycosides appear to kill lateral line hair cells with distinctly nonlinear time parameters, cisplatin has more or less predictable effects. There is a log-linear relationship between dose and time for loss of cisplatin-treated hair cells, suggesting that cisplatin damage is related to total intracellular cisplatin accumulation (Ou et al., 2007). There is little work to date on cell death signaling cascade activation in cisplatin-treated lateral line hair cells. Ton and Parng (2005) found that several antioxidants, including *N*-acetyl L-cysteine and D-methionine, protected hair cells in cisplatin-treated zebrafish, suggesting that cisplatin may induce oxidative stress pathways in damaged hair cells. Oxidative stress pathways have been implicated in both cisplatin and aminoglycoside ototoxicity, suggesting some conservation of cell death mechanisms between different classes of ototoxic drugs (Sha & Schacht, 2000; Rybak & Ramkumar, 2007).

Cisplatin-treated cells in the zebrafish lateral line also show signs of apoptotic cell death including nuclear condensation and mitochondrial swelling (Ou et al., 2007; Giari et al., 2011). Nuclear condensation and altered distribution of mitochondrial proteins such as cytochrome *c* are also evident in cochlear hair cells from cisplatin-treated guinea pigs, suggesting that these are conserved features of cisplatin-induced hair cell death in vertebrates (Wang et al., 2004). More studies are needed to dissect fully the contribution of specific cell death pathways to cisplatin ototoxicity.

3.1.3 Copper

Several studies have shown that copper is toxic to zebrafish lateral line hair cells (Hernandez et al., 2006; Linbo et al., 2006; Olivari et al., 2008). Copper concentrations from 1 to 50 μM rapidly kill hair cells, with the first signs of damage apparent within 5 minutes of exposure to 5 μM copper sulfate (Olivari et al., 2008). Copper-damaged hair cells show similar morphological changes to those seen in

hair cells damaged with other ototoxins, including signs of both apoptosis and necrosis (Hernandez et al., 2006; Olivari et al., 2008).

Copper is not considered an ototoxic threat in humans, so at first pass it appears that these studies are more important from a toxicology perspective than a biomedical one. However, evidence in mammalian inner ear suggests that cisplatin may be taken up by hair cells via a copper transporter (More et al., 2010). Therefore, some investigators have ironically suggested using copper as a competitive inhibitor of cisplatin entry into hair cells as a means of preventing cisplatin ototoxicity (More et al., 2010; Ding et al., 2011). These zebrafish studies highlight the potentially ototoxic nature of copper and suggest that copper is probably not a therapeutically viable protective strategy.

3.1.4 Discovery of Novel Ototoxins

Although there are incidental case reports of ototoxic side effects linked to several different drugs, as prescription drug use is greatest in older humans, ototoxic effects may be masked by or misdiagnosed as age-related hearing loss, or considered part of disease onset rather than an effect of treatment (Seligmann et al., 1996). Currently there are no systematic mechanisms in place to ensure identification of potential ototoxic side effects of therapeutic drugs. To tease out ototoxic effects that might have been overlooked, the zebrafish lateral line is now being used to screen for potential ototoxic drugs (Chiu et al., 2008; Hirose et al., 2011). The first screen was performed with the National Institute of Nervous Diseases and Stroke (NINDS) Custom Collection II library, which is composed of 1040 FDA-approved drugs and bioactive compounds (Chiu et al., 2008). Twenty-one potentially ototoxic compounds were identified in this screen, including known ototoxins such as neomycin and cisplatin, demonstrating that the screening paradigm is sensitive enough to pick up known toxins. Several novel candidate ototoxins were also detected from diverse drug classes, including multiple nonaminoglycoside antibiotics, antiprotozoal medications, and anticholinergic compounds. Two candidate ototoxins, propantheline and pentamidine, were tested for ototoxicity in mammals and both were found to kill hair cells in mouse utricle cultures, highlighting the utility of zebrafish lateral line as a first-pass screen for ototoxicity in other hair cell systems (Chiu et al., 2008).

Given the known ototoxicity of antineoplastic drugs such as cisplatin, an additional screen was performed of the 88 compounds in the National Cancer Institute (NCI) Approved Oncology Drugs Set to determine whether other anticancer drugs act as hair cell toxins (Hirose et al., 2011). As with the NINDS library screen described in the preceding paragraph, the NCI library screen detected several known ototoxins, demonstrating the robust nature of the screening paradigm. This NCI screen also identified four suspected ototoxins (those with hearing loss noted in occasional case reports) and five novel putative ototoxins, as well as ototoxic combinations of chemotherapy cocktails (see Table 1 for a complete list).

This study suggests that many neoplastic agents may have unrecognized ototoxic effects and that multidrug chemotherapy regimens are an area of concern.

There is currently no FDA requirement for ototoxicity testing during the drug development process, nor in the development of multidrug regimens. These drug screens demonstrate that many currently approved drugs may have unrecognized ototoxic potential and that the zebrafish lateral line is a powerful model system for discovery of novel ototoxins already in clinical use as well as for identifying the potential ototoxic effects of combinatorial drug regimens.

4 Protecting Hair Cells from Chemical Toxins

As discussed in Section 3, the larval zebrafish lateral line system has aided in the identification of potentially ototoxic drugs and the cell death mechanisms activated by these compounds. It is also well suited for screening for genes and small molecules that protect hair cells from ototoxic agents. Protective genes and compounds discovered in zebrafish are listed in Table 2.

Table 2 Hair cell protectants identified in zebrafish

(C) Compound/(G) gene	Target/gene ID	Protects against ^a	Reference
(C) PROTO1	unknown	Neomycin	Owens et al., 2008
(C) PROTO2	unknown	Neomycin	Owens et al., 2008
(G) Sentinel	Human CC2D2A gene	Neomycin	Owens et al., 2008
(G) Persephone	Chloride-bicarbonate exchanger	Neomycin	Owens et al., 2008
(G) Merovingian	unknown	Neomycin	Owens et al., 2008
(G) Bane	unknown	Neomycin	Owens et al., 2008
(G) Trainman	unknown	Neomycin	Owens et al., 2008
(C) Glutathione	Antioxidant	Cisplatin	Ton & Parng, 2005
(C) Allopurinol	xanthine oxidase inhibitor	Cisplatin	Ton & Parng, 2005
(C) <i>N</i> -acetyl-L-cysteine	Antioxidant	Cisplatin	Ton & Parng, 2005
(C) 2-Oxothiazolidine-4-carboxylate	Antioxidant, cysteine precursor	Cisplatin	Ton & Parng, 2005
(C) D-Methionine	Antioxidant	Cisplatin	Ton & Parng, 2005
(C) Epicatechin	Antioxidant	Cisplatin	Kim et al., 2008
(C) Amsacrine	Topoisomerase 2 poison	Neomycin	Ou et al., 2009
(C) Carvedilol	Beta-2 adrenergic blocker	Neomycin	Ou et al., 2009
(C) Cepharanthine	Plasma membrane stabilizer	Neomycin	Ou et al., 2009
(C) Drofenine	Acetylcholinesterase inhibitor	Neomycin	Ou et al., 2009
(C) Hexamethylenamiloride	Na/H exchange inhibitor	Neomycin	Ou et al., 2009
(C) Phenoxybenzamine	α -1 Adrenergic blocker	Neomycin	Ou et al., 2009
(C) Tacrine	Acetylcholinesterase inhibitor	Neomycin	Ou et al., 2009

^aThis indicates what ototoxin was used to identify the protective agent. Protection against other ototoxins has not yet been confirmed.

4.1 *Uncovering Genes that Modulate Hair Cell Loss*

Several kinds of evidence demonstrate that genetic differences can modulate responses to ototoxic challenges. For example, certain mitochondrial mutations increase patient susceptibility to aminoglycoside-induced hearing loss (Prezant et al., 1992; Guan et al., 2000). Another important example is the demonstration of a major genetic contribution to the age and severity of age related hearing loss (Gates et al., 1999). However, aside from the mitochondrial DNA mutations, few of these genetic modifiers have been identified. A zebrafish ENU mutagenesis screen (see Section 2) to identify genes that alter susceptibility to neomycin-induced hair cell death discovered five simple recessive mutations and five mutations with more complex genetics, each of which protected hair cells from neomycin damage (Owens et al., 2008). Linkage mapping and DNA sequencing in a protective mutant, known as *sentinel*, uncovered a premature stop codon in a novel gene of unknown function that was later linked to the human CC2D2A gene mutated in Joubert syndrome (Gorden et al., 2008). The *sentinel* mutation does not protect hair cells from cisplatin damage, further supporting the suggestion of segregation of cell death and protective pathways between ototoxic insults (Owens et al., 2008).

4.2 *Discovery of Otoprotective Drugs*

The zebrafish lateral line has been used to discover drugs and drug-like small molecules that protect hair cells from exposure to ototoxic conditions. Several research groups have undertaken targeted testing of candidate compounds based on a priori assumptions about hair cell death processes. For example, Ton and Parng (2005) showed that multiple antioxidants protected lateral line hair cells from cisplatin exposure (see Section 3.1.2). Kim et al. (2008) reported that the green tea extract epicatechin also protected lateral line hair cells from cisplatin-induced death.

The alternative approach of large- or medium-scale screening of known drugs or small, drug-like molecules offers the possibility for the discovery of novel protective drugs. The first such screen identified two novel protective compounds now named PROTO1 and PROTO2 (Owens et al., 2008). These PROTO compounds, both urea thiophene carboxamides, robustly protect zebrafish hair cells from neomycin damage but not cisplatin toxicity. Further experiments show that PROTO1 also provided robust protection of mammalian hair cells from aminoglycoside exposure (Owens et al., 2008). PROTO1 does not prevent uptake of fluorescently tagged aminoglycoside, suggesting that this compound may target specific intracellular processes activated after aminoglycoside entry into hair cells and further, that these processes are not acting in cisplatin-mediated hair cell death. Importantly, the PROTO compounds do not interfere with the antibacterial action of aminoglycosides.

Discovery of a novel small molecule compound is the first step in the clinical development of new otoprotective drugs. Additional steps include the development

and testing of alternative chemical formulations, assessment of pharmacokinetics, and evaluation of cell-based actions of the drugs. However, development of a novel drug is an exceedingly long and expensive process, requiring several years and millions of dollars (Chong & Sullivan, 2007; Boguski et al., 2009). Therefore, repurposing of existing drugs for new uses offers an attractive alternative to *de novo* drug development. Using this logic, Ou et al. (2009) screened the NINDS Custom Collection II library (see Section 3.1.4) for new otoprotective compounds. Using the same drug library for multiple screen purposes (e.g., ototoxicity and otoprotection) further improves the efficiency and cost-effectiveness of the screening process.

Seven confirmed “hits” were obtained, with candidates belonging to multiple diverse drug classes including acetylcholinesterase inhibitors (Drofenine and Tacrine), diuretics (Hexamethylenamiloride), and a plasma membrane stabilizer (Cepharanthine), all of which are currently in clinical use. All seven drugs provided dose-dependent protection against neomycin-induced hair cell loss (see Table 2). Tacrine also protected mouse utricular hair cells from neomycin damage in vitro, again suggesting that protective compounds discovered in zebrafish screens may be clinically useful (Ou et al., 2009). Collectively, these results offer robust proof-of-concept for the zebrafish lateral line system as a model for hearing-related drug discovery and highlight some important areas for future research on ototoxicity using drugs already approved for clinical use.

5 Hair Cell Regeneration

5.1 *Hair Cell Regeneration in Diverse Vertebrate Taxa and Epithelia: A Brief Primer*

Hair cell regeneration in amphibians was first documented in the 1930s as a component of larger studies on limb and tail regeneration. When tails are amputated from larval salamanders (*Amblystoma punctatum*), the regenerated tail contains a new lateral line, complete with neuromasts and hair cells (Stone, 1933, 1937). Since then, lateral line hair cell regeneration, in response to damage ranging from tail amputation to cell-specific ablation, has been routinely seen in a variety of larval amphibians, broadly including anurans (frogs and toads) and urodeles (salamanders) reviewed in Chapter 8 of *The Mechanosensory Lateral Line* (Coombs et al., 1989). Similarly, regeneration of lateral line hair cells has been well documented in the zebrafish (reviewed in Brignull et al., 2009). Many more studies have examined hair cell regeneration in the inner ear of anamniotes (fishes and amphibians). For example, the vestibular system of mature bullfrogs (*Rana catesbeiana*: Baird et al., 1993), and the sensory epithelia of mature oscars (*Astronotus ocellatus*: Lombarte et al., 1993), goldfish (*Carassius auratus*: Smith et al., 2006), and Atlantic cod (*Gadus morhua*: Faucher et al., 2009) all regenerate hair cells after damage. In cases where it has been examined, anamniotes combine

the ability to regenerate hair cells after damage with the continuous production of new hair cells in undamaged tissue in the ear (reviewed in Corwin, 1992; Lombarbte & Popper, 1994; Lanford et. al., 1996) or the lateral line (Jørgensen, 1991). The continued production of hair cells is generally thought to replace hair cells lost to aging and to maintain the density of hair cells in sensory epithelia that continue to grow throughout an animal's lifetime.

Regenerative capacity appears slightly more limited in nonmammalian amniotes such as birds and reptiles, in which studies on hair cells are, by necessity, limited to the ear. Hair cell regeneration occurs in the vestibular system of the lizard *Podaris sicula* (Avallone et al., 2003, 2008), and in the vestibular system of mature avian models in response to ototoxin-induced damage and as a part of normal cell turnover (reviewed in Stone & Cotanche, 2007). However, the distinction between regeneration and replacement as a result of turnover is important in avian models because the auditory and vestibular tissues behave differently. Whereas hair cell replacement is ongoing in the vestibular sensory epithelia (Jørgensen & Mathiesen, 1988; Weisleder & Rubel, 1993), there is no ongoing replacement of hair cells in the auditory epithelia and new hair cells are produced only during regeneration after sound- or ototoxin-induced damage (Corwin & Cotanche, 1988; Ryals & Rubel, 1988; Oesterle & Rubel, 1993). Despite the lack of regular turnover, regenerated hair cells in the auditory epithelia regain full morphological and functional maturity, even in adults. These and other studies in the extensive literature on regeneration in avian models are reviewed in Chapter 4 of *Hair Cell Regeneration, Repair, and Protection* (Salvi et al., 2008 [SHAR Vol. 33]). It remains to be determined whether the auditory epithelium in the lizard is similarly limited, with regeneration after damage but not ongoing hair cell production as a result of cell turnover.

Mammals, in contrast to other vertebrates, lack robust regeneration of inner ear hair cells, and hair cell production in mammals is typically limited to very early stages of life (reviewed in Warchol, 2011). In humans, the maximum number of hair cells found in the inner ear occurs during gestation (Ashmore, 2008). After birth, the number of hair cells in the inner ear begins a slow, steady decline that continues throughout life and the irreversible nature of human hearing loss indicated that hair cell replacement after damage is not possible. A more extensive overview of hair cell regeneration can be found in *Hair Cell Regeneration, Repair, and Protection* (Salvi et al., 2008 [SHAR Vol. 33]).

5.2 *Understanding Regeneration*

Hair cell regeneration has a rich history as part of tail and limb regeneration studies on amphibians (Stone, 1933; Speidel, 1947; Wright, 1947). These studies identified several stages in the process of limb regeneration that are conserved in fin regeneration studies in zebrafish (reviewed in Akimenko et al., 2003). The first two stages of limb or fin regeneration include wound healing and blastema formation. The blastema is a specialized tissue formed of de-differentiated cells that migrate

into the wound. Cells within the blastema proliferate and eventually migrate out again to form regenerating bone and muscle (Akimenko et al., 2003; Odelberg, 2005). Once the basic structure of the fin or limb has been reestablished, the lateral line begins the process of regenerating. Unlike in fin or limb regeneration, the blastema plays little to no role in replacing hair cells. Instead, cells in the last intact neuromast, closest to the plane of amputation, begin to proliferate and eventually a migrating primordium buds off (Stone, 1933; Dufourcq et al., 2006). In both zebrafish and axolotl salamanders, the primordium migrates along the length of the regenerated fin or limb and deposits new neuromasts. Although this process of regeneration closely mimics normal lateral line development, hair cell regeneration in neuromasts after hair cell-specific ablation follows a distinctly different regenerative process.

Initial studies of lateral line hair cell regeneration in zebrafish used the aminoglycoside antibiotic neomycin to kill hair cells simply by introducing it into water surrounding the fish (see Section 3.1.1) (Song et al., 1995; Harris et al., 2003). This is followed by an increase in the rate of proliferation in the surrounding supporting cells as determined by assaying for markers specific to phases of the cell cycle. Two markers commonly used in zebrafish studies are bromodeoxyuridine (BrdU), a thymidine analog that is taken up by cells in S-phase, and antibodies against phosphohistone3 (PH3) that identify cells in M-phase. High levels of reentry to the cell cycle, or proliferation, as indicated by either BrdU or PH3, persist for 18–20 hours after hair cell ablation and the majority of hair cells that develop are mitotically derived. By 48 hours, recovery is 80% complete and regenerated hair cells are able to take up FM1-43, indicating functional mechanotransduction. Recovery is complete within 72 hours of hair cell ablation, and regeneration was observed at all dosages of aminoglycoside antibiotics tested.

Importantly, studies utilizing aminoglycosides for regeneration experiments must take into account the developmental sensitivity of hair cells to the ototoxins. Studies have shown that immature hair cells, those hair cells that are unable to uptake mechanotransduction-dependent dyes, are largely insensitive to aminoglycosides (Murakami et al., 2003; Santos et al., 2006). Because there is some evidence for a constant, low level of turnover and hair cell replacement in larval neuromasts (Williams & Holder, 2000), this complicates analysis of the mechanism of hair cell regeneration. Studies in the lateral line clearly show that the majority of regenerated hair cells are mitotically derived from supporting cells (Hernandez et al., 2006; Ma et al., 2008; Wibowo et al., 2011). However, nearly all regeneration studies in the lateral line also show a few hair cells appearing during regeneration that are not labeled by indicators of mitosis. It is most likely that these cells are the now mature hair cells that were young enough to be insensitive to aminoglycosides during treatment. However, the possibility that these cells regenerated without undergoing mitosis cannot be ruled out.

The presence of nonmitotically labeled hair cells after regeneration in the zebrafish lateral line has been a matter of great interest because in mature chicken and amphibian models there are two distinct modes of hair cell regeneration (reviewed in Stone & Cotanche, 2007). In the chick auditory epithelia, initial phases

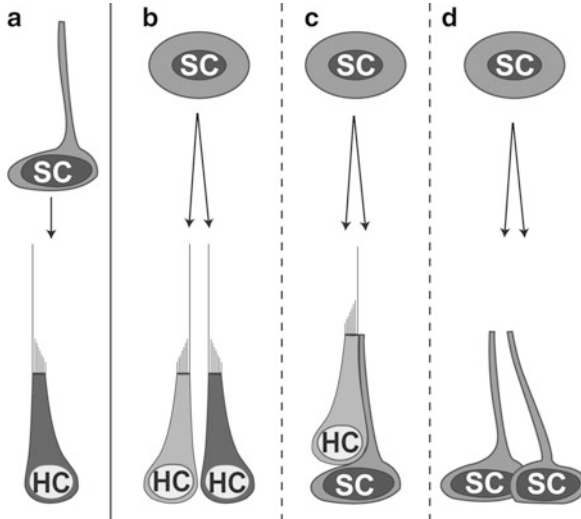


Fig. 3 Multiple mechanisms for hair cell (HC) regeneration have been observed. (a) Direct transdifferentiation, as seen in chicks, occurs when a fully differentiated support cell (SC) is converted into a HC without undergoing mitosis. (b–d) HC regeneration via mitosis of a de-differentiated SC is the major mode of regeneration in zebrafish and the second phase of HC regeneration in chicks. Live cell imaging in zebrafish indicates that the majority of SC division during regeneration is symmetric, producing two HCs and rapidly replenishing HCs while depleting SCs (b). Asymmetric SC division, producing one HC and one SC, occurs infrequently during the first 24 hours of HC regeneration but provides a mechanism for retaining the relative ratios of SCs to HCs (c). Symmetric division of SC into two SCs allows for the replenishment of the SC population following the rapid regeneration of HCs by symmetric division although it remains to be well documented

of hair cell regeneration occur by transdifferentiation in which supporting cells take on hair cell fates without undergoing mitosis (Fig. 3a). Direct transdifferentiation accounts for 30–50% of hair cell regeneration in the chick auditory organ. During later phases, the remainder of hair cells regeneration occurs via a mitotically-dependent mechanism (reviewed in Stone & Cotanche, 2007 and Fig. 3b–d). Hair cell production during development (Lopez-Schier et al., 2004) and regeneration (Lopez-Schier & Hudspeth, 2006) in the zebrafish lateral line is primarily symmetric: a single support cell becomes two hair cells (Fig. 3b). Currently, there is no evidence for nonsymmetric division of support cells during regeneration. However, the robust regenerative capacity of the lateral line neuromasts implies that some mechanism to replace support cells lost during regeneration exists. Candidate mechanisms for support cell replenishment may be via asymmetric cell division to produce one hair cell and one support cell (Fig. 3c), or via symmetric support cell division, resulting in two support cells (Fig. 3d).

Although there is no clear reason for the one-step process in zebrafish versus the two-step regeneration process in birds, it is tempting to speculate that it is related to the different time frames of regeneration in the respective model organisms.

Whereas zebrafish lateral line hair cells regenerate fully within 72 hours, regeneration in the avian inner ear takes several weeks. In starlings (*Sturnus vulgaris*), partial functional recovery appears at 3–4 weeks and is nearly complete at 2 months (Marean et al., 1993, 1998). Regeneration by transdifferentiation begins within 15 hours of gentamicin-induced hair cell ablation and typical hair cell morphology emerges after 2 days of recovery. The first evidence of supporting cells reentering the cell cycle begins 2–3 days after hair cell ablation. In addition to producing hair cells, mitotic division of supporting cells serves to replace the supporting cell population depleted during transdifferentiation (Fig. 3).

Copper, which is generally toxic to fish, is specifically toxic to the zebrafish lateral line (Hernandez et al., 2006; Linbo et al., 2006; Olivari et al., 2008) (see Section 3.1.3). Although copper kills hair cells of the lateral line in a dosage-dependent manner, at higher dosages copper also kills supporting cells. At concentrations where there is no apparent cell death in the supporting cells, hair cells regenerate within a few days. At high concentrations, however, hair cells fail to regenerate (Hernandez et al., 2006). These data further support the idea that the supporting cells may be the elusive stem-cell population that replaces lost hair cells.

Regardless of the cause of cell death, functional regeneration requires more than simply replacing lost cells. The organization and innervation of neuromasts must be reestablished as well. One important component of neuromast organization is the highly stereotyped organization of hair bundles. Each individual hair cell demonstrates planar polarity; cilia are arranged by height from the tallest kinocilium on one side to the shortest stereocilia on the other side (Fig. 4a). Each neuromast displays a specific internal organization with two populations of oppositely oriented hair cells aligned along a single, bidirectional axis (Fig. 4b) (see also the chapters by Webb and by Chagnaud & Coombs in this volume). The bidirectional axis of a neuromast is most clear in the 24 hours following regeneration, during which there is typically a clear, central demarcation in the neuromast with a strict mirror-image orientation of hair cells. However, as regeneration progresses, hair cells intermingle and the mirror-image organization is lost although organization relative to the bidirectional axis is maintained (Lopez-Schier & Hudspeth, 2006). Finally, the lateral line displays a specific organization in which subsets of neuromasts are oriented with hair cells aligned in parallel with, or perpendicular to, the lateral line of neuromasts down the trunk (Fig. 4c) (Flock & Wersäll, 1962; Lopez-Schier et al., 2004; Wibowo et al., 2011). All aspects of organization are maintained during regeneration.

Maintaining polarity during regeneration is critical for the sensory function of the lateral line, but also for the reestablishment of innervation. In the lateral line of larval zebrafish, a single afferent nerve makes contact with hair cells of the same polarity only, despite innervating multiple hair cells and in some cases, even multiple neuromasts (Lopez-Schier & Hudspeth, 2006; Nagiel et al., 2008). Therefore, during regeneration, neuromast polarity is reestablished before reinnervation (Lopez-Schier & Hudspeth, 2006). The mechanism by which a single neuron identifies and synapses with multiple hair cells of identical polarity in regenerating neuromasts remains unclear.

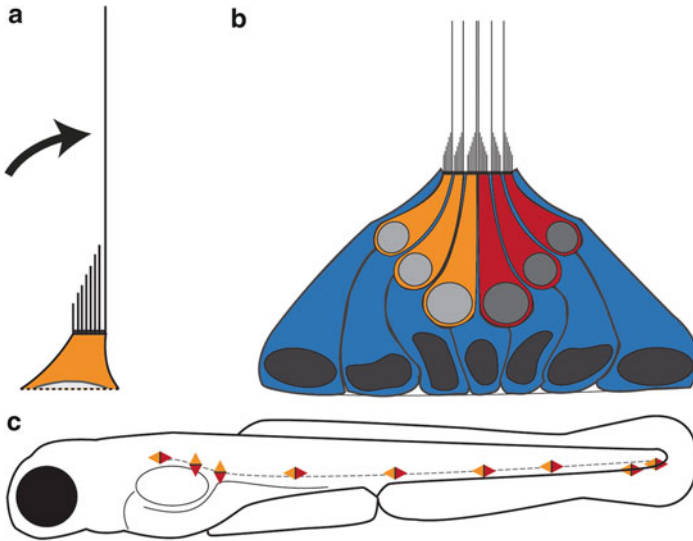


Fig. 4 (a) Polarity in a single hair cell is determined by the orientation of cilia in the classic stair-step fashion. Stimuli that move cilia in the direction indicated by the arrow, toward the large kinocilium, open mechanotransduction channels that initiate a signaling cascade. Stimuli out of alignment with hair cell polarity open fewer mechanotransduction channels, making the cell less responsive to those stimuli. (b) Neuromasts also have polarity, established by the organization of hair cells within each neuromast. In this simplified schematic, support cells (blue) surround two populations of hair cells depicting mirror image polarity, typical during the first few hours of hair cell regeneration. This organization defines a neuromast's sensitivity to stimuli along a single axis in early stages of development and regeneration. At later stages the two populations of oppositely oriented hair cells later become spatially intermingled such that the two populations are no longer segregated into two well-demarcated halves of the neuromast. (c) Neuromasts along the primary lateral line have specific placements with regard to their polarity. In a zebrafish at 4 dpf, the majority of neuromasts are aligned to respond to anterior-posterior stimuli (◀▶) while a subset located near the swim bladder respond to dorsal-ventral stimuli (◊). During the process of hair cell regeneration, the polarity must be reestablished at the level of individual cells (a), within a neuromast (b), and at the level of the entire animal (c). (Figure based on Nagiel et al., 2008)

Recent work has revealed that the mirror-symmetry of adjacent hair cells within a neuromast is the result of localized Notch signaling (Wibowo et al., 2011). In regenerating neuromasts, two areas of repressed Notch signaling, organized perpendicularly to the axis of planar polarity, allow for differentiation of supporting cells into hair cells. These two polar compartments essentially define the internal plane for mirror symmetry. Hair cell precursors in these low-Notch signaling compartments divide symmetrically and the sister cells consistently develop hair bundles with opposite polarities. The method by which the polar compartments are localized appropriately in relationship to the neuromast's planar polarity is an area of open investigation.

A second structural property of neuromasts that must be reestablished during regeneration is size. As described in Chapter 5 of *Fish Bioacoustics*

(Webb et al., 2008), neuromasts within the lateral line are highly stereotyped in both position and size. The number of hair cells per neuromast varies in relation to its position. For example, in animals at 4–5 dpf, the first supraorbital (SO1) neuromast of the anterior lateral line is small (7.4 ± 1.9 hair cells) compared to the second (SO2) neuromast (14.7 ± 4.0) (Harris et al., 2003). When neuromasts are ablated at 5 dpf, they regenerate to the appropriate size (Ma et al., 2008). Again, the Notch pathway seems a likely contributor to specification of neuromast size.

Notch signaling pathways are an appealing avenue for investigation owing to their extensive role in hair cell development (reviewed in Cotanche & Kaiser, 2010). Briefly stated, Notch is important in later stages of development for cell fate determination, leading to the specification of sensory versus nonsensory tissue within the inner ear. During development, cells expressing Notch adopt a sensory fate while signaling surrounding cells to adopt a nonsensory fate—a classic example of lateral inhibition. In these surrounding cells, pro-sensory genes are downregulated, notably *Atoh1*, a basic helix–loop–helix protein required for hair cell specification. Those cells expressing Notch become hair cells, while the neighboring cells that have down regulated pro-sensory genes become supporting cells.

During regeneration in the zebrafish lateral line, the same complement of genes is activated as in the developing inner ear. *Atoh1*, normally expressed at low levels in a few developing hair cells, is broadly upregulated in neuromasts undergoing regeneration for at least the first 24 hours and then returns to normal, low levels by 48 hours post-damage (Ma et al., 2008). This time frame is consistent with the observation that the highest levels of supporting cell proliferation in regenerating neuromasts occurs within the first 24 hours. Components of the Notch signaling pathway are similarly upregulated during regeneration of the damaged avian auditory epithelium (Cafaro et al., 2007; Daudet et al., 2009).

Conversely, the process of slowing down or stopping the production of new hair cells at the appropriate time is also regulated by Notch signaling. In the zebrafish lateral line, pharmacologically inhibiting Notch results in overproduction of hair cells during regeneration after hair cell ablation (Ma et al., 2008). These data are consistent with those from the mature chick auditory epithelium; pharmacological inhibition of Notch signaling pathways results in hair cell overproduction during damage-induced regeneration (Daudet et al., 2009). Significantly, downregulation of Notch signaling pathways in undamaged tissue, either in the developing lateral line or the avian auditory epithelia, has no effect (Ma et al., 2008; Daudet et al., 2009). These results contrast with those from mammalian models where repression of Notch signaling during normal development does result in the overproduction of hair cells in auditory epithelia (reviewed in Cotanche & Kaiser, 2010).

Although the overall effect of downregulating Notch signaling during damage-induced regeneration in avian or fish models is the overproduction of hair cells, it is important to note a potentially significant difference. In zebrafish, for which regeneration is primarily mitotic, repressing Notch signaling increases the rate and extends the duration of supporting cell proliferation causing the overproduction of hair cells (Ma et al., 2008). These data indicate that Notch signaling normally functions to halt mitotic activity in neuromasts at the appropriate time, thus acting

as a negative regulator of proliferation. This differs from the response of avian models to Notch repression in which hair cells are overproduced without increasing the rate of cell division. Increased production of hair cells in the absence of increased mitosis suggests that Notch signaling in avian models plays a role in regulating direct transdifferentiation of supporting cells into hair cells. These studies provide an example of how a similar outcomes, overproduction of hair cells during regeneration, in two model systems may be regulated differently at the molecular level, even via the same genes. Further, it highlights the importance of comparative studies in model organisms, especially in the case of regeneration, where one of the fundamental goals is to understand how regenerative capacity has been lost en route to mammals.

5.3 Modulating Regeneration

Not surprisingly, there is great interest in screening for agents that stimulate hair cell regeneration and promising results are just beginning to emerge from such studies. Two complementary approaches to this end are chemical and genetic screens. In this section we review the outcomes of several screens, chemical and genetic, and discuss the implications and further directions for this area of research.

5.3.1 Chemical Screens for Regeneration

The same characteristics that make the zebrafish lateral line useful in screening for hair cell protectants (see Section 4.2) makes it an effective model in screening for modulators of hair cell regeneration. Following regeneration in the presence of candidate drugs, it is possible to rapidly assay lateral line hair cell regeneration by simple visual examination. Of more than 2000 drugs screened to date, several inhibitors and just three enhancers of hair cell regeneration have been identified.

Low molecular weight fucoidan (LMWF) was identified as an accelerant of hair cell regeneration in the lateral line (Moon et al., 2011). Following neomycin-induced hair cell ablation, the presence of LMWF significantly increases the rate of hair cell regeneration during the first 12 hours. However, this accelerated regeneration slows over the following 12 hours such that treated and untreated animals achieve similar hair cell numbers after 24 hours. BrdU labeling reveals that that increased hair cell regeneration is accompanied by increased rates of proliferation in supporting cells. Despite the increase in proliferation and regeneration, LMWF did not induce overproliferation of hair cells either during regeneration or during normal developmental addition of hair cells. It is important to note that there was no increase in the rate of cell death in regenerating neuromasts during the final 12 hours of regeneration. Therefore, LMWF accelerates the initial phases of regeneration, which results in a slower-than-normal rate of hair cell regeneration during the latter phases. This indicates that the activity of LMWF has a limited time

window in which to increase regeneration—perhaps as a result of habituation or to a limiting reagent, such as the number of stem cells or hair cell precursors available.

Fucoidan is a highly sulfated polysaccharide derived from brown seaweed that has a long history in traditional Asian medicine. More recent studies have revealed that fucoidans modulate immune system function and act as antitumor, anti-inflammatory, antibacterial, and antiviral agents, among other attributes (Kusaykin et al., 2008). Although the many known actions of fucoidan provide ample opportunity for speculation, the mechanism by which it stimulates hair cell production in regenerating, but not normally developing, neuromasts remains to be determined.

A larger screen examined a library of nearly 1700 FDA-approved drugs and bioactive compounds for modulation of hair cell regeneration in the lateral line of the *brn3C* transgenic line. This screen identified two enhancers and six inhibitors of regeneration (Namdaran et al., 2012). The enhancers of regeneration, prednisolone and dexamethasone, are both synthetic glucocorticoids (SGs) that act in a dosage-dependent manner to stimulate hair cell regeneration by increasing the rate of supporting cell proliferation. Interestingly, the SGs that enhance lateral line hair cell regeneration significantly inhibit fin regeneration. In the fin, the presence of exogenous glucocorticoids, via specific activation of the glucocorticoid receptors, decreases levels of proliferation in the plane of the amputation and inhibit formation of wound epithelium and blastema critical to fin regeneration (Mathew et al., 2007). These data suggest that enhanced lateral line hair cell regeneration in the presence of SGs is not a direct result of glucocorticoid-receptor activity. These data also highlight the importance of the cellular environment in regenerative processes. Neomycin induces hair cell-specific ablation that is enhanced by SGs. In contrast, hair cells lost as a result of fin amputation are dependent on multiple stages of tissue regeneration including wound healing, blastema formation, and finally redeposition of neuromasts (see Section 5.2).

It is interesting to note that both types of regeneration enhancing drugs that were identified in drug library screens stimulate excess proliferation by supporting cells. Despite this similarity, the outcomes of treatment with LMWF versus SGs are very different. LMWF accelerated initial stages of regeneration at the expense of later stages of regeneration without increasing the total number of hair cells. In contrast, SGs cause a sustained increase in the rate of supporting cell proliferation, leading to an increase in the total number of hair cells regenerated. Although the SGs provided a 10–25% increase in the total number of hair cells regenerated, it is a relatively modest increase in comparison to the doubling of hair cells regenerated when stimulated by DAPT, an inhibitor of the Notch signaling pathway (Ma et al., 2008). Clearly, neither the rate nor extent of proliferation-mediated hair cell regeneration is maximized by the introduction of SGs. It would be interesting to determine whether DAPT and SGs might be additive or synergistic as potential means of establishing the upper limits of hair cell regeneration in the lateral line.

A second significant difference between these hair cell regeneration enhancers is their effects on lateral line hair cell addition during normal development and hair cell turnover. DAPT treatment has little effect during 48 hours of treatment.

Similarly, LMWF does not increase the rate of hair cell development during the 24 hours tested. In contrast, SGs stimulate the rate of hair cell addition during both normal development and regeneration. The unique profile of each compound in regulating hair cell production during development and/or regeneration suggests at least partially distinct mechanisms of action for each of the drugs identified. Precisely what those mechanisms are remains to be determined.

Another open question arising from these studies is how altering the regenerative process, or the rate of development, affects the long-term survival of hair cells in these neuromasts with excess hair cells. Although in the short term (48–72 hours) DAPT and SGs result in neuromasts with unusually high numbers of hair cells, it is not known whether all the hair cells are appropriately innervated, have the correct polarity, or display altered rates of turnover or sensitivity. In essence, does the neuromast integrate additional hair cells into its overall organization or are they effectively ectopic? If they are fully integrated, does that change depend on whether extra hair cells are added during development or during regeneration? Studies in mammalian models have succeeded in inducing the production of ectopic hair cells, highlighting the exciting potential for initiating regeneration in mammals. However, the hair cells induced in these experiments were short lived, possibly owing to the absence of innervation and/or supporting cells. Examining the survival of lateral line hair cells resulting from drug-induced overproduction may provide suggestions of how the surrounding environment regulates hair cells survival and integration into functioning neuromasts that normally have well established size limits. This in turn may lead to a better understanding of the environmental cues required for hair cell survival in tissues that do not normally regenerate.

The recent screening study of FDA-approved drugs and bioactive compounds also identified several inhibitors of lateral line hair cell regeneration (Namdaran et al., 2012). The six inhibitors identified were those that provided a dosage-dependent inhibition of hair cell regeneration at concentrations that were not toxic to hair cells. The inhibitors fell into two general categories: those that slowed regeneration and those that completely blocked it. The two drugs that appeared to block regeneration also blocked proliferation of the supporting cells as indicated by a significant decrease in BrdU labeling.

The drugs identified as modulators of lateral line hair cell regeneration from drug screens may provide tools for a molecular dissection of hair cell growth and regeneration. Inhibitors in particular provide a method for isolating hair cells at various stages of regeneration in order to enrich the environment for analysis of stage-specific critical genes. Enhancers of regeneration provide molecular tools for understanding the process by which regeneration is halted when hair cells are at the appropriate density. Together, the drugs identified by large-scale screen provide new techniques for studying the basic biology of hair cells. They are also attractive as candidate approaches to stimulating hair cell regeneration in the mammalian inner ear as we work toward the ability to assist hearing impaired patients.

5.3.2 Genetic Screens for Regeneration

A genetic approach to stimulating hair cell regeneration requires identifying and thoroughly characterizing target genes for manipulation. A variety of genetic approaches have been used to identify novel genes critical to hair cell regeneration. The *phoenix* mutant, with decreased hair cell regeneration, was identified in an insertional-mutagenesis screen where a modified retrovirus was randomly integrated into the zebrafish genome, disrupting the function of endogenous genes (Behra et al., 2009). The disrupted gene was identified by sequencing the region of the insertion based on the presence of the known retrovirus sequence. The gene identified in this mutant was a truly novel gene with no previously described function and no known homologs. The gene is expressed strongly in the supporting cells of neuromasts and discrete patches in the inner ear beginning around 2 dpf and persisting through at least 12 dpf. Based on conserved structures, the authors suggest *phoenix* is a structural protein with enzymatic ATPase activity.

Although much remains to be learned about the normal function of *phoenix*, when it is mutated in zebrafish, hair cell regeneration is impaired as a result of decreased proliferation in the supporting cell population. These data suggest that *phoenix* is active in regulating the ability of supporting cells to either recognize or respond to the loss of hair cells. *Phoenix* may play a role in allowing supporting cells to successfully enter or progress through the cell cycle and then differentiate either as supporting cells or as hair cells. This study provides an example of the potential use of zebrafish to identify novel genes that regulate hair cell regeneration independently of development. Although the lack of mammalian orthologs makes the analysis of *phoenix* challenging, it is also tempting to speculate that the absence of a gene in mammals, one that remains present in nonmammalian vertebrates, may account for differences in regenerative capacity.

6 Summary

The small size of the zebrafish and the large experimental toolbox available for zebrafish studies, combined with the external location and ease of manipulation of the lateral line, makes the zebrafish an ideal model for studies of hair cell death, protection, and regeneration. Targeted studies have uncovered some of the critical events that occur during the stages leading up to aminoglycoside- or cisplatin-induced hair cell death, and provide evidence that mitochondrial changes may be of central importance. More recently, studies in zebrafish that combine genetic and chemical screens have uncovered several genes and drugs that modulate hair cell death and regeneration.

It is important to note that, with few exceptions, regeneration studies utilizing the zebrafish lateral line have used larval animals. Although clear trends are

emerging in how regeneration occurs, it remains to be determined if they hold true in adult animals.

These studies land at the intersection of fundamental and applied research. Work using the zebrafish lateral line as a model is providing new insights into the molecular and genetic regulation of hair cell turnover and regeneration, and survival or death of hair cells in the face of stresses. In addition to contributing to our fundamental understanding of hair cell biology, the lateral line is providing a model for translational studies: drug screens to assay the impact of current and future pharmaceuticals in the zebrafish may provide hints as to their effect on mammalian hair cell survival and regeneration. Someday, the combination of basic and translational research available in the zebrafish could lead to therapies for humans struggling with hearing loss. Well before then, zebrafish lateral line research increases our fundamental understanding of basic cellular processes in such fields as intracellular signaling and stem cell biology. This junction between fundamental biology and translational studies lends additional power to the zebrafish lateral line as both a sensory system worthy of study in its own right and a model system for hearing habilitation.

References

- Akimenko, M. A., Mari-Beffa, M., Becerra, J., & Geraudie, J. (2003). Old questions, new tools, and some answers to the mystery of fin regeneration. *Developmental Dynamics*, 226(2), 190–201.
- Ashmore, J. (2008). Cochlear outer hair cell motility. *Physiological Reviews*, 88(1), 173–210.
- Avallone, B., Fascio, U., Balsamo, G., & Marmo, F. (2008). Gentamicin ototoxicity in the sacculle of the lizard *Podarcis sicula* induces hair cell recovery and regeneration. *Hearing Research*, 235(1–2), 15–22.
- Avallone, B., Porritiello, M., Esposito, D., Mutone, R., Balsamo, G., & Marmo, F. (2003). Evidence for hair cell regeneration in the crista ampullaris of the lizard *Podarcis sicula*. *Hearing Research*, 178(1–2), 79–88.
- Baird, R. A., Torres, M. A., & Schuff, N. R. (1993). Hair cell regeneration in the bullfrog vestibular otolith organs following aminoglycoside toxicity. *Hearing Research*, 65(1–2), 164–174.
- Bang, P. I., Sewell, W. F., & Malicki, J. J. (2001). Morphology and cell type heterogeneities of the inner ear epithelia in adult and juvenile zebrafish (*Danio rerio*). *Journal of Comparative Neurology*, 438(2), 173–190.
- Barin, K., & Dodson, E. E. (2011). Dizziness in the elderly. *Otolaryngologic Clinics of North America*, 44(2), 437–454.
- Behra, M., Bradsher, J., Sougrat, R., Gallardo, V., Allende, M. L., & Burgess, S. M. (2009). Phoenix is required for mechanosensory hair cell regeneration in the zebrafish lateral line. *PLoS Genetics*, 5(4), e1000455.
- Boguski, M. S., Mandl, K. D., & Sukhatme, V. P. (2009). Drug discovery: Repurposing with a difference. *Science*, 324(5933), 1394–1395.
- Brignull, H. R., Raible, D. W., & Stone, J. S. (2009). Feathers and fins: Non-mammalian models for hair cell regeneration. *Brain Research*, 1277, 12–23.

- Brown, A. D., Mussen, T. D., Sisneros, J. A., & Coffin, A. B. (2011) Reevaluating the use of aminoglycoside antibiotics in behavioral studies of the lateral line. *Hearing Research*, 272(1–2), 1–4.
- Burket, C. T., Montgomery, J. E., Thummel, R., Kassen, S. C., LaFave, M. C., Langenau, D. M., Zon, L. I., & Hyde, D. R. (2008). Generation and characterization of transgenic zebrafish lines using different ubiquitous promoters. *Transgenic Research*, 17(2), 265–279.
- Cafaro, J., Lee, G. S., & Stone, J. S. (2007). Atoh1 expression defines activated progenitors and differentiating hair cells during avian hair cell regeneration. *Developmental Dynamics*, 236(1), 156–170.
- Chiu, L. L., Cunningham, L. L., Raible, D. W., Rubel, E. W., & Ou, H. C. (2008). Using the zebrafish lateral line to screen for ototoxicity. *Journal of the Association for Research in Otolaryngology*, 9(2), 178–190.
- Chong, C. R., & Sullivan, D. J., Jr. (2007). New uses for old drugs. *Nature*, 448(7154), 645–646.
- Coffin, A. B., Dabdoub, A., Kelley, M. W., & Popper, A. N. (2007). Myosin VI and VIIa distribution among inner ear epithelia in diverse fishes. *Hearing Research*, 224(1–2), 15–26.
- Coffin, A. B., Reinhart, K. E., Owens, K. N., Raible, D. W., & Rubel, E. W. (2009). Extracellular divalent cations modulate aminoglycoside-induced hair cell death in the zebrafish lateral line. *Hearing Research*, 253(1–2), 42–51.
- Coffin, A. B., Rubel, E. W., & Raible, D. W. (2013). Bax, Bcl2, and p53 differentially regulate neomycin- and gentamicin-induced hair cell death in the zebrafish lateral line. *Journal of the Association for Research in Otolaryngology*. doi: [10.1007/s10162-013-0404-1](https://doi.org/10.1007/s10162-013-0404-1).
- Coombs, S., Görner, P., & Münz, H. (1989). *The mechanosensory lateral line: Neurobiology and evolution*. New York: Springer-Verlag.
- Coombs, S., Braun, C. B., & Donovan, B. (2001). The orienting response of Lake Michigan mottled sculpin is mediated by canal neuromasts. *Journal of Experimental Biology*, 204, 337–348.
- Corey, D. P., & Hudspeth, A. J. (1983). Kinetics of the receptor current in bullfrog saccular hair cells. *Journal of Neuroscience*, 3(5), 962–976.
- Corwin, J. T. (1992). Regeneration in the auditory system. *Experimental Neurology*, 115(1), 7–12.
- Corwin, J. T., & Cotanche, D. A. (1988). Regeneration of sensory hair cells after acoustic trauma. *Science*, 240(4860), 1772–1774.
- Cotanche, D. A., & Kaiser, C. L. (2010). Hair cell fate decisions in cochlear development and regeneration. *Hearing Research*, 266(1–2), 18–25.
- Cunningham, L. L., Cheng, A. G., & Rubel, E. W. (2002). Caspase activation in hair cells of the mouse utricle exposed to neomycin. *Journal of Neuroscience*, 22(19), 8532–8540.
- Cunningham, L. L., Matsui, J. I., Warchol, M. E., & Rubel, E. W. (2004). Overexpression of Bcl-2 prevents neomycin-induced hair cell death and caspase-9 activation in the adult mouse utricle in vitro. *Journal of Neurobiology*, 60(1), 89–100.
- Dalton, D. S., Cruickshanks, K. J., Klein, B. E., Klein, R., Wiley, T. L., & Nondahl, D. M. (2003). The impact of hearing loss on quality of life in older adults. *Gerontologist*, 43(5), 661–668.
- Daudet, N., Gibson, R., Shang, J., Bernard, A., Lewis, J., & Stone, J. (2009). Notch regulation of progenitor cell behavior in quiescent and regenerating auditory epithelium of mature birds. *Developmental Biology*, 326(1), 86–100.
- David, N. B., Sapede, D., Saint-Etienne, L., Thisse, C., Thisse, B., Dambly-Chaudiere, C., Rosa, F. M., & Ghysen, A. (2002). Molecular basis of cell migration in the fish lateral line: Role of the chemokine receptor CXCR4 and of its ligand, SDF1. *Proceedings of the National Academy of Sciences of the USA*, 99(25), 16297–16302.
- Ding, D., He, J., Allman, B. L., Yu, D., Jiang, H., Seigel, G. M., & Salvi, R. J. (2011). Cisplatin ototoxicity in rat cochlear organotypic cultures. *Hearing Research*.
- Doyon, Y., McCammon, J. M., Miller, J. C., Faraji, F., Ngo, C., Katibah, G. E., Amora, R., Hocking, T. D., Zhang, L., Rebar, E. J., Gregory, P. D., Urmov, F. D., & Amacher, S. L. (2008). Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. *Nature Biotechnology*, 26(6), 702–708.

- Dufourcq, P., Roussigne, M., Blader, P., Rosa, F., Peyrieras, N., & Vriza, S. (2006). Mechano-sensory organ regeneration in adults: The zebrafish lateral line as a model. *Molecular and Cellular Neurosciences*, 33(2), 180–187.
- Engeszer, R. E., Patterson, L. B., Rao, A. A., & Parichy, D. M. (2007). Zebrafish in the wild: A review of natural history and new notes from the field. *Zebrafish*, 4(1), 21–40.
- Eppsteiner, R. W., & Smith, R. J. (2011). Genetic disorders of the vestibular system. *Current Opinion in Otolaryngology & Head and Neck Surgery*, 19(5), 397–402.
- Ernest, S., Rauch, G. J., Haffter, P., Geisler, R., Petit, C., & Nicolson, T. (2000). Mariner is defective in myosin VIIA: A zebrafish model for human hereditary deafness. *Human Molecular Genetics*, 9(14), 2189–2196.
- Faucher, K., Aas-Hansen, O., Damsgard, B., Laukli, E., & Stenklev, N. C. (2009). Damage and functional recovery of the Atlantic cod (*Gadus morhua*) inner ear hair cells following local injection of gentamicin. *International Journal of Audiology*, 48(7), 456–464.
- Flock, Å., & Wersäll, J. (1962). A study of the orientation of the sensory hairs of the receptor cells in the lateral line organ of fish, with special reference to the function of the receptors. *Journal of Cell Biology*, 15, 19–27.
- Foley, J. E., Yeh, J. R., Maeder, M. L., Reyon, D., Sander, J. D., Peterson, R. T., & Joung, J. K. (2009). Rapid mutation of endogenous zebrafish genes using zinc finger nucleases made by Oligomerized Pool ENgineering (OPEN). *PLoS One*, 4(2), e4348.
- Gates, G. A., Couropmitree, N. N., & Myers, R. H. (1999). Genetic associations in age-related hearing thresholds. *Archives of Otolaryngology - Head and Neck Surgery*, 125(6), 654–659.
- Germana, A., Abbate, F., Gonzalez-Martinez, T., del Valle, M. E., de Carlos, F., Germana, G., & Vega, J. A. (2004). S100 protein is a useful and specific marker for hair cells of the lateral line system in postembryonic zebrafish. *Neuroscience Letters*, 365(3), 186–189.
- Ghysen, A., & Dambly-Chaudiere, C. (2004). Development of the zebrafish lateral line. *Current Opinion in Neurobiology*, 14(1), 67–73.
- Giari, L., Dezfuli, B. S., Astolfi, L., & Martini, A. (2011). Ultrastructural effects of cisplatin on the inner ear and lateral line system of zebrafish (*Danio rerio*) larvae. *Journal of Applied Toxicology*.
- Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K. A., Antonio, M., Beisel, K. W., Steel, K. P., & Brown, S. D. (1995). A type VII myosin encoded by the mouse deafness gene shaker-1. *Nature*, 374(6517), 62–64.
- Gorden, N. T., Arts, H. H., Parisi, M. A., Coene, K. L., Letteboer, S. J., van Beersum, S. E., Mans, D. A., Hikida, A., Eckert, M., Knutzen, D., Alswaid, A. F., Ozyurek, H., Dibooglu, S., Otto, E. A., Liu, Y., Davis, E. E., Hutter, C. M., Bammler, T. K., Farin, F. M., Dorschner, M., Topcu, M., Zackai, E. H., Rosenthal, P., Owens, K. N., Katsanis, N., Vincent, J. B., Hildebrandt, F., Rubel, E. W., Raible, D. W., Knoers, N. V., Chance, P. F., Roepman, R., Moens, C. B., Glass, I. A., & Doherty, D. (2008). CC2D2A is mutated in Joubert syndrome and interacts with the ciliopathy-associated basal body protein CEP290. *American Journal of Human Genetics*, 83(5), 559–571.
- Granato, M., van Eeden, F. J., Schach, U., Trowe, T., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C. P., Jiang, Y. J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J., & Nusslein-Volhard, C. (1996). Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva. *Development*, 123, 399–413.
- Grunwald, D. J., & Eisen, J. S. (2002). Headwaters of the zebrafish – emergence of a new model vertebrate. *Nature Reviews: Genetics*, 3(9), 717–724.
- Guan, M. X., Fischel-Ghodsian, N., & Attardi, G. (2000). A biochemical basis for the inherited susceptibility to aminoglycoside ototoxicity. *Human Molecular Genetics*, 9(12), 1787–1793.
- Haffter, P., Granato, M., Brand, M., Mullins, M. C., Hammerschmidt, M., Kane, D. A., Odenthal, J., van Eeden, F. J., Jiang, Y. J., Heisenberg, C. P., Kelsh, R. N., Furutani-Seiki, M., Vogelsang, E., Beuchle, D., Schach, U., Fabian, C., & Nusslein-Volhard, C. (1996). The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development*, 123, 1–36.

- Harris, J. A., Cheng, A. G., Cunningham, L. L., MacDonald, G., Raible, D. W., & Rubel, E. W. (2003). Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (*Danio rerio*). *Journal of the Association for Research in Otolaryngology*, 4(2), 219–234.
- Hernandez, P. P., Moreno, V., Olivari, F. A., & Allende, M. L. (2006). Sub-lethal concentrations of waterborne copper are toxic to lateral line neuromasts in zebrafish (*Danio rerio*). *Hearing Research*, 213(1–2), 1–10.
- Hirose, K., Westrum, L. E., Stone, J. S., Zirpel, L., & Rubel, E. W. (1999). Dynamic studies of ototoxicity in mature avian auditory epithelium. *Annals of the New York Academy of Sciences*, 884, 389–409.
- Hirose, Y., Simon, J. A., & Ou, H. C. (2011). Hair cell toxicity in anti-cancer drugs: Evaluating an anti-cancer drug library for independent and synergistic toxic effects on hair cells using the zebrafish lateral line. *Journal of the Association for Research in Otolaryngology*, 12(6), 719–728.
- Jiang, H., Sha, S. H., Forge, A., & Schacht, J. (2006). Caspase-independent pathways of hair cell death induced by kanamycin in vivo. *Cell Death and Differentiation*, 13(1), 20–30.
- Jørgensen, J. M. (1991). Regeneration of lateral line and inner ear vestibular cells. *Ciba Foundation Symposium*, 160, 151–163.
- Jørgensen, J. M., & Mathiesen, C. (1988). The avian inner ear. Continuous production of hair cells in vestibular sensory organs, but not in the auditory papilla. *Naturwissenschaften*, 75(6), 319–320.
- Kaufman, C. K., White, R. M., & Zon, L. (2009). Chemical genetic screening in the zebrafish embryo. *Nature Protocols*, 4(10), 1422–1432.
- Kaus, S. (1987). The effect of aminoglycoside antibiotics on the lateral line organ of *Aplocheilichthys lineatus* (Cyprinodontidae). *Acta Oto-Laryngologica*, 103(5–6), 291–298.
- Kaus, S. (1992). The influence of calcium on the ototoxicity of aminoglycosides. *Acta Oto-Laryngologica*, 112(1), 83–87.
- Kawakami, K., Takeda, H., Kawakami, N., Kobayashi, M., Matsuda, N., & Mishina, M. (2004). A transposon-mediated gene trap approach identifies developmentally regulated genes in zebrafish. *Developmental Cell*, 7(1), 133–144.
- Keats, B. J. B., Fay, R. R., & Popper, A. N. (2002). *Genetics of Auditory Disorders*. New York: Springer-Verlag.
- Kerstetter, A. E., Azodi, E., Marrs, J. A., & Liu, Q. (2004). Cadherin-2 function in the cranial ganglia and lateral line system of developing zebrafish. *Developmental Dynamics*, 230(1), 137–143.
- Kim, C. H., Kang, S. U., Pyun, J., Lee, M. H., Hwang, H. S., & Lee, H. (2008). Epicatechin protects auditory cells against cisplatin-induced death. *Apoptosis*, 13(9), 1184–1194.
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253–310.
- Kral, A., & O'Donoghue, G. M. (2010). Profound deafness in childhood. *New England Journal of Medicine*, 363(15), 1438–1450.
- Kroese, A., & van den Bercken, J. (1980). Dual action of ototoxic antibiotics on sensory hair cells. *Nature*, 283(5745), 395–397.
- Kroese, A., & van den Bercken, J. (1982). Effects of ototoxic antibiotics on sensory hair cell functioning. *Hearing Research*, 6(2), 183–197.
- Kusaykin, M., Bakunina, I., Sova, V., Ermakova, S., Kuznetsova, T., Besednova, N., Zaporozhets, T., & Zvyagintseva, T. (2008). Structure, biological activity, and enzymatic transformation of fucoidans from the brown seaweeds. *Biotechnology Journal*, 3(7), 904–915.
- Lanford, P. J., Presson, J. C., & Popper, A. N. (1996). Cell proliferation and hair cell addition in the ear of the goldfish, *Carassius auratus*. *Hearing Research*, 100(1–2), 1–9.
- Linbo, T. L., Stehr, C. M., Incardona, J. P., & Scholz, N. L. (2006). Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. *Environmental Toxicology and Chemistry*, 25(2), 597–603.

- Liu, X. Z., Walsh, J., Mburu, P., Kendrick-Jones, J., Cope, M. J., Steel, K. P., & Brown, S. D. (1997a). Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nature Genetics*, 16(2), 188–190.
- Liu, X. Z., Walsh, J., Tamagawa, Y., Kitamura, K., Nishizawa, M., Steel, K. P., & Brown, S. D. (1997b). Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. *Nature Genetics*, 17(3), 268–269.
- Lombarte, A., & Popper, A. N. (1994). Quantitative analyses of postembryonic hair cell addition in the otolithic endorgans of the inner ear of the European hake, *Merluccius merluccius* (Gadiformes, Teleostei). *Journal of Comparative Neurology*, 345(3), 419–428.
- Lombarte, A., Yan, H. Y., Popper, A. N., Chang, J. S., & Platt, C. (1993). Damage and regeneration of hair cell ciliary bundles in a fish ear following treatment with gentamicin. *Hearing Research*, 64(2), 166–174.
- Lopez-Schier, H., & Hudspeth, A. J. (2006). A two-step mechanism underlies the planar polarization of regenerating sensory hair cells. *Proceedings of the National Academy of Sciences of the USA*, 103(49), 18615–18620.
- Lopez-Schier, H., Starr, C. J., Kappler, J. A., Kollmar, R., & Hudspeth, A. J. (2004). Directional cell migration establishes the axes of planar polarity in the posterior lateral-line organ of the zebrafish. *Developmental Cell*, 7(3), 401–412.
- Lord, E. M., & Gates, W. H. (1929). Shaker, a new mutation of the house mouse. *American Naturalist*, 63(688), 435–442.
- Ma, E. Y., Rubel, E. W., & Raible, D. W. (2008). Notch signaling regulates the extent of hair cell regeneration in the zebrafish lateral line. *Journal of Neuroscience*, 28(9), 2261–2273.
- Malicki, J., Schier, A. F., Solnica-Krezel, L., Stemple, D. L., Neuhauss, S. C., Stainier, D. Y., Abdellilah, S., Rangini, Z., Zwartkruis, F., & Driever, W. (1996). Mutations affecting development of the zebrafish ear. *Development*, 123, 275–283.
- Mangiardi, D. A., McLaughlin-Williamson, K., May, K. E., Messana, E. P., Mountain, D. C., & Cotanche, D. A. (2004). Progression of hair cell ejection and molecular markers of apoptosis in the avian cochlea following gentamicin treatment. *Journal of Comparative Neurology*, 475(1), 1–18.
- Marcotti, W., van Netten, S. M., & Kros, C. J. (2005). The aminoglycoside antibiotic dihydrostreptomycin rapidly enters mouse outer hair cells through the mechano-electrical transducer channels. *Journal of Physiology*, 567(Pt 2), 505–521.
- Marean, G. C., Burt, J. M., Beecher, M. D., & Rubel, E. W. (1993). Hair cell regeneration in the European starling (*Sturnus vulgaris*): Recovery of pure-tone detection thresholds. *Hearing Research*, 71(1–2), 125–136.
- Marean, G. C., Burt, J. M., Beecher, M. D., & Rubel, E. W. (1998). Auditory perception following hair cell regeneration in European starling (*Sturnus vulgaris*): Frequency and temporal resolution. *Journal of the Acoustical Society of America*, 103(6), 3567–3580.
- Marot, M., Uziel, A., & Romand, R. (1980). Ototoxicity of kanamycin in developing rats: Relationship with the onset of the auditory function. *Hearing Research*, 2(2), 111–113.
- Mathew, L. K., Sengupta, S., Kawakami, A., Andreasen, E. A., Lohr, C. V., Loynes, C. A., Renshaw, S. A., Peterson, R. T., & Tanguay, R. L. (2007). Unraveling tissue regeneration pathways using chemical genetics. *Journal of Biological Chemistry*, 282(48), 35202–35210.
- Matsui, J. I., Gale, J. E., & Warchol, M. E. (2004). Critical signaling events during the aminoglycoside-induced death of sensory hair cells in vitro. *Journal of Neurobiology*, 61(2), 250–266.
- McDermott, B. M., Jr., Asai, Y., Baucom, J. M., Jani, S. D., Castellanos, Y., Gomez, G., McClintock, J. M., Starr, C. J., & Hudspeth, A. J. (2010). Transgenic labeling of hair cells in the zebrafish acousticolateralis system. *Gene Expression Patterns*, 10(2–3), 113–118.
- McHenry, M. J., Feitl, K. E., Strother, J. A., & Van Trump, W. J. (2009). Larval zebrafish rapidly sense the water flow of a predator's strike. *Biology Letters*, 5(4), 477–479.
- Meng, X., Noyes, M. B., Zhu, L. J., Lawson, N. D., & Wolfe, S. A. (2008). Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases. *Nature Biotechnology*, 26(6), 695–701.

- Metcalfe, W. K., Kimmel, C. B., & Schabtach, E. (1985). Anatomy of the posterior lateral line system in young larvae of the zebrafish. *Journal of Comparative Neurology*, 233(3), 377–389.
- Meyers, J. R., MacDonald, R. B., Duggan, A., Lenzi, D., Standaert, D. G., Corwin, J. T., & Corey, D. P. (2003). Lighting up the senses: FM1-43 loading of sensory cells through nonselective ion channels. *Journal of Neuroscience*, 23(10), 4054–4065.
- Montgomery, J. C., Baker, C. F., & Carton, A. G. (1997). The lateral line can mediate rheotaxis in fish. *Nature*, 389, 960–963.
- Moon, I. S., So, J. H., Jung, Y. M., Lee, W. S., Kim, E. Y., Choi, J. H., Kim, C. H., & Choi, J. Y. (2011). Fucoidan promotes mechanosensory hair cell regeneration following amino glycoside-induced cell death. *Hearing Research*, 282(1–2), 236–42.
- More, S. S., Akil, O., Ianculescu, A. G., Geier, E. G., Lustig, L. R., & Giacomini, K. M. (2010). Role of the copper transporter, CTR1, in platinum-induced ototoxicity. *Journal of Neuroscience*, 30(28), 9500–9509.
- Mullins, M. C., Hammerschmidt, M., Haffter, P., & Nusslein-Volhard, C. (1994). Large-scale mutagenesis in the zebrafish: In search of genes controlling development in a vertebrate. *Current Biology*, 4(3), 189–202.
- Murakami, S. L., Cunningham, L. L., Werner, L. A., Bauer, E., Pujol, R., Raible, D. W., & Rubel, E. W. (2003). Developmental differences in susceptibility to neomycin-induced hair cell death in the lateral line neuromasts of zebrafish (*Danio rerio*). *Hearing Research*, 186(1–2), 47–56.
- Nagiel, A., Andor-Ardo, D., & Hudspeth, A. J. (2008). Specificity of afferent synapses onto plane-polarized hair cells in the posterior lateral line of the zebrafish. *Journal of Neuroscience*, 28(34), 8442–8453.
- Namdaran, P., Reinhart, K. E., Owens, K. N., Raible, D. W., & Rubel, E. W. (2012). Identification of modulators of hair cell regeneration in the zebrafish lateral line. *Journal of Neuroscience*, 32(10), 3516–3528.
- Nasevicius, A., & Ekker, S. C. (2000). Effective targeted gene 'knockdown' in zebrafish. *Nature Genetics*, 26(2), 216–220.
- Nicolson, T. (2005). The genetics of hearing and balance in zebrafish. *Annual Review of Genetics*, 39, 9–22.
- Nicolson, T., Rusch, A., Friedrich, R. W., Granato, M., Ruppertsberg, J. P., & Nusslein-Volhard, C. (1998). Genetic analysis of vertebrate sensory hair cell mechanosensation: The zebrafish circler mutants. *Neuron*, 20(2), 271–283.
- Obholzer, N., Wolfson, S., Trapani, J. G., Mo, W., Nechiporuk, A., Busch-Nentwich, E., Seiler, C., Sidi, S., Sollner, C., Duncan, R. N., Boehland, A., Nicolson, T. (2008). Vesicular glutamate transporter 3 is required for synaptic transmission in zebrafish hair cells. *Journal of Neuroscience*, 28(9), 2110–2118.
- Odelberg, S. J. (2005). Cellular plasticity in vertebrate regeneration. *Anatomical Record. Part B, New anatomist.*, 287(1), 25–35.
- Oesterle, E.C., & Rubel, E.W (1993). Postnatal production of supporting cells in the chick cochlea. *Hearing Research* 66(2), 213–224
- Olivari, F. A., Hernandez, P. P., & Allende, M. L. (2008). Acute copper exposure induces oxidative stress and cell death in lateral line hair cells of zebrafish larvae. *Brain Research*, 1244, 1–12.
- Ou, H. C., Raible, D. W., & Rubel, E. W. (2007). Cisplatin-induced hair cell loss in zebrafish (*Danio rerio*) lateral line. *Hearing Research*, 233(1–2), 46–53.
- Ou, H. C., Cunningham, L. L., Francis, S. P., Brandon, C. S., Simon, J. A., Raible, D. W., & Rubel, E. W. (2009). Identification of FDA-approved drugs and bioactives that protect hair cells in the zebrafish (*Danio rerio*) lateral line and mouse (*Mus musculus*) utricle. *Journal of the Association for Research in Otolaryngology*, 10(2), 191–203.
- Owens, K. N., Cunningham, D. E., MacDonald, G., Rubel, E. W., Raible, D. W., & Pujol, R. (2007). Ultrastructural analysis of aminoglycoside-induced hair cell death in the zebrafish lateral line reveals an early mitochondrial response. *Journal of Comparative Neurology*, 502(4), 522–543.

- Owens, K. N., Santos, F., Roberts, B., Linbo, T., Coffin, A. B., Knisely, A. J., Simon, J. A., Rubel, E. W., & Raible, D. W. (2008). Identification of genetic and chemical modulators of zebrafish mechanosensory hair cell death. *PLoS Genetics*, 4(2), e1000020.
- Owens, K. N., Coffin, A. B., Hong, L. S., Bennett, K. O., Rubel, E. W., & Raible, D. W. (2009). Response of mechanosensory hair cells of the zebrafish lateral line to aminoglycosides reveals distinct cell death pathways. *Hearing Research*, 253(1–2), 32–41.
- Parinov, S., Kondrichin, I., Korzh, V., & Emelyanov, A. (2004). Tol2 transposon-mediated enhancer trap to identify developmentally regulated zebrafish genes in vivo. *Developmental Dynamics*, 231(2), 449–459.
- Peterson, R. T., Link, B. A., Dowling, J. E., & Schreiber, S. L. (2000). Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proceedings of the National Academy of Sciences of the USA*, 97(24), 12965–12969.
- Postlethwait, J. H., Johnson, S. L., Midson, C. N., Talbot, W. S., Gates, M., Ballinger, E. W., Africa, D., Andrews, R., Carl, T., Eisen, J. S., Horne, S., Kimmel, C. B., Mutchinson, M., Johnson, M., & Rodriguez, A. (1994). A genetic linkage map for the zebrafish. *Science*, 264(5159), 699–703.
- Prezant, R. T., Shohat, M., Jaber, L., Pressman, S., & Fischel-Ghodsian, N. (1992). Biochemical characterization of a pedigree with mitochondrially inherited deafness. *American Journal of Medical Genetics*, 44(4), 465–472.
- Raible, D. W., & Kruse, G. J. (2000). Organization of the lateral line system in embryonic zebrafish. *Journal of Comparative Neurology*, 421(2), 189–198.
- Raviv, D., Dror, A. A., & Avraham, K. B. (2010). Hearing loss: A common disorder caused by many rare alleles. *Annals of the New York Academy of Sciences*, 1214, 168–179.
- Ricci, A. J., & Fettiplace, R. (1998). Calcium permeation of the turtle hair cell mechanotransducer channel and its relation to the composition of endolymph. *Journal of Physiology*, 506 (Pt 1), 159–173.
- Richardson, G. P., Forge, A., Kros, C. J., Fleming, J., Brown, S. D., & Steel, K. P. (1997). Myosin VIIA is required for aminoglycoside accumulation in cochlear hair cells. *Journal of Neuroscience*, 17(24), 9506–9519.
- Rihel, J., Prober, D. A., Arvanites, A., Lam, K., Zimmerman, S., Jang, S., Haggarty, S. J., Kokel, D., Rubin, L. L., Peterson, R. T., & Schier, A. F. (2010). Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*, 327(5963), 348–351.
- Ruben, R. J. (2000). Redefining the survival of the fittest: Communication disorders in the 21st century. *Laryngoscope*, 110(2 Pt 1), 241–245.
- Ryals, B. M., & Rubel, E. W. (1988). Hair cell regeneration after acoustic trauma in adult Coturnix quail. *Science*, 240(4860), 1774–1776.
- Rybak, L. P., & Ramkumar, V. (2007). Ototoxicity. *Kidney International*, 72(8), 931–935.
- Salvi, R. J., Popper, A. N., & Fay, R. R. (2008). *Hair Cell Regeneration, Repair, and Protection*. New York: Springer.
- Santos, F., MacDonald, G., Rubel, E. W., & Raible, D. W. (2006). Lateral line hair cell maturation is a determinant of aminoglycoside susceptibility in zebrafish (*Danio rerio*). *Hearing Research*, 213(1–2), 25–33.
- Schacht, J., & Hawkins, J. E. (2006). Sketches of otohistory. Part 11: Ototoxicity: Drug-induced hearing loss. *Audiology and Neuro-Otology*, 11(1), 1–6.
- Schacht, J., Popper, A. N., & Fay, R. R. (2008). *Auditory Trauma, Protection, and Repair*. New York: Springer.
- Schier, A. F., Neuhauss, S. C., Harvey, M., Malicki, J., Solnica-Krezel, L., Stainier, D. Y., Zwartkruis, F., Abdelilah, S., Stemple, D. L., Rangini, Z., Yang, H., & Driever, W. (1996). Mutations affecting the development of the embryonic zebrafish brain. *Development*, 123, 165–178.
- Seiler, C., Ben-David, O., Sidi, S., Hendrich, O., Rusch, A., Burnside, B., Avraham, K. B., & Nicolson, T. (2004). Myosin VI is required for structural integrity of the apical surface of sensory hair cells in zebrafish. *Developmental Biology*, 272(2), 328–338.

- Self, T., Mahony, M., Fleming, J., Walsh, J., Brown, S. D., & Steel, K. P. (1998). Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. *Development*, 125(4), 557–566.
- Seligmann, H., Podoshin, L., Ben-David, J., Fradis, M., & Goldsher, M. (1996). Drug-induced tinnitus and other hearing disorders. *Drug Safety*, 14(3), 198–212.
- Sha, S. H., & Schacht, J. (2000). Antioxidants attenuate gentamicin-induced free radical formation in vitro and ototoxicity in vivo D-Methionine is a potential protectant. *Hearing Research*, 142(1–2), 34–40.
- Smith, M. E., Coffin, A. B., Miller, D. L., & Popper, A. N. (2006). Anatomical and functional recovery of the goldfish (*Carassius auratus*) ear following noise exposure. *Journal of Experimental Biology*, 209(Pt 21), 4193–4202.
- Solnica-Krezel, L., Stemple, D. L., Mountcastle-Shah, E., Rangini, Z., Neuhauss, S. C., Malicki, J., Schier, A. F., Stainier, D. Y., Zwartkruis, F., Abdelilah, S., & Driever, W. (1996). Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish. *Development*, 123, 67–80.
- Song, J., Yan, H. Y., & Popper, A. N. (1995). Damage and recovery of hair cells in fish canal (but not superficial) neuromasts after gentamicin exposure. *Hearing Research*, 91(1–2), 63–71.
- Speidel, C. C. (1947). Correlated studies of sense organs and nerves of the lateral-line in living frog tadpoles. *Journal of Comparative Neurology*, 87, 29–55.
- Stemple, D. L., Solnica-Krezel, L., Zwartkruis, F., Neuhauss, S. C., Schier, A. F., Malicki, J., Stainier, D. Y., Abdelilah, S., Rangini, Z., Mountcastle-Shah, E., & Driever, W. (1996). Mutations affecting development of the notochord in zebrafish. *Development*, 123, 117–128.
- Steyger, P. S., Peters, S. L., Rehling, J., Hordichok, A., & Dai, C. F. (2003). Uptake of gentamicin by bullfrog saccular hair cells in vitro. *Journal of the Association for Research in Otolaryngology*, 4(4), 565–578.
- Stone, J. S., & Cotanche, D. A. (2007). Hair cell regeneration in the avian auditory epithelium. *International Journal of Developmental Biology*, 51(6–7), 633–647.
- Stone, L. (1933). The development of lateral-line sense organs in amphibians observed in living and vital-stained preparations. *Journal of Comparative Neurology*, 57, 507–540.
- Stone, L. (1937). Further experimental studies of the development of lateral-line sense organs in amphibians observed in living preparations. *Journal of Comparative Neurology*, 68, 83–115.
- Suli, A., Watson, G. M., Rubel, E. W., & Raible, D. W. (2012). Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells. *PLoS One*, 7(2), e29727.
- Suster, M. L., Kikuta, H., Urasaki, A., Asakawa, K., & Kawakami, K. (2009). Transgenesis in zebrafish with the tol2 transposon system. *Methods in Molecular Biology*, 561, 41–63.
- Ton, C., & Parg, C. (2005). The use of zebrafish for assessing ototoxic and otoprotective agents. *Hearing Research*, 208(1–2), 79–88.
- Trapani, J. G., & Nicolson, T. (2011). Mechanism of spontaneous activity in afferent neurons of the zebrafish lateral-line organ. *Journal of Neuroscience*, 31(5), 1614–1623.
- Van Trump, W. J., Coombs, S., Duncan, K., & McHenry, M. J. (2010). Gentamicin is ototoxic to all hair cells in the fish lateral line system. *Hearing Research*, 261 (1–2), 42–50.
- Wang, J., Ladrech, S., Pujol, R., Brabet, P., Van De Water, T. R., & Puel, J. L. (2004). Caspase inhibitors, but not c-Jun NH2-terminal kinase inhibitor treatment, prevent cisplatin-induced hearing loss. *Cancer Research*, 64(24), 9217–9224.
- Warchol, M. E. (2011). Sensory regeneration in the vertebrate inner ear: Differences at the levels of cells and species. *Hearing Research*, 273(1–2), 72–79.
- Webb, J. F., Montgomery, J. C., & Mogdans, J. (2008). Bioacoustics and the lateral line system of fishes. In J. F. Webb, R. R. Fay, & A. N. Popper (Eds.), *Fish bioacoustics* (pp. 145–182). New York: Springer.
- Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M. D., Kelley, P. M., Kimberling, W. J., Wagenaar, M., Levi-Acobas, F., Larget-Piet, D., Munnich, A., Steel, K. P., Brown, S. D. M., & Petit, C. (1995). Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature*, 374(6517), 60–61.

- Weisleder, P., & Rubel, E. W. (1993). Hair cell regeneration after streptomycin toxicity in the avian vestibular epithelium. *Journal of Comparative Neurology*, 331(1), 97–110.
- Wersäll, J., & Flock, Å. (1964). Suppression and restoration of the microphonic output from the lateral line organ after local application of streptomycin. *Life Sciences*, 3, 1151–1155.
- Whitfield, T. T. (2002). Zebrafish as a model for hearing and deafness. *Journal of Neurobiology*, 53(2), 157–171.
- Whitfield, T. T., Granato, M., van Eeden, F. J., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C. P., Jiang, Y. J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J., & Nusslein-Volhard, C. (1996). Mutations affecting development of the zebrafish inner ear and lateral line. *Development*, 123, 241–254.
- Whitfield, T. T., Riley, B. B., Chiang, M. Y., & Phillips, B. (2002). Development of the zebrafish inner ear. *Developmental Dynamics*, 223(4), 427–458.
- Wibowo, I., Pinto-Teixeira, F., Satou, C., Higashijima, S., & Lopez-Schier, H. (2011). Compartmentalized Notch signaling sustains epithelial mirror symmetry. *Development*, 138(6), 1143–1152.
- Williams, J. A., & Holder, N. (2000). Cell turnover in neuromasts of zebrafish larvae. *Hearing Research*, 143(1–2), 171–181.
- World Health Organization (2012). Deafness and Hearing Impairment Fact Sheet, 2012. Retrieved from <http://www.who.int/mediacentre/factsheets/fs300/en/> (Accessed March 15, 2012).
- Wright, M. R. (1947). Regeneration and degeneration experiments on lateral line nerves and sense organs in anurans. *Journal of Experimental Zoology*, 105, 221–257.
- Xiao, T., Roeser, T., Staub, W., & Baier, H. (2005). A GFP-based genetic screen reveals mutations that disrupt the architecture of the zebrafish retinotectal projection. *Development*, 132(13), 2955–2967.
- Zingler, V. C., Cnyrim, C., Jahn, K., Weintz, E., Fernbacher, J., Frenzel, C., Brandt, T., & Strupp, M. (2007). Causative factors and epidemiology of bilateral vestibulopathy in 255 patients. *Annals of Neurology*, 61(6), 524–532.