Published in final edited form as:
*Wiley Interdiscip Rev Dev Biol*. 2015 January ; 4(1): 1–16. doi:10.1002/wdev.160.

# There and Back Again: Development and Regeneration of the Zebrafish Lateral Line System

**Eric D. Thomas**[1,3], **Ivan A. Cruz**[2,3], **Dale W. Hailey**[3], and **David W. Raible**[1,2,3,*]

[1]Graduate Program in Neurobiology and Behavior, University of Washington, Seattle, WA 98195-7420, USA

[2]Molecular and Cellular Biology Graduate Program, University of Washington, Seattle, WA 98195-7420, USA

[3]Department of Biological Structure, University of Washington, Seattle, WA 98195-7420, USA

## Abstract

The zebrafish lateral line is a sensory system used to detect changes in water flow. It is comprised of clusters of mechanosensory hair cells called neuromasts. The lateral line is initially established by a migratory group of cells, called a primordium, that deposits neuromasts at stereotyped locations along the surface of the fish. Wnt, FGF, and Notch signaling are all important regulators of various aspects of lateral line development, from primordium migration to hair cell specification. As zebrafish age, the organization of the lateral line becomes more complex in order to accommodate the fish's increased size. This expansion is regulated by many of the same factors involved in the initial development. Furthermore, unlike mammalian hair cells, lateral line hair cells have the capacity to regenerate after damage. New hair cells arise from the proliferation and differentiation of surrounding support cells, and the molecular and cellular pathways regulating this are beginning to be elucidated. All in all, the zebrafish lateral line has proven to be an excellent model in which to study a diverse array of processes, including collective cell migration, cell polarity, cell fate, and regeneration.

## INTRODUCTION

Aquatic vertebrates use the lateral line system to detect changes in surrounding water currents, enabling them to detect movements in their environment. This mechanoreceptive sense, "touch at a distance", allows them to orient in currents, detect prey and avoid predators[1,2]. The lateral line system is comprised of clusters of mechanosensory hair cells and support cells called neuromasts (Figure 1) which lie on the surface of the body. Neuromasts are located in stereotyped locations on the head, comprising the anterior lateral line system (aLL), or along the body, forming the posterior lateral line system (pLL; Figure 2). The zebrafish lateral line has attracted considerable interest as a model system to understand fundamental principles about sensory development, collective cell migration, growth, and regeneration.

*Author for correspondence (draible@uw.edu).

The development of the lateral line system has fascinated embryologists for over a hundred years. Harrison[3] and Stone[4,5] demonstrated that the lateral line system is formed by a migrating primordium that deposits neuromasts in its wake. The coordinated movement of cells, known as collective migration, has recently been recognized as a distinct mode of cell movement[6]. In this review, we will discuss recent progress in understanding the signals that pattern the zebrafish lateral line primordium, control its migration and promote the differentiation of mechanosensory neuromasts.

We will also discuss growth and regeneration of the zebrafish lateral line system. Mechanosensory hair cells of the lateral line system share many properties with inner ear hair cells, which are used for hearing and balance[7]. These similarities include genes that encode for functional mechanotransduction, many of which are disrupted in inherited human deafness. Like mechanosensory hair cells of the inner ear, lateral line hair cells are innervated by peripheral neurons with central projections to the hindbrain. Lateral line hair cells are also susceptible to the same agents that damage human hair cells[8]. However, in contrast to human inner ear hair cells, lateral line hair cells can regenerate after damage. The location of lateral line hair cells on the surface of the body makes them easily accessible, and thus an excellent system in which to study the mechanisms underlying damage and regeneration.

## THE POSTERIOR LATERAL LINE PRIMORDIUM: PATTERNING AND MIGRATION

The zebrafish pLL primordium (pLLP) is generated from placodal cells adjacent to the ear. The primordium migrates down the flank of the fish, along the horizontal myoseptum of the somites and beneath the skin (Figure 3; Movie 1). The primordium is organized along its migratory axis, from leading to trailing zones. This axis reflects relative maturity, with higher proliferation occurring in the leading zone. While in the trailing zone, cells are organized into rosettes, or protoneuromasts, which then separate from the primordium and are deposited as neuromasts (Fig. 3, Movie 1). This axial organization also regulates migration along the flank, and is coordinately controlled by distinct signaling pathways. In this section we will discuss the molecular mechanisms of primordium development and migration.

### Specification of the posterior lateral line placode and primordium

The pLL originally derives from the lateral line placode, located just posterior to the otic vesicle. The lateral line placode can first be distinguished at approximately 18 hours post fertilization (hpf), and derives from a pre-placodal region in which the genes *six1b* and *eya1* are uniformly expressed[9,10]. Prior to placode formation, these pre-placodal cells are designated to become neural or epithelial. At approximately 14 hpf, the *six1b* expression domain retracts, and the proneural transcription factors *neurog1* and *neuroD* are upregulated in the rostral-most quarter of the region; these cells will develop into the posterior lateral line ganglion (pLLG). The cells in the caudal three-quarters of the pre-placodal region will eventually regain *six1b* expression and form the pLLP. The rostral-most cells forming the pLLG are restricted to a proneural fate through Notch signaling. Inhibition of Notch

signaling, by either morpholino injection or mutation, results in a significant expansion of the *neuroD* expression domain, leading to an increase in the number of neurons at the expense of primordium cells. Conversely, overexpression of the Notch intracellular domain (NICD) further restricts *neuroD* expression and decreases the number of neurons[9]. Therefore, activation of Notch signaling in the caudal pre-placodal region drives cells to adopt a sensory epithelial fate, while restricting a neural fate to the rostral-most region via lateral inhibition.

## Migration of the lateral line primordium

Migration of the pLLP is controlled by the chemokine Sdf1a (alternately named Cxcl12a) and its receptors Cxcr4b and Cxcr7b. At 20 hpf, the pLLP begins migrating from just posterior to the otic vesicle toward the tip of the tail[11]. A stripe of cells along the horizontal myoseptum, the path along which the pLLP migrates, expresses the chemokine *sdf1a*, whereas the pLLP expresses the chemokine receptors *cxcr4b* and *cxcr7b*[12–14]. Disrupting Sdf1a or either of its receptors prevents pLLP migration[12,14]. However, unlike other contexts in which an expression gradient of guidance cues regulates migratory behavior, *sdf1a* is expressed uniformly across the horizontal myoseptum[15]. It is the asymmetric distribution of the chemokine receptors within the primordium, rather than of *sdf1a*, that is responsible for the directional migration of the primordium. *Cxcr4b* expression is restricted to the posterior two-thirds of the traveling primordium (hereafter known as the leading zone), whereas *cxcr7b* expression is limited to the remaining third (called the trailing zone; Figure 4A). Both *cxcr4b* and *cxcr7b* morphants show an expansion in their respective expression regions, indicating antagonistic interactions between the two receptors[15]. This is further supported by the fact that ectopic expression of *cxcr4b* suppresses *cxcr7b* expression. The heterotrimeric G protein G1β, a downstream target of Cxcr4b, is also active in the leading zone, and promotes actin polymerization in the direction of migration[16].

Regulated restriction of the two chemokine receptors to complementary expression domains is critical for primordium migration. Expression of the homeobox gene *hoxb8a* is also restricted to the leading zone of the primordium (Fig. 4A). Morpholino-mediated knockdown of *hoxb8a* results in migration defects as well as in a significant reduction in *cxcr4b* expression. Conversely, overexpression of *hoxb8a* diminishes *cxcr7b* expression, suggesting that Hoxb8a regulates chemokine receptor expression in both the leading and trailing zone[17]. Regulation of *cxcr4b* expression in the trailing zone is inhibited by the estrogen receptor 1 (*esr1): cxcr4b* expression expands throughout the entire primordium in *esr1* morphants, whereas ectopic *esr1* expression significantly reduces or ablates *cxcr4b* expression[18]. However, whether *esr1* is regulated directly by Cxcr7b or by some other downstream factor remains unknown (Fig. 4A).

How does this asymmetric receptor expression convert a uniformly expressed chemokine signal into a directional migration signal? It has been hypothesized that, because Cxcr7b binds Sdf1 with a much higher affinity than Cxcr4b, it serves as a molecular sink at the trailing edge of the primordium[15]. Binding of Sdf1 to Cxcr7b results in its internalization and degradation, thus preventing any Cxcr4b receptors expressed near the trailing zone from binding the chemokine. This sink activity would create a gradient of chemokine activity

across the primordium, with a high level of Cxcr4b-Sdf1a binding at the leading edge of the primordium and a low level towards the trailing zone. Several recent studies have provided evidence for such a localized gradient; utilizing fluorescently-tagged receptor internalization and turnover as a proxy for Sdf1a binding, these studies show that Sdf1a activity is highest at the leading edge and declines linearly across the primordium[19,20]. Furthermore, expression of *cxcr7b* within the lateral line nerve, which migrates along with the pLLP, was sufficient to rescue this gradient as well as migration defects in *cxcr7b* mutants, indicating that *cxcr7b* sink activity is sufficient to drive the collective migration of the pLLP[19].

## Primordium patterning

The organization of cells within the migrating primordium is determined by the interplay between the canonical Wnt and FGF signaling pathways. Canonical Wnt signaling, as measured by *lef1* expression, is high in the leading zone of the pLLP, whereas FGF signaling, as measured by expression of the downstream target *pea3*, is high in the trailing region[21–23] (Fig. 4B). Wnt signaling serves to establish FGF signaling in the trailing zone, as well as to drive proliferation in the leading zone, whereas FGF signaling drives the formation of the epithelial rosettes which will ultimately form the neuromasts. As with the expression of the chemokine receptors, the localization of these signaling pathways is determined through mutual inhibition. While Wnt signaling drives the expression of the ligands Fgf3 and Fgf10a, it also induces local expression of the FGF inhibitor *sef*, thus preventing FGF signaling from occurring in the leading zone[21]. In the absence of Wnt signaling, Fgf3 and Fgf10a are active in the trailing zone, where they are able to activate Fgfr1and initiate FGF signaling. FGF signaling in the trailing zone also induces the expression of Dkk1b, an inhibitor of Wnt signaling, thus restricting Wnt signaling to the leading zone of the pLLP (Fig. 4B). In *apc* mutants, in which Wnt signaling is constitutively active, *sef* expression expands into the trailing zone, but is significantly downregulated after overexpression of *dkk1b*. Conversely, expression of *pea3* and *dkk1b* expands into the leading zone in *sef* morphants[21]. Perturbing either of these signaling pathways results in defects in either pLLP migration or in rosette morphogenesis[21–23]. Thus, the balance between Wnt and FGF signaling is essential for proper lateral line development.

While it is well established that Wnt and Fgf signaling play important roles within the primordium, it is still not clear to what degree each signaling pathway is active within domains along the leading-trailing axis. While expression of downstream targets can serve as a proxy for signaling activity, the actual levels of Wnt and FGF activity relative to each other remains unknown. The problem is complicated by unknown factors such as rates or extent of diffusion, relative activities of inhibitors and interactions across signaling pathways. Note that, although there is significant overlap between regions, the leading zone as established by *cxcr4b* expression is not synonymous with a Wnt domain, and likewise for the trailing zone, *cxcr7b*, and an FGF domain. Clarification of these issues awaits further quantitative studies coupled with mathematical modeling.

## Morphogenesis of epithelial rosettes

Neuromast precursors are organized in epithelial rosettes that mature as they progress towards the trailing end of the primordium for deposition[22,23]. Upon their exit from the

leading zone, cells begin to develop apicobasal polarity, which is imposed by well-characterized regulators of this process, such as atypical protein kinase C and Lgl[24]. Rosette formation is initiated by apical constriction of cells through the actions of non-muscle myosin II and the actin binding protein Shroom3[24,25]. Rosette maturation continues with the redistribution of Cadherin2 into adherens junctions in the apical zone through the actions of dynamic microtubules[26]. Together, these changes in polarity coordinate the transition between leading and trailing zones within the primordium and shape the formation of the neuromast organelles.

FGF signaling is responsible for the morphogenesis of the epithelial rosettes that will ultimately form the mature neuromasts. Within the trailing region of the pLLP, *fgf10a* expression is limited to a few individual cells, whereas *fgfr1* is expressed throughout the trailing zone, but excluded from those few *fgf10a*-expressing cells[22,23]. These *fgf10a*-expressing cells will form the center of epithelial rosettes and will lead to the activation of Fgfr1 in the surrounding cells. In turn, Fgfr1 activation initiates apical constriction as cells leave the leading zone, causing the cells to develop a columnar morphology. More specifically, apical constriction is mediated by FGF-Ras-MAPK signaling, which results in the apical localization of the Rho-activated kinase, Rock2a (Figure 5). Rock2a then phosphorylates the myosin regulatory light chain (MRLC) and induces apical constriction. Inhibition of FGF-Ras-MAPK signaling does not repress Rock2a expression, but it does prevent its apical localization, which in turn prevents MRLC phosphorylation and apical constriction, thus preventing rosette formation[27]. The apical localization of Rock2a is mediated by FGF-dependent expression of Shroom3[25] (Fig. 5). Rosette formation is prevented in *shroom3* morphants, and *shroom3* overexpression prevents the loss of rosettes following inhibition of FGF signaling. The working model in Fig. 5 is likely to be refined as a clearer picture of the quality and extent of FGF signaling along with interaction among other signaling pathways is clarified.

## Proliferation and neuromast deposition

As neuromasts are deposited from the trailing zone of the pLLP, new rosettes form in the leading zone to replace them. Fate mapping experiments demonstrate that a small number of cells in the leading zone expand to contribute to multiple neuromasts[23]. The rate of cell proliferation varies across the migrating primordium, with higher levels of cell division in the leading zone than in the trailing zone[28]. These observations are consistent with the idea that the leading zone consists of proliferating neuromast progenitors. The rate of cell proliferation has been suggested to regulate the rate of neuromast deposition[23]. Aman *et al.* noted that, as the primordium migrates toward the tail, the leading zone, which lacks *cxcr7b* expression, remains constant in size, whereas the *cxcr7b* domain in the trailing edge fluctuates in size as new protoneuromasts are deposited[29]. The authors posited that the rate of cell addition within the leading edge determines how quickly newly-formed rosettes enter the *cxcr7b* domain for deposition, and thus determines the rate of neuromast deposition. Consistent with this hypothesis, decreases in proliferation or increases in apoptosis significantly reduce the rates of deposition, leading to a decrease in the total number of neuromasts deposited along the trunk[29].

Although both Wnt and FGF signaling regulate proliferation within the pLLP, Wnt signaling is the primary regulator at the leading edge. Inhibition of the Wnt-regulated transcription factor *lef1* strongly reduces proliferation in the leading edge and results in the premature termination of pLLP migration and in altered neuromast patterning[30–32]. McGraw *et al.* suggest that *lef1* is responsible for maintaining a progenitor identity within cells of the leading zone. Fate mapping experiments revealed that a subset of cells remain in the leading zone throughout pLLP migration in wild type fish, but those cells exited the leading zone prematurely in *lef1* mutants[31]. Valdivia *et al.*, on the other hand, posit that *lef1* regulates the size of the migratory leading column as well as how far from the leading edge new rosettes are formed, and that loss of *lef1* decreases the length of the migratory column until the leading zone is depleted and migration halts[32].

Inhibition of *rspo3*, a facilitator of Wnt signaling, results in a reduction in proliferation comparable to that of *lef1* knockdown[33]. However, unlike in *lef1* mutants, reduction in *rspo3* levels did not result in premature termination of migration or alter the rate of neuromast deposition. This suggests that the migration and deposition defects seen in *lef1* mutants are not dependent upon proliferation *per se*, but rather upon some other function of *lef1*. This study found that *lef1* promotes the expression of another FGF inhibitor, the MAPK phosphatase *dusp6* (also known as *mkp3*), and that inhibition of *dusp6* results in protoneuromast formation closer to the leading edge as well as alteration of migration and neuromast deposition as seen in *lef1* mutants[33]. Thus, Lef1 mediates the rate of neuromast deposition by balancing the Wnt and FGF signaling domains via Dusp6 (Fig. 4B).

## NEUROMAST ORGANIZATION AND DEVELOPMENT

The neuromast forms the unit structure of the lateral line sensory system. It is composed of centrally positioned hair cells that project their mechanosensory bundles, enclosed in a gelatinous cupula, out into the water. The hair cells are surrounded by supporting cells (Fig. 1), which are positioned basally and send fine interdigitating processes between the hair cells. The neuromast surface is covered by mantle cells. At their basal surface, hair cells contain ribbon synapses. These synapses are innervated by projections from afferent neurons (Fig. 1) with cell bodies in the lateral line ganglia. The hair cells also have efferent connections with cell bodies in the hindbrain. In this section we review the development and organization of cell types within the neuromast.

### Specification of hair cell precursors

The bHLH transcription factor *atoh1a* is required for hair cell development, both in the inner ear and the lateral line[34]. Within the migrating pLLP, expression of *atoh1a* becomes restricted to hair cell precursors in the central cell of each rosette as morphogenesis begins[35,36]. Like other aspects of protoneuromast formation, expression of *atoh1a* is initially regulated by FGF signaling[22,23]. Within the central cell of each rosette, *atoh1a* not only drives the continued expression of *fgf10a*, thus establishing this cell as an FGF signaling center, but also mediates the expression of *deltaD*[36] (Figure 6). Activation of Fgfr1 in the surrounding cells results in the expression of *notch3*. Interaction between the centrally-expressed DeltaD ligand and the Notch3 receptor represses expression of *atoh1a* in surrounding cells. *Atoh1a* also drives the expression of *atoh1b*, which maintains *atoh1a*

expression in central cells; the loss of *atoh1b* severely reduces *atoh1a* expression in developing protoneuromasts[34,36]. The expression of *atoh1a* in the central cell specifies it as a hair cell precursor, whereas the lack of *atoh1a* expression in the surrounding cells specifies them as support cells (Fig. 6). This process of lateral inhibition is necessary for proper neuromast development; when Notch signaling is blocked, *atoh1a* is upregulated in surrounding cells and hair cells are generated at the expense of supporting cells[34,36]. The interplay between Notch and FGF signaling must be properly regulated for proper morphogenesis of the pLLP. When Notch signaling is eliminated, primordium migration and neuromast deposition is disrupted—a phenotype that can be partially relieved by concomitant reduction in *atoh1a*[36].

## Planar polarization of lateral line neuromasts

Lateral line hair cells have an inherent polarity, and respond to mechanical stimuli in primarily one direction. Hair bundles, comprised of rows of actin-based stereocilia and a larger, microtubule-based kinocilium, are located at the apical surface of the hair cell, with the kinocilium placed asymmetrically to the stereocilia. This arrangement determines the directionality of stimulus sensitivity: displacement of stereocilia toward the kinocilium results in depolarization of the hair cell, whereas deflection away from the kinocilium results in hyperpolarization. The axis along which these bundles are arranged determines the axis of planar cell polarity (PCP): bundles can be arranged along the anteroposterior axis (AP) or the dorsoventral axis (DV) of the body (Figure 7).

Within an individual neuromast, all hair cells have bundles oriented along the same axis (either AP or DV), but in opposing directions, so that half of the hair cells respond to stimuli from one direction (*i.e.* deflection toward the posterior) and the other half respond to stimuli from the other (deflection toward the anterior). Hair cells of opposing directionality are initially arranged along an axis perpendicular to the PCP axis, creating an axis of mirror symmetry throughout the neuromast. Disruption of genes important for maintenance of PCP, such as *vangl2*, results in a random orientation of hair cells with respect to one another[37]. The PCP axis of an individual neuromast depends upon its developmental origin (a more complete description of the distinct sets of lateral line precursors and their contribution to postembryonic patterning is covered in a following section). Neuromasts that are deposited from the first primordium (primI) have AP polarity, whereas neuromasts deposited by the second primordium and dorsal primordium (primII and primD) have DV polarity[37,38]. When the direction of primordium migration is altered, for example in mutants with abnormal somites, neuromast polarity is also altered, suggesting that migration direction predetermines neuromast orientation[37,38]. However, later-developing intercalary neuromasts have the same polarity as primI-derived neuromasts despite having distinct migration histories[39], suggesting that inherent cues establish polarity independent of migration.

Studies to determine the origin of PCP organization have shown that hair cell precursors divide to give rise to two new hair cells. This supports the hypothesis that mirror symmetry within the neuromast arises from the coordinated division of hair cell precursors along the PCP axis[37]. However, live imaging of regenerating neuromasts has revealed that precursors do not inherently divide along the PCP axis, but instead undergo extensive rearrangements

to ensure their proper final orientation[40]. In *vangl2* mutants, the number of rearrangements that follow misaligned precursor division is significantly reduced, so that hair cells remain randomly oriented within the neuromast. Vangl2 thus ensures the proper alignment of hair cell precursors after cell division. It is unknown if other core PCP proteins, such as Disheveled, Frizzled, and Prickle, also play a role in this process.

### Neuromast innervation

Neuromast hair cells are innervated by afferent neurons of the pLLG[11,41,42] (Fig. 1). Individual afferent neurons innervate multiple hair cells within a neuromast, and may also innervate multiple neuromasts. However, the afferent will only form lasting synapses with hair cells of matching polarity, even across multiple neuromasts[43,44]. Afferent neurons that do make connections with hair cells of differing polarities soon cull one of those connections to maintain consistent selection of hair cell polarity. This specificity is even maintained after hair cell damage and regeneration[43,44]. The preference of afferent neurons for hair cells of particular polarity appears to be mediated by evoked hair cell activity. While neurons still prefer a specific hair cell polarity in the absence of mechanotransduction[45], afferent arborization becomes more complex, neurites are more transient, and neurons display decreased affinity for the appropriate hair cells[46]. Lateral line afferents project centrally to the hindbrain, where they display a somatotopic organization according to the neuromasts they innervate in the periphery[47,48]. This peripheral and central somatotopy is dependent, in part, on the birth order of the neurons[49,50].

The initial extension of lateral line ganglion axons follows pLLP migration. As the pLLP migrates from the otic vesicle to the tip of the tail, some growth cones of lateral line neurons migrate along with it[11]. Inhibition of primordium migration, via inhibition of *cxcr4b*, also inhibits the extension of the lateral line nerve[51]. This co-migration of the primordium and nerve is dependent upon glial cell line-derived neurotrophic factor (GDNF) signaling. The migrating primordium produces GDNF, whereas the lateral line neurons express both GFRα1, a GDNF receptor, as well as *ret1*, a downstream target of GDNF[52]. Reduction of *gdnf* or *ret1* activity has no effect on primordium migration, but significantly reduces the extension of the lateral line nerve[53]. After initial leader axons have extended along with the primordium, other axons follow, with both leaders and followers showing distinct somatotopy[54]. Specification of leader and follower axons is determined by different levels of expression of the transcription factor *neuroD;* overexpression of *neuroD* turns followers into leaders[55]. These results support the idea that organization of neuronal projections is independent of peripheral targets[41].

## LATERAL LINE EXPANSION AND GROWTH

The lateral line system becomes larger and more elaborate as the zebrafish grows to adulthood. Instead of a single posterior line containing seven to eight neuromasts, the adult zebrafish has four posterior lines extending along the trunk and tail that are organized into groups of neuromasts called stitches[39,56–59] (Figure 8). The pattern of the aLL, which is initially established at the same time as the pLL, also becomes more elaborate, with some neuromasts becoming incorporated into bony canals while others remain superficial[60,61] (Fig. 8). As the caudal fin grows, the terminal neuromasts elaborate to give rise to four lines

of neuromasts along the rays of the fin, forming the caudal lateral line (cLL)[62,63]. There are three different mechanisms by which new neuromasts are added as the zebrafish grows: migration of additional primordia, expansion of latent precursors, and budding from existing neuromasts. In this section we will review recent studies exploring how new lateral line organs are added.

The major anatomical features of the pLL organs of the trunk are defined by the migration of distinct primordia[39]. The L line is established by the migration of the initial pLL primordium (primI) described earlier, as well as that of the second primordium (primII), which follows the path of primI along the horizontal myoseptum[58]. The D line is established by a third primordium (primD) that migrates along the dorsal midline. The distinct primordia develop at different times from adjacent placodes, along with the neurons that innervate them[10]. During juvenile stages, the neuromasts of the L and D lines are displaced ventrally and two new lines are established, forming a total of four lines of neuromasts. These new lines, called L′ and D′, derive from the interneuromast cells that are deposited by primII and primD, respectively.

In addition to the primary neuromasts deposited by migrating primordia, intercalary neuromasts develop *in situ* from primordium-derived cells laid down between each primary neuromast[64]. These interneuromast cells are latent precursors that proliferate and coalesce into new neuromasts as the zebrafish develops. The development of these new neuromasts is regulated by signals from glial cells associated with the adjacent lateral line nerve. In mutant fish that lack glia, such as *erbb2b* mutants, or in fish where the nerves are physically removed, neuromasts develop precociously. These data indicate that the glial cells are responsible for maintaining interneuromast quiescence[64,65] As is the case with primary neuromasts, the development of intercalary neuromasts is regulated by canonical Wnt and Fgf signaling[65].

The budding of new accessory neuromasts from existing primary neuromasts results in stitch formation, as well as the growth of new lines along the caudal fin rays and the gill operculum[39,59,63] (Fig. 8). The formation of accessory neuromasts is regulated by underlying dermal structures such as bones or scales[59]. Stitches are normally innervated by the same nerve, and this innervation is necessary for proper stitch formation and maintenance[10,66]. The size of new accessory neuromasts is controlled by Wnt signaling[67]. When new neuromasts begin to bud, Wnt signaling is activated in cells near the previously deposited neuromast. The Wnt activator R-spondin (*rspo2*), which can stabilize Wnt signaling by binding to the LGR receptor, is expressed in axons that innervate budding neuromasts, whereas *lgr6* is expressed in the neuromasts. Inhibition of Wnt signaling by *dkk2* overexpression prevents the formation of cLL neuromasts, and ablation of the afferent nerve diminishes Wnt signaling. If neuromasts are denervated they degenerate over time, indicating that innervation is also required for long-term maintenance of neuromasts[66]. Together, these data indicate that both innervation and Wnt signaling are required for the budding and maintenance of accessory neuromasts.

## REGENERATION OF LATERAL LINE HAIR CELLS

In addition to the expanding growth of the lateral line system during juvenile phases, the neuromasts can undergo repair after damage. Lateral line hair cells, like inner ear hair cells, are susceptible to chemical exposure. Aminoglycoside antibiotic drugs[68,69], chemotherapeutics[70,71] and metal ions[72–74] have all been shown to cause lateral line hair cell death. After damage by any of these toxins, hair cells regenerate and are restored to their original number[69,75,76] (Figure 9). Neuromast polarity is also restored after hair cell regeneration[37], along with specificity of afferent innervation[44]. Larval behaviors that require lateral line function, such as predator avoidance[77] or orientation in flow (rheotaxis)[78], are restored as hair cells are regenerated, with behavior restored before regeneration is complete. In this section we will review mechanisms underlying lateral line regeneration.

### Hair cell regeneration by cell division

Zebrafish lateral line hair cells are rapidly replaced after damage[37,68,69,73,76]. Following treatment with the aminoglycoside neomycin, complete hair cell recovery occurs within 72 hours (Fig. 9), as measured by FM1-43 dye uptake[76]. Hair cell addition can be observed earlier using transgenic markers for immature hair cells[73]. To better understand how hair cells regenerate, transgenic fish with fluorescently-labeled hair cells and other cell types have been used for live time-lapse analyses. These studies have shown that symmetric divisions of support cells produce two daughter hair cells[37,40,79]. Using BrdU or phospho-histone H3 antibodies to identify cells in the cell cycle, the majority of divisions occur within the first 24 hours following damage[76]. While there is evidence of some new hair cells forming without proliferation[75]. However, it is most likely that these cells reflect developmental hair cell addition that is ongoing even in the absence of damage. Consistent with the idea that the vast majority of new hair cells are generated through cell division, mitotic inhibitors block regeneration[40,80,81].

### Identity of hair cell progenitors

Efforts have been made to identify stem cells within the support cell population by examining proliferation during regeneration[37,40,76,79]. Time-lapse analyses have revealed that dividing precursors localize to poles of each neuromast, perpendicular to the axis of hair cell polarity. However, these precursors migrate into the pole position from elsewhere in the neuromast, suggesting that the polar compartment is not a stem cell niche[40]. Since hair cell precursors divide symmetrically, with both daughters generating new hair cells, other cells must be responsible for renewal of the precursors if the neuromast is to retain the ability to regenerate hair cells. The identity or location of these cells is currently unknown.

Distinctions among supporting cell populations within the neuromast have only recently been established. Lateral line specific transgenes have been discovered through GFP enhancer trap screens that label various support cell populations[82–84], and have been used to profile gene expression in support cell populations[83,85–87]. These studies have provided a source of both support cell markers and genetic regulators of support cell function.

## Genes regulating hair cell regeneration

While hair cell regeneration shares many characteristics with development, it is hypothesized that there are some genes that are regeneration-specific. These genes would regulate processes that include allocation of latent precursor cells, or the trigger mechanisms that initiate regeneration. The molecular mechanisms underlying these processes are essentially unknown at this time. Researchers are performing forward genetic screens in order to identify pathways and genes that are important for lateral line hair cell regeneration. So far, only one mutant has been identified affecting hair cell regeneration. In a screen, Behra *et al*. identified a retroviral integration into a previously uncharacterized gene, *phoenix*. Lateral line hair cells develop normally in these mutants, but show a severe reduction in the ability to regenerate after ablation[82]. This study demonstrates that forward genetic screens are likely to identify other genes and pathways that play specific roles in hair cell regeneration.

As discussed previously, Notch and Wnt signaling regulate many aspects of lateral line development, and both of these signaling pathways have been shown to modulate hair cell maintenance and regeneration. *In situ* hybridization experiments have shown that Notch signaling components are upregulated in lateral line neuromasts following hair cell damage. Furthermore, inhibition of Notch signaling with the γ-secretase inhibitor DAPT increases the number of proliferating precursors and the number of regenerated hair cells[76]. However, DAPT treatment has no effect on hair cell number in the absence of damage. These results suggest that Notch signaling is initiated during regeneration to help ensure that the correct number of hair cells are regenerated and restore the system to a quiescent state.

Pharmacological activation or inhibition of canonical Wnt signaling supports the idea that this signaling pathway is an equally important regulator of support cell proliferation during hair cell regeneration, as it is during development[88]. Neuromast size is controlled by a balance between Wnt signaling activity in proliferating support cells and the Wnt-inhibiting Dkk activity produced by differentiated lateral line hair cells[67]. This negative feedback loop regulates the proliferation of support cells as well as the size of the neuromast. Transcript analysis after damage supports the idea that Wnt and Notch may be regulating later steps in hair cell regeneration, such as the return to quiescence, but are not involved in the earliest steps in triggering regeneration[86].

Analysis of gene regulation during regeneration of the adult zebrafish inner ear in response to noise exposure identified the *stat3/socs3a* signaling pathway as a potential regulator of hair cell regeneration[89]. This study also implicated *stat3/socs3a* in lateral line hair cell regeneration, as phosphorylated Stat3 accumulates in support cell nuclei following hair cell death, and pharmacological inhibition of Stat3 appeared to accelerate the regenerative process. Transcript analysis supports the idea that *stat3* may play a role in lateral line hair cell regeneration as it is one of the earliest genes to be upregulated after damage[86]; however, this observation is somewhat paradoxical with respect to the effects of Stat3 inhibition. Further studies are needed to determine how this signaling pathway is activated during regeneration and the stages at which it might regulate the process.

## CONCLUSIONS

The zebrafish lateral line system has emerged as a model for studying the fundamental processes of development, including tissue patterning, morphogenesis, and growth. Its position on the surface of the body makes it well-suited to visualization and manipulation. The regeneration of functional mechanosensory cells after damage offers the potential to uncover processes involved in the maintenance, proliferation, and differentiation of sensory precursors. Future studies have the potential for understanding circuit development regulating fundamental behaviors[90] and the evolution of tissue patterning[91,92]. The lateral line system has bright prospects for future studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We acknowledge Heather Brignull for critical reading and Arminda Suli for providing the Tg(*ribeyeA*:tRFP) fish. EDT and IAC were supported by the Developmental Biology Predoctoral Training Grant (T32HD007183) from NICHD. Supported in part by NIH DC005987 and DC011269 to DWR.

## References

1. Dijkgraaf S. The functioning and significance of the lateral-line organs. Biol Rev Camb Philos Soc. 1963; 38:51–105. [PubMed: 14027866]
2. Coombs, S.; Bleckmann, H.; Fay, RR.; Popper, AN. The Lateral Line System. Springer; New York: 2014.
3. Harrison RG. Experimentelle Untersuchungen Über die Entwicklung der Sinnesorgane der Seitenlinie bei den Ampkibien. Arch Für Mikrosk Anat. 1903; 63:35–149.
4. Stone LS. Primitive lines in Amblystoma and their relation to the migratory lateral-line primordia. J Comp Neurol. 1928; 45:169–190.
5. Stone LS. The development of lateral-line sense organs in amphibians observed in living and vital-stained preparations. J Comp Neurol. 1933; 57:507–540.
6. Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol. 2009; 10:445–457. [PubMed: 19546857]
7. Nicolson T. The Genetics of Hearing and Balance in Zebrafish. Annu Rev Genet. 2005; 39:9–22. [PubMed: 16285850]
8. Ou H, Simon JA, Rubel EW, Raible DW. Screening for chemicals that affect hair cell death and survival in the zebrafish lateral line. Hear Res. 2012; 288:58–66. [PubMed: 22310494]
9. Mizoguchi T, Togawa S, Kawakami K, Itoh M. Neuron and sensory epithelial cell fate is sequentially determined by Notch signaling in zebrafish lateral line development. J Neurosci Off J Soc Neurosci. 2011; 31:15522–15530.
10. Sarrazin AF, Nuñez VA, Sapède D, Dambly-Chaudière C, Ghysen A. Origin and early development of the posterior lateral line system of zebrafish. J Neurosci Off J Soc Neurosci. 2010; 30:8234–8244.
11. Metcalfe WK, Kimmel CB, Schabtach E. Anatomy of the posterior lateral line system in young larvae of the zebrafish. J Comp Neurol. 1985; 233:377–389. [PubMed: 3980776]
12. David NB, Sapède D, Saint-Etienne L, Thisse C, Thisse B, Dambly-Chaudière C, Rosa FM, Ghysen A. Molecular basis of cell migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. Proc Natl Acad Sci U S A. 2002; 99:16297–16302. [PubMed: 12444253]

13. Haas P, Gilmour D. Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. Dev Cell. 2006; 10:673–680. [PubMed: 16678780]

14. Valentin G, Haas P, Gilmour D. The chemokine SDF1a coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. Curr Biol CB. 2007; 17:1026–1031.

15. Dambly-Chaudière C, Cubedo N, Ghysen A. Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. BMC Dev Biol. 2007; 7:23. [PubMed: 17394634]

16. Xu H, Ye D, Behra M, Burgess S, Chen S, Lin F. Gβ1 controls collective cell migration by regulating the protrusive activity of leader cells in the posterior lateral line primordium. Dev Biol. 2014; 385:316–327. [PubMed: 24201188]

17. Breau MA, Wilkinson DG, Xu Q. A Hox gene controls lateral line cell migration by regulating chemokine receptor expression downstream of Wnt signaling. Proc Natl Acad Sci U S A. 2013; 110:16892–16897. [PubMed: 24082091]

18. Gamba L, Cubedo N, Ghysen A, Lutfalla G, Dambly-Chaudière C. Estrogen receptor ESR1 controls cell migration by repressing chemokine receptor CXCR4 in the zebrafish posterior lateral line system. Proc Natl Acad Sci U S A. 2010; 107:6358–6363. [PubMed: 20308561]

19. Donà E, Barry JD, Valentin G, Quirin C, Khmelinskii A, Kunze A, Durdu S, Newton LR, Fernandez-Minan A, Huber W, et al. Directional tissue migration through a self-generated chemokine gradient. Nature. 2013; 503:285–289. [PubMed: 24067609]

20. Venkiteswaran G, Lewellis SW, Wang J, Reynolds E, Nicholson C, Knaut H. Generation and dynamics of an endogenous, self-generated signaling gradient across a migrating tissue. Cell. 2013; 155:674–687. [PubMed: 24119842]

21. Aman A, Piotrowski T. Wnt/beta-catenin and Fgf signaling control collective cell migration by restricting chemokine receptor expression. Dev Cell. 2008; 15:749–761. [PubMed: 19000839]

22. Lecaudey V, Cakan-Akdogan G, Norton WHJ, Gilmour D. Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. Dev Camb Engl. 2008; 135:2695–2705.

23. Nechiporuk A, Raible DW. FGF-dependent mechanosensory organ patterning in zebrafish. Science. 2008; 320:1774–1777. [PubMed: 18583612]

24. Hava D, Forster U, Matsuda M, Cui S, Link BA, Eichhorst J, Wiesner B, Chitnis A, Abdelilah-Seyfried S. Apical membrane maturation and cellular rosette formation during morphogenesis of the zebrafish lateral line. J Cell Sci. 2009; 122:687–695. [PubMed: 19208766]

25. Ernst S, Liu K, Agarwala S, Moratscheck N, Avici ME, Dalle Nogare D, Chitnis AB, Ronneberger O, Lecaudey V. Shroom3 is required downstream of FGF signalling to mediate proneuromast assembly in zebrafish. Dev Camb Engl. 2012; 139:4571–4581.

26. Revenu C, Streichan S, Donà E, Lecaudey V, Hufnagel L, Gilmour D. Quantitative cell polarity imaging defines leader-to-follower transitions during collective migration and the key role of microtubule-dependent adherens junction formation. Dev Camb Engl. 2014; 141:1282–1291.

27. Harding MJ, Nechiporuk AV. Fgfr-Ras-MAPK signaling is required for apical constriction via apical positioning of Rho-associated kinase during mechanosensory organ formation. Dev Camb Engl. 2012; 139:3130–3135.

28. Laguerre L, Ghysen A, Dambly-Chaudière C. Mitotic patterns in the migrating lateral line cells of zebrafish embryos. Dev Dyn Off Publ Am Assoc Anat. 2009; 238:1042–1051.

29. Aman A, Nguyen M, Piotrowski T. Wnt/β-catenin dependent cell proliferation underlies segmented lateral line morphogenesis. Dev Biol. 2011; 349:470–482. [PubMed: 20974120]

30. Gamba L, Cubedo N, Lutfalla G, Ghysen A, Dambly-Chaudiere C. Lef1 controls patterning and proliferation in the posterior lateral line system of zebrafish. Dev Dyn Off Publ Am Assoc Anat. 2010; 239:3163–3171.

31. McGraw HF, Drerup CM, Culbertson MD, Linbo T, Raible DW, Nechiporuk AV. Lef1 is required for progenitor cell identity in the zebrafish lateral line primordium. Dev Camb Engl. 2011; 138:3921–3930.

32. Valdivia LE, Young RM, Hawkins TA, Stickney HL, Cavodeassi F, Schwarz Q, Pullin LM, Villegas R, Moro E, Argenton F, et al. Lef1-dependent Wnt/β-catenin signalling drives the

proliferative engine that maintains tissue homeostasis during lateral line development. Dev Camb Engl. 2011; 138:3931–3941.

33. Matsuda M, Nogare DD, Somers K, Martin K, Wang C, Chitnis AB. Lef1 regulates Dusp6 to influence neuromast formation and spacing in the zebrafish posterior lateral line primordium. Dev Camb Engl. 2013; 140:2387–2397.
34. Millimaki BB, Sweet EM, Dhason MS, Riley BB. Zebrafish atoh1 genes: classic proneural activity in the inner ear and regulation by Fgf and Notch. Dev Camb Engl. 2007; 134:295–305.
35. Itoh M, Chitnis AB. Expression of proneural and neurogenic genes in the zebrafish lateral line primordium correlates with selection of hair cell fate in neuromasts. Mech Dev. 2001; 102:263–266. [PubMed: 11287207]
36. Matsuda M, Chitnis AB. Atoh1a expression must be restricted by Notch signaling for effective morphogenesis of the posterior lateral line primordium in zebrafish. Dev Camb Engl. 2010; 137:3477–3487.
37. López-Schier H, Hudspeth AJ. A two-step mechanism underlies the planar polarization of regenerating sensory hair cells. Proc Natl Acad Sci U S A. 2006; 103:18615–18620. [PubMed: 17124170]
38. López-Schier H, Starr CJ, Kappler JA, Kollmar R, Hudspeth AJ. Directional cell migration establishes the axes of planar polarity in the posterior lateral-line organ of the zebrafish. Dev Cell. 2004; 7:401–412. [PubMed: 15363414]
39. Nuñez VA, Sarrazin AF, Cubedo N, Allende ML, Dambly-Chaudière C, Ghysen A. Postembryonic development of the posterior lateral line in the zebrafish. Evol Dev. 2009; 11:391–404. [PubMed: 19601973]
40. Wibowo I, Pinto-Teixeira F, Satou C, Higashijima S, López-Schier H. Compartmentalized Notch signaling sustains epithelial mirror symmetry. Dev Camb Engl. 2011; 138:1143–1152.
41. Gompel N, Dambly-Chaudière C, Ghysen A. Neuronal differences prefigure somatotopy in the zebrafish lateral line. Dev Camb Engl. 2001; 128:387–393.
42. Raible DW, Kruse GJ. Organization of the lateral line system in embryonic zebrafish. J Comp Neurol. 2000; 421:189–198. [PubMed: 10813781]
43. Faucherre A, Pujol-Martí J, Kawakami K, López-Schier H. Afferent neurons of the zebrafish lateral line are strict selectors of hair-cell orientation. PloS One. 2009; 4:e4477. [PubMed: 19223970]
44. Nagiel A, Andor-Ardó D, Hudspeth AJ. Specificity of afferent synapses onto plane-polarized hair cells in the posterior lateral line of the zebrafish. J Neurosci Off J Soc Neurosci. 2008; 28:8442–8453.
45. Nagiel A, Patel SH, Andor-Ardó D, Hudspeth AJ. Activity-independent specification of synaptic targets in the posterior lateral line of the larval zebrafish. Proc Natl Acad Sci U S A. 2009; 106:21948–21953. [PubMed: 19996172]
46. Faucherre A, Baudoin JP, Pujol-Martí J, López-Schier H. Multispectral four-dimensional imaging reveals that evoked activity modulates peripheral arborization and the selection of plane-polarized targets by sensory neurons. Dev Camb Engl. 2010; 137:1635–1643.
47. Alexandre D, Ghysen A. Somatotopy of the lateral line projection in larval zebrafish. Proc Natl Acad Sci U S A. 1999; 96:7558–7562. [PubMed: 10377454]
48. Liao JC. Organization and physiology of posterior lateral line afferent neurons in larval zebrafish. Biol Lett. 2010; 6:402–405. [PubMed: 20181553]
49. Pujol-Martí J, Baudoin JP, Faucherre A, Kawakami K, López-Schier H. Progressive neurogenesis defines lateralis somatotopy. Dev Dyn Off Publ Am Assoc Anat. 2010; 239:1919–1930.
50. Pujol-Martí J, Zecca A, Baudoin JP, Faucherre A, Asakawa K, Kawakami K, López-Schier H. Neuronal birth order identifies a dimorphic sensorineural map. J Neurosci Off J Soc Neurosci. 2012; 32:2976–2987.
51. Gilmour D, Knaut H, Maischein HM, Nüsslein-Volhard C. Towing of sensory axons by their migrating target cells in vivo. Nat Neurosci. 2004; 7:491–492. [PubMed: 15097993]
52. Shepherd IT, Beattie CE, Raible DW. Functional Analysis of Zebrafish GDNF. Dev Biol. 2001; 231:420–435. [PubMed: 11237470]

53. Schuster K, Dambly-Chaudière C, Ghysen A. Glial cell line-derived neurotrophic factor defines the path of developing and regenerating axons in the lateral line system of zebrafish. Proc Natl Acad Sci U S A. 2010; 107:19531–19536. [PubMed: 20974953]
54. Sato A, Koshida S, Takeda H. Single-cell analysis of somatotopic map formation in the zebrafish lateral line system. Dev Dyn Off Publ Am Assoc Anat. 2010; 239:2058–2065.
55. Sato A, Takeda H. Neuronal subtypes are specified by the level of neurod expression in the zebrafish lateral line. J Neurosci Off J Soc Neurosci. 2013; 33:556–562.
56. Haehnel M, Taguchi M, Liao JC. Heterogeneity and dynamics of lateral line afferent innervation during development in zebrafish (Danio rerio). J Comp Neurol. 2012; 520:1376–1386. [PubMed: 22102005]
57. Ledent V. Postembryonic development of the posterior lateral line in zebrafish. Dev Camb Engl. 2002; 129:597–604.
58. Sapède D, Gompel N, Dambly-Chaudière C, Ghysen A. Cell migration in the postembryonic development of the fish lateral line. Dev Camb Engl. 2002; 129:605–615.
59. Wada H, Ghysen A, Satou C, Higashijima S, Kawakami K, Hamaguchi S, Sakaizumi M. Dermal morphogenesis controls lateral line patterning during postembryonic development of teleost fish. Dev Biol. 2010; 340:583–594. [PubMed: 20171200]
60. Webb JF, Shirey JE. Postembryonic development of the cranial lateral line canals and neuromasts in zebrafish. Dev Dyn Off Publ Am Assoc Anat. 2003; 228:370–385.
61. Wada H, Iwasaki M, Kawakami K. Developmental of the lateral line canal system through a bone remodeling process in zebrafish. Dev Biol. 2014; 392:1–14. [PubMed: 24836859]
62. Dufourcq P, Roussigné M, Blader P, Rosa F, Peyrieras N, Vriz S. Mechano-sensory organ regeneration in adults: the zebrafish lateral line as a model. Mol Cell Neurosci. 2006; 33:180–187. [PubMed: 16949838]
63. Wada H, Hamaguchi S, Sakaizumi M. Development of diverse lateral line patterns on the teleost caudal fin. Dev Dyn Off Publ Am Assoc Anat. 2008; 237:2889–2902.
64. Grant KA, Raible DW, Piotrowski T. Regulation of latent sensory hair cell precursors by glia in the zebrafish lateral line. Neuron. 2005; 45:69–80. [PubMed: 15629703]
65. Lush ME, Piotrowski T. ErbB expressing Schwann cells control lateral line progenitor cells via non-cell-autonomous regulation of Wnt/β-catenin. eLife. 2014; 3:e01832. [PubMed: 24642408]
66. Wada H, Dambly-Chaudière C, Kawakami K, Ghysen A. Innervation is required for sense organ development in the lateral line system of adult zebrafish. Proc Natl Acad Sci U S A. 2013; 110:5659–5664. [PubMed: 23509277]
67. Wada H, Ghysen A, Asakawa K, Abe G, Ishitani T, Kawakami K. Wnt/Dkk negative feedback regulates sensory organ size in zebrafish. Curr Biol CB. 2013; 23:1559–1565.
68. Harris JA, Cheng AG, Cunningham LL, MacDonald G, Raible DW, Rubel EW. Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (Danio rerio). J Assoc Res Otolaryngol JARO. 2003; 4:219–234.
69. Williams JA, Holder N. Cell turnover in neuromasts of zebrafish larvae. Hear Res. 2000; 143:171–181. [PubMed: 10771194]
70. Ou HC, Raible DW, Rubel EW. Cisplatin-induced hair cell loss in zebrafish (Danio rerio) lateral line. Hear Res. 2007; 233:46–53. [PubMed: 17709218]
71. Ton C, Parng C. The use of zebrafish for assessing ototoxic and otoprotective agents. Hear Res. 2005; 208:79–88. [PubMed: 16014323]
72. Faucher K, Floriani M, Gilbin R, Adam-Guillermin C. Uranium-induced sensory alterations in the zebrafish Danio rerio. Aquat Toxicol Amst Neth. 2012; 124–125:94–105.
73. Hernández PP, Moreno V, Olivari FA, Allende ML. Sub-lethal concentrations of waterborne copper are toxic to lateral line neuromasts in zebrafish (Danio rerio). Hear Res. 2006; 213:1–10. [PubMed: 16386394]
74. Linbo TL, Stehr CM, Incardona JP, Scholz NL. Dissolved copper triggers cell death in the peripheral mechanosensory system of larval fish. Environ Toxicol Chem SETAC. 2006; 25:597–603.

75. Hernández PP, Olivari FA, Sarrazin AF, Sandoval PC, Allende ML. Regeneration in zebrafish lateral line neuromasts: expression of the neural progenitor cell marker sox2 and proliferation-dependent and-independent mechanisms of hair cell renewal. Dev Neurobiol. 2007; 67:637–654. [PubMed: 17443814]
76. Ma EY, Rubel EW, Raible DW. Notch Signaling Regulates the Extent of Hair Cell Regeneration in the Zebrafish Lateral Line. J Neurosci. 2008; 28:2261–2273. [PubMed: 18305259]
77. McHenry MJ, Feitl KE, Strother JA, Van Trump WJ. Larval zebrafish rapidly sense the water flow of a predator's strike. Biol Lett. 2009; 5:477–479. [PubMed: 19324627]
78. Suli A, Watson GM, Rubel EW, Raible DW. Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells. PloS One. 2012; 7:e29727. [PubMed: 22359538]
79. Mirkovic I, Pylawka S, Hudspeth AJ. Rearrangements between differentiating hair cells coordinate planar polarity and the establishment of mirror symmetry in lateral-line neuromasts. Biol Open. 2012; 1:498–505. [PubMed: 23213442]
80. Mackenzie SM, Raible DW. Proliferative regeneration of zebrafish lateral line hair cells after different ototoxic insults. PloS One. 2012; 7:e47257. [PubMed: 23094043]
81. Namdaran P, Reinhart KE, Owens KN, Raible DW, Rubel EW. Identification of modulators of hair cell regeneration in the zebrafish lateral line. J Neurosci Off J Soc Neurosci. 2012; 32:3516–3528.
82. Behra M, Bradsher J, Sougrat R, Gallardo V, Allende ML, Burgess SM. Phoenix is required for mechanosensory hair cell regeneration in the zebrafish lateral line. PLoS Genet. 2009; 5:e1000455. [PubMed: 19381250]
83. Behra M, Gallardo VE, Bradsher J, Torrado A, Elkahloun A, Idol J, Sheehy J, Zonies S, Xu L, Shaw KM, et al. Transcriptional signature of accessory cells in the lateral line, using the Tnk1bp1:EGFP transgenic zebrafish line. BMC Dev Biol. 2012; 12:6. [PubMed: 22273551]
84. Scott EK, Baier H. The cellular architecture of the larval zebrafish tectum, as revealed by gal4 enhancer trap lines. Front Neural Circuits. 2009; 3:13. [PubMed: 19862330]
85. Gallardo VE, Liang J, Behra M, Elkahloun A, Villablanca EJ, Russo V, Allende ML, Burgess SM. Molecular dissection of the migrating posterior lateral line primordium during early development in zebrafish. BMC Dev Biol. 2010; 10:120. [PubMed: 21144052]
86. Jiang L, Romero-Carvajal A, Haug JS, Seidel CW, Piotrowski T. Gene-expression analysis of hair cell regeneration in the zebrafish lateral line. Proc Natl Acad Sci U S A. 2014; 111:E1383–1392. [PubMed: 24706903]
87. Steiner AB, Kim T, Cabot V, Hudspeth AJ. Dynamic gene expression by putative hair-cell progenitors during regeneration in the zebrafish lateral line. Proc Natl Acad Sci U S A. 2014; 111:E1393–1401. [PubMed: 24706895]
88. Head JR, Gacioch L, Pennisi M, Meyers JR. Activation of canonical Wnt/β-catenin signaling stimulates proliferation in neuromasts in the zebrafish posterior lateral line. Dev Dyn Off Publ Am Assoc Anat. 2013; 242:832–846.
89. Liang J, Wang D, Renaud G, Wolfsberg TG, Wilson AF, Burgess SM. The stat3/socs3a pathway is a key regulator of hair cell regeneration in zebrafish. [corrected]. J Neurosci Off J Soc Neurosci. 2012; 32:10662–10673.
90. Pujol-Martí J, López-Schier H. Developmental and architectural principles of the lateral-line neural map. Front Neural Circuits. 2013; 7:47. [PubMed: 23532704]
91. Ghysen A, Dambly-Chaudière C, Coves D, de la Gandara F, Ortega A. Developmental origin of a major difference in sensory patterning between zebrafish and bluefin tuna. Evol Dev. 2012; 14:204–211. [PubMed: 23189756]
92. Piotrowski T, Baker CVH. The development of lateral line placodes: Taking a broader view. Dev Biol. 2014; 389:68–81. [PubMed: 24582732]

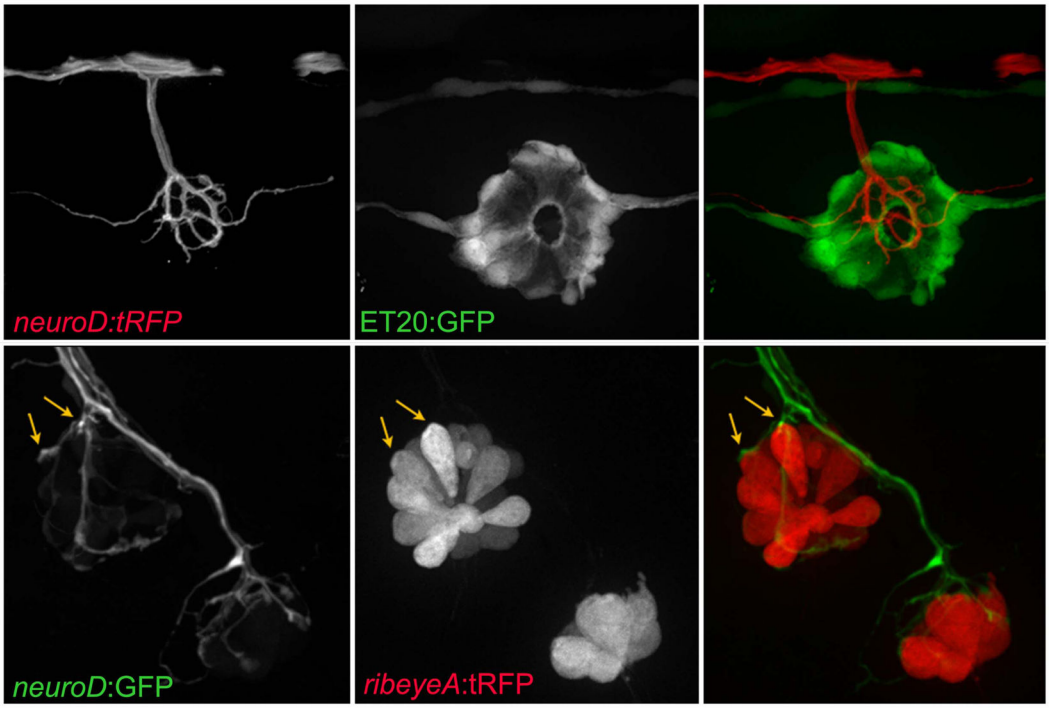


**Figure 1.**

Anatomy of lateral line neuromasts. **Top Panel)** Image of an individual neuromast at 5 dpf. The lateral line nerve and afferent terminals are labeled in red by *neuroD:*tRFP (left). Support cells are labeled in green by ET20:GFP (middle). **Bottom Panel)** 5 dpf neuromast, with afferent terminals labeled in green marked by *neuroD:*GFP (left), and the hair cells in red, marked by *ribeyeA:*tRFP (middle). Yellow arrows indicate afferent terminals (left) and their synaptic partners (middle).

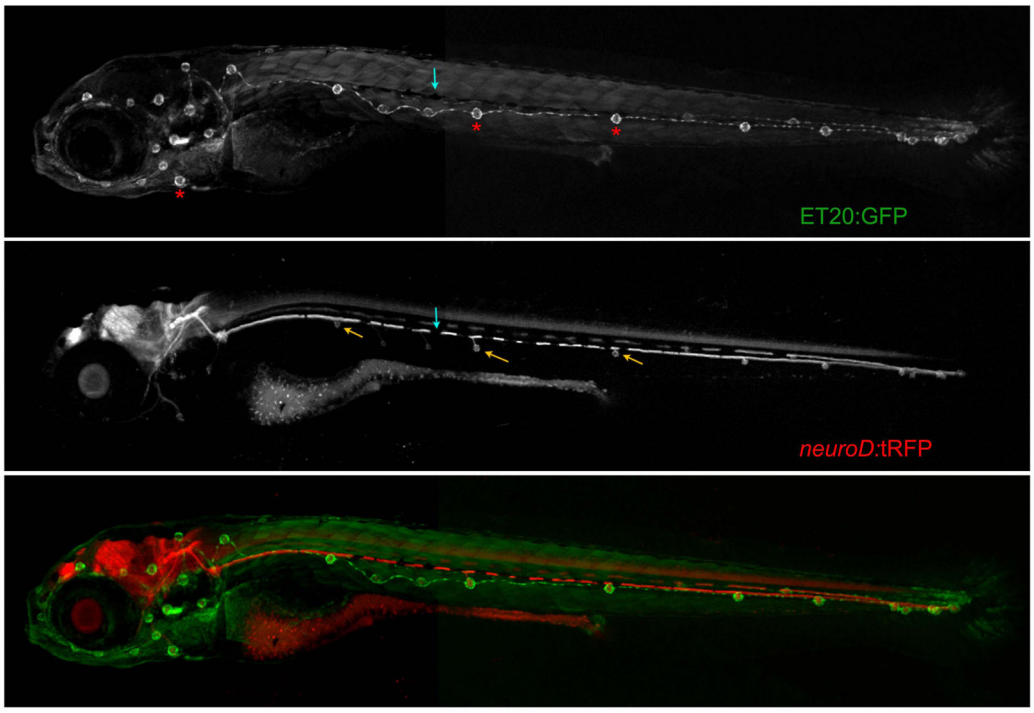


**Figure 2.**

Organization of the zebrafish lateral line. **Top Panel)** Image of a 5 dpf zebrafish larva in which the neuromasts and interneuromast cells are labeled by the transgenic marker ET20:GFP. Red asterisks indicate both aLL and pLL neuromasts. Blue arrow indicates a melanocyte located in the horizontal myoseptum. **Middle Panel)** The lateral line nerve, labeled by *neuroD:*tRFP. Blue arrow indicates the same melanocyte as in the top panel, which obscures signal from the submerged nerve. Yellow arrows indicate individual afferent fibers projecting from the central bundle and innervating neuromasts. **Bottom Panel)** Composite image of the top and middle panels.

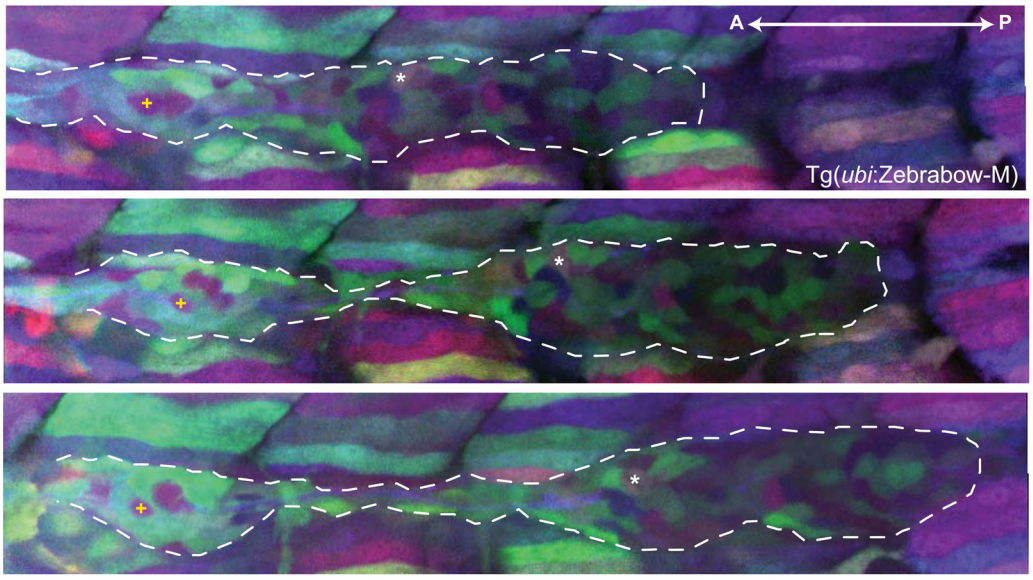


**Figure 3.**

Migration of the pLLP. **Top Panel)** Image of the migrating pLLP in the Zebrabow transgenic line. The primordium is outlined by the dashed white line, and is traveling posteriorly (to the right). **Middle Panel)** Deposition of a mature protoneuromast at the trailing edge of the primordium. **Bottom Panel)** After deposition, the primordium continues its migration, leaving a string of interneuromast cells in its wake. Due to the random colorization of cells in the Zebrabow line, individual cells can be followed over time. As the pLLP migrates, the tan cell marked by the white asterisk is displaced anteriorly as it moves out of the leading zone and into the trailing zone. Conversely, the red cell marked by the gold plus remains relatively immobile, having already been incorporated into a protoneuromast. Anterior and posterior are marked in the top panel.

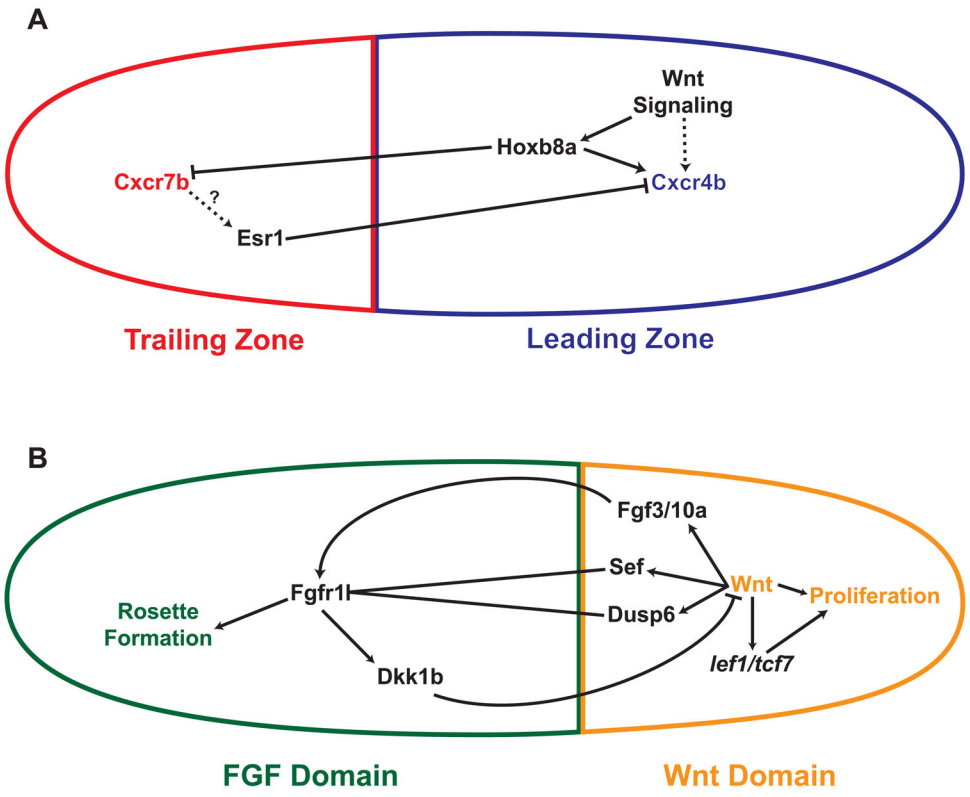


**Figure 4.**

Signaling pathways regulating pLLP patterning. **A)** Primordium migration is regulated by asymmetric expression of chemokine receptors. *Cxcr4b* is expressed in the leading zone, whereas *cxcr7b* is expressed in the trailing zone. *Cxcr4b* expression is activated by Wnt signaling and the homeobox gene *hoxb8a*, which itself is activated by Wnt signaling. *Hoxb8a* also represses expression of *cxcr7b*, thus limiting its expression to the trailing zone. Conversely, the estrogen receptor Esr1 is expressed in the trailing zone and restricts expression of *cxcr4b* to the leading zone. Dashed arrows indicate suggested, yet unproven, interactions. **B)** Localization of Wnt and FGF signaling within the pLLP. Wnt signaling mediates proliferation near the leading edge, in part through the effectors *lef1* and *tcf7*, whereas FGF signaling regulates the formation of epithelial rosettes closer to the trailing edge. Wnt-induced expression of the inhibitors Sef and Dusp6 restricts FGF signaling to the trailing zone, where the ligands Fgf3 and Fgf10a, themselves induced by Wnt signaling, can activate the Fgfr1 receptor. FGF signaling also drives the expression of the Wnt-inhibitor Dkk1b, which prevents Wnt activity from occurring in the trailing zone. Note that, while there is significant overlap between domains, the Wnt and FGF domains are not equivalent to the leading and trailing zones, respectively.

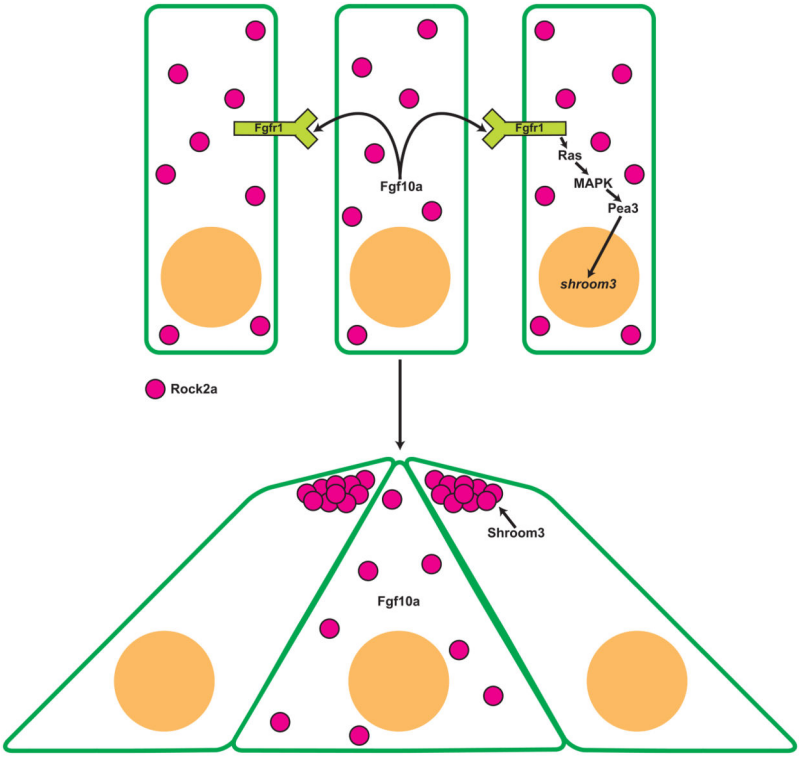


**Figure 5.**

FGF signaling regulates apical constriction of protoneuromasts. Localized Fgf10a expression drives FGF signaling in surrounding cells. Activation of FGF-Ras-MAPK signaling drives the expression of the actin binding protein Shroom3, which in turn mediates the apical localization of Rock2a, which induces apical constriction through its interaction with non-muscle myosin.

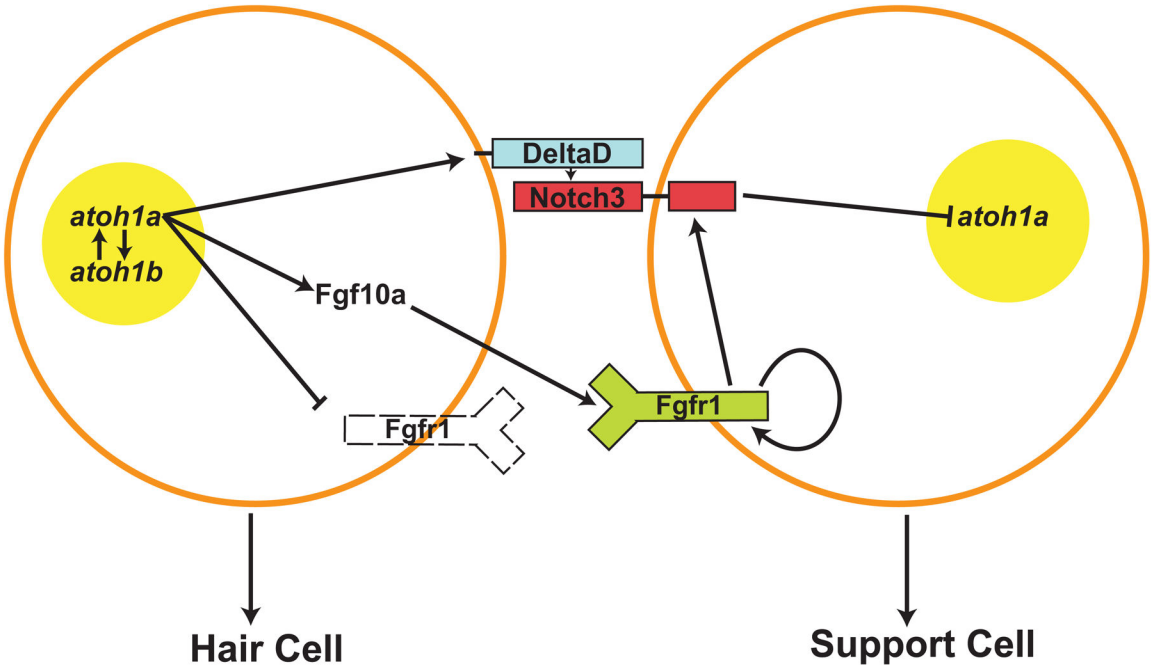


**Figure 6.**

Specification of hair cell precursors. Expression of *atoh1a* becomes restricted to centralized cells within developing protoneuromasts. *Atoh1a* expression activates *atoh1b*, which itself maintains expression of *atoh1a*. *Atoh1a* continues to drive expression of *fgf10a* and also activates expression of *deltaD*. Fgf10a diffuses to surrounding cells and, through activation of Fgfr1, maintains expression of *notch3*. This is prevented in the central cells by *atoh1a*-induced repression of *fgfr1* expression. Binding of Notch3 to DeltaD represses the expression of *atoh1a* in surrounding cells. *Atoh1a*-expressing cells will ultimately differentiate into hair cells, whereas the surrounding cells lacking *atoh1a* expression will become support cells.

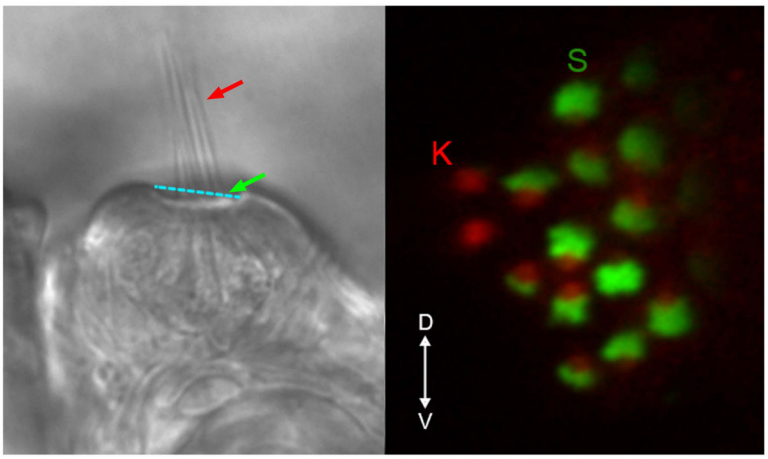


**Figure 7.**

Planar polarization of lateral line neuromasts. **Left Panel)** Lateral DIC image of a neuromast at 5 dpf. Red arrow indicates kinocilia, whereas the green arrow indicates the location of the stereocilia (which are not visible in this image). Dashed blue line indicates the oblique transection across the apical surface of the hair cells shown in the right panel. **Right Panel)** The stereocilia (S, labeled in green) are localized dorsally or ventrally to their respective kinocilia (K, labeled in red), indicating that this neuromast has dorsoventral planar polarity.

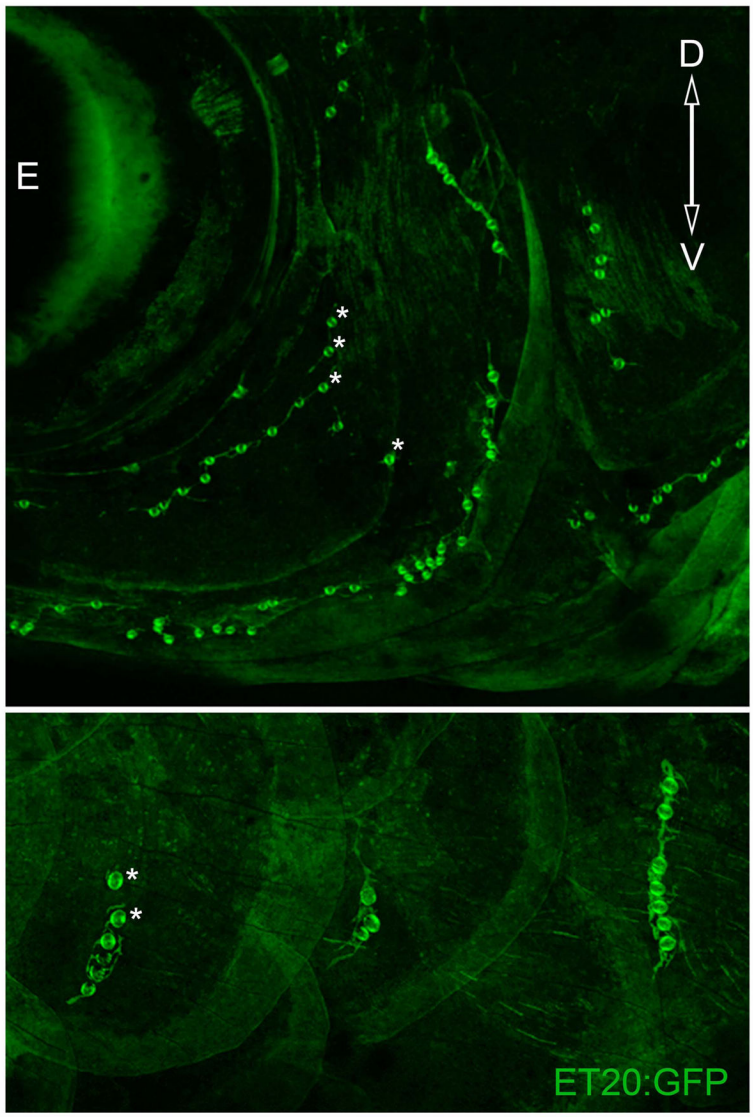


**Figure 8.**

Lateral line organization in adult zebrafish. **Top Panel)** Image of the anterior lateral line in an ET20:GFP transgenic adult fish. The eye is marked by "E." Neuromasts are indicated by white asterisks. Note the expansion of neuromast patterning compared to that of Figure 1. **Bottom Panel)** Image of stitch patterning in the adult posterior L line. New neuromasts bud dorsally and ventrally off of existing neuromasts. Scales included for scale.

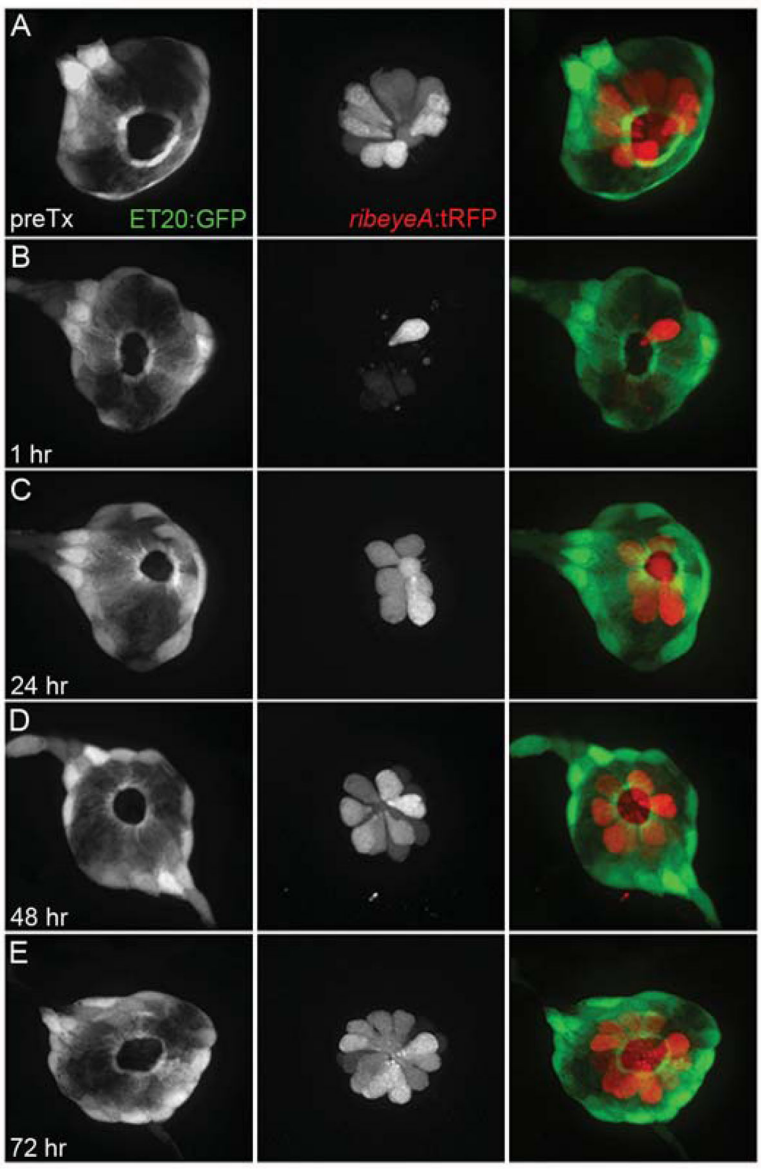


**Figure 9.**

Time course of hair cell regeneration. **A)** A 5 dpf neuromast, with support cells labeled in green (ET20) and hair cells labeled in red (*ribeyeA*), prior to neomycin treatment. **B)** At 1 hour post neomycin treatment, the vast majority of the hair cells are gone, while the support cells are unaffected. **C)** At 24 hours post treatment, some hair cells have been restored. **D)** By 48 hours post treatment, most hair cells have regenerated. **E)** After 72 hours, the number of hair cells has returned to pre-treatment levels.