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Building the posterior lateral line system in zebrafish

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Abstract

The posterior lateral line in zebrafish has emerged as an excellent system to study how a sensory organ system develops. Here we review recent studies that illustrate how interactions between multiple signaling pathways coordinate cell fate, morphogenesis and collective migration of cells in the posterior lateral line primordium. These studies also illustrate how the posterior lateral line system is contributing much more broadly to our understanding of mechanisms operating during the growth, regeneration and self-organization of other organ systems during development and disease.

INTRODUCTION

The mechanosensory lateral line system is a sensory network that evolved to detect the pattern of water movement over the bodies of fish and some amphibians (Coombs and van Netten, 2006). It is made up of mechanosensory organs called neuromasts, distributed in a stereotyped pattern over the surface of the animal's body, afferent neurons of the lateral line ganglia that carry information from these sensory organs back to the brain, and efferent neurons that carry information from the brain to the neuromasts. Each neuromast has sensory hair cells at its center. While the neuromast is embedded in the epidermis, each hair cell is exposed and extends a ciliary bundle into the water. These ciliary bundles are covered with a gelatinous cupula and water movement results in simultaneous shearing movement of the ciliary bundles causing individual hair cells to alter their firing rate when they move in a specific direction. The hair cells are innervated by sensory neurons in the lateral nerve ganglion, located adjacent to the otic vesicle. Hair cells at the center of the neuromast are surrounded by support cells, which both provide support and serve as a progenitor population from which more sensory hair cells will be generated during growth and regeneration of the sensory hair cells following injury. Mantle cells surround the support cells and they are thought to secrete the gelatinous substance in the cupula that surrounds ciliary bundles (Ghyssen and Dambly-Chaudiere, 2007).

Named for the prominent pits, grooves and lines on the body surface in which some of the neuromasts are embedded, this relatively simple sensory system has attracted the attention of biologists for over a hundred and fifty years (Dijkgraaf, 1989). The canals of the lateral line system were initially thought to be mucous secreting organs, until the German anatomist Franz Leydig discovered neuromasts in very wide canals in 1850. By 1861 Franz Eilhard Schulze had examined fish and amphibians under the microscope and recognized free neuromasts with their cupulae for the first time. He noticed movement of cupulae caused by water impact and suggested that gross water movements or low frequency vibrations may

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stimulate them. Once the anatomical similarity of neuromast hair cells to that of hair cells in the vertebrate ear had been recognized, the notion that they are sensory organs for perception of water vibrations or low frequency sound came to dominate. Subsequent work by Bruno Hofer and Karl von Frisch showed that the lateral line was not critical for sound perception and Sven Dijkgraaf coined the term *ferntastinn* to describe its function when his pioneering work suggested that the mechanosensory lateral line system mediates the unique sensation of “touch at a distance”. In adult fish of a variety of species, the lateral line mediates behavioral orientation to water currents or rheotaxis (Montgomery et al., 1997). It is now thought to contribute to surface feeding, schooling behavior, obstacle avoidance, subsurface detection of prey, and is involved in the detection of relative movement between the fish and surrounding water within a few centimeters. Recent studies suggest that the distributed network of neuromasts over the surface of the fish may be particularly effective at interpreting patterns of water turbulence surrounding the fish (Yang et al., 2006; Liao, 2007). In zebrafish larvae, however, the repertoire of behavior mediated by lateral line sensory input is likely to be smaller; thus far convincing evidence has been presented that induction of an escape response triggered by water flow depends on the lateral line system (McHenry et al., 2009). Other behaviors dependent on the lateral line may not emerge until later in development (Van Trump and McHenry, 2008). The function of the lateral line remains the subject of active study and debate by sensory ecologists and physiologists who continue to refine our understanding of how it influences behavior and how its function differs from the ear, to which it is often compared (Mogdans et al., 1999; Braun and Coombs, 2000; Coombs et al., 2000; Bleckmann, 2008).

The foundation for our understanding of the mechanosensory lateral line system has come from pioneering work done in a range of amphibians and fish (Coombs et al., 1989). In recent years, however, analysis of its development in the zebrafish model system has led to a renaissance in lateral line biology and to the recognition that the lateral line primordium can contribute to a deeper understanding of broad questions such as how interactions between multiple signaling pathways direct the coordination of cell fate and morphogenesis during development, how collective migration is controlled, how progenitor cells are maintained and how an organ system responds to injury and replaces damaged components.

The establishment of zebrafish as a model system for studying the lateral line began with the foresight of a small number of labs that developed detailed descriptions of its early development in zebrafish well before the broader value of this seemingly obscure system had been recognized (Metcalf, 1985; Metcalfe et al., 1985; Gompel et al., 2001). The superficial location of the lateral line system under the skin makes it easy to monitor its development, and the sensory hair cells are selectively labelled with vital dyes like Yo-Pro1 (Santos et al., 2006) or DASPEI (Balak et al., 1990) dissolved in rearing medium, making it easy to visualize the distribution of neuromasts as hair cells differentiate. In addition, the establishment of transgenic lines expressing fluorescent proteins in hair cells (ET4) or Mantle cells (ET20) (Parinov et al., 2004), subsets of hair cells, support cells and mantle cells (McDermott et al., 2010), or afferent neurons (Faucher et al., 2009) in the lateral line system has dramatically improved our ability to record dynamic events in its development. Of these, perhaps the CldnB::lynGFP line created by Darren Gilmour in which membrane localized GFP is expressed in the lateral line system and epidermis has had the greatest impact (Haas and Gilmour, 2006). The availability of tools to examine and manipulate gene function in zebrafish embryos has made it a particularly powerful system to understand the genetic regulatory networks operating during lateral line development. While new lines are continuously being developed, there already are a number of transgenic lines in which the expression of specific proteins or signaling systems can be monitored or manipulated in zebrafish. For example, lines have been developed to activate Notch signaling (Scheer et al., 2001), inhibit Wnt or Lef/TCF signaling (Lewis et al., 2004; Stoick-Cooper et al., 2007),

express FGF3, a constitutively active or dominant negative FGF receptor (Lee et al., 2005; Lecaudey et al., 2008; Marques et al., 2008), or to express the chemokine Sdf1a (Knaut et al., 2005). Fluorescent Reporter lines also exist for visualizing the pattern of Notch (Yeo et al., 2007; Parsons et al., 2009), FGF (Molina et al., 2007), and Lef/Tcf signaling (Dorsky et al., 2002; Valdivia et al., In Press). It should be noted, however, that in many of these transgenic lines, though reporter transcripts for fluorescent proteins are expressed, fluorescence is often not initiated fast enough or at high enough levels to be seen effectively in the migrating lateral line primordium, nevertheless it is often seen in deposited neuromasts. A notable exception is a line used in Valdivia et al in that reports Lef1 dependent signaling in the migrating pLLP (Valdivia et al., In Press).

OVERVIEW OF POSTERIOR LATERAL LINE DEVELOPMENT IN ZEBRAFISH

The lateral line system in zebrafish has two main parts, an anterior Lateral Line (aLL) system, including neuromasts of head, and the posterior Lateral Line (pLL) system, which includes neuromasts of the trunk and tail (Metcalfe et al., 1985; Gompel et al., 2001). Most recent studies in zebrafish have focused on the pLL system, which is derived from the pLL placode, an epidermal thickening formed just caudal to the otic vesicle. The caudal cells of this placode detach from the underlying basement membrane to form the posterior Lateral Line Primordium (pLLP), a cohesive cluster of about a hundred cells that migrates under the skin along the horizontal myoseptum to the tip of the tail, periodically depositing neuromasts (Figure 1). Approximately twenty cells of the original placode remain to form the neurons of the lateral line ganglion. Bipolar sensory cells in the lateral line ganglion extend growth cones that follow the migrating pLLP and innervate sensory hair cells of deposited neuromasts to establish the lateral line sensory system (Metcalfe, 1985).

The migrating pLLP is a dynamic structure with progressive changes in the behavior and morphology of its cells from its leading end to its trailing end (Figure 2). Cells toward the leading end are flatter and have a mesenchymal morphology. In an adjacent trailing domain the cells become taller and acquire prominent apical and basal polarity as they assume a columnar epithelial morphology and begin to form center-oriented epithelial rosettes (Figure 3) (Lecaudey et al., 2008). These changes in cell morphology are coordinated with mechanisms that specify formation of a central hair cell progenitor (Figure 4) and together the cells begin to form a nascent neuromast or “proto-neuromast”. Cell proliferation at the leading end of the pLLP displaces the newly formed proto-neuromast to a more trailing position (Nechiporuk and Raible, 2008). Once enough new cells are added a new proto-neuromast is generated at the leading end. The migrating pLLP eventually contains 3–4 sequentially formed proto-neuromasts at progressive stages of maturation. During this maturation the epithelial rosettes become more stable and expression of the basic helix-loop-helix transcription factor, Atoh1a, becomes established in a central cell of the proto-neuromast, which specifies it as a sensory hair cell progenitor (Itoh and Chitnis, 2001; Sarrazin et al., 2006). Eventually, cells at the trailing end cease forward migration, lose their cohesive interactions with neighboring cells, and are deposited along the horizontal myoseptum. As cells are left behind, the ones that have been incorporated into a stable epithelial rosette are deposited as neuromasts, while the remaining cells are deposited in the wake of the migrating pLLP as interneuromast cells. These cells serve as progenitors for the generation of additional neuromasts as the zebrafish continues to grow (Grant et al., 2005).

Prior to deposition of the trailing proto-neuromast, its central sensory hair cell progenitor undergoes a final division to form a pair of sensory hair cells (see Figure 9). The resulting sibling hair cells acquire opposing planar polarity (Rouse and Pickles, 1991) and are eventually stimulated by water movement in opposing directions. Their planar polarity and directional sensitivity is parallel to the original direction of migration of the primordium

along the rostral-caudal axis of the embryo (Lopez-Schier et al., 2004). As the neuromasts continue to mature, additional sensory hair cell progenitors are periodically specified from surrounding support cells, and they divide to form more sensory hair cell pairs with opposing polarity. In a similar manner the support cells can also help replace hair cells lost through age and injury, giving the system the remarkable ability to recover from damage following exposure to drugs like the aminoglycosides, environmental toxins like copper ions, and noise, all of which are also known to cause irreversible injury to the hair cells in our inner ear (Balak et al., 1990; Harris et al., 2003; Dufourcq et al., 2006; Hernandez et al., 2007; Behra et al., 2009; Coffin et al., 2010). This makes the lateral line system particularly attractive both for understanding development, and for understanding mechanisms that contribute to regeneration following injury.

While the pLLP lays down the initial or “primary” neuromasts in the midline of the trunk and tail, a number of independent mechanisms contribute to the growth of the lateral line system. These mechanisms include the formation of a secondary posterior lateral line primordium, which starts migration later and migrates much more slowly depositing additional neuromasts, as well as the formation of additional neuromasts by the proliferation of inter-neuromast cells or by the growth and budding of preexisting neuromasts (Grant et al., 2005; Nunez et al., 2009; Wada et al., 2010). An excellent review describes the growth and post-larval development of the lateral line system (Ghyssen and Dambly-Chaudiere, 2007).

Having provided a broad outline of some of the key features of embryonic development of the pLL, the following sections will review some recent studies that discuss how integration of information from multiple signaling pathways coordinates cell fate and morphogenesis, and how collective migration is regulated in the pLLP. The purpose of these sections is to review current thinking about these questions and to highlight issues that remain unresolved. The review also draws comparisons to other systems, where similar mechanisms operate, as they are both likely to benefit from insights obtained from the lateral line and provide clues to some of its unresolved questions.

THE SELF-ORGANIZATION OF THE PLLP DURING DEVELOPMENT

A Wnt-dependent FGF signaling system initiates proto-neuromast formation

The distinctive changes in the morphology and behavior of cells from its leading end to its trailing end are thought to be determined by the polarized and partially antagonistic function of a number of signaling systems in the pLLP (Aman and Piotrowski, 2008). The leading end of the pLLP is characterized by relatively high levels of Wnt signaling activity, while the trailing end is dominated by FGF signaling (Figure 2A, B) (Lecaudey et al., 2008; Nechiporuk and Raible, 2008). Though it remains unclear how Wnt signaling is established or which Wnt ligands are critical for this function, a Wnt signaling center at the leading end of the pLLP initiates formation of an FGF signaling system in an adjacent trailing domain (Figure 2C). The establishment of this Wnt-dependent FGF signaling system then coordinates establishment of a new proto-neuromast (Aman and Piotrowski, 2008). In this context, Wnt signaling promotes expression of the FGF ligands, FGF3 and FGF10, while at the same time promoting the expression of an intracellular antagonist of FGF receptor signaling, Sef (Tsang et al., 2002). As a consequence, FGFs, expressed in response to Wnt signaling at the leading end, cannot locally activate FGF receptor signaling. Instead, these FGFs diffuse to an adjacent trailing domain, where they are able to effectively activate FGF signaling. Activation of FGF signaling in this adjacent domain drives the expression of Dkk1, a diffusible Wnt antagonist (Glinka et al., 1998; Hashimoto et al., 2000). Dkk1 facilitates stable establishment of the FGF signaling center by protecting it from significant inhibition by the Wnt signaling system. In this manner, two adjacent Wnt and FGF signaling

centers are established. While they are mutually antagonistic locally, establishment of the FGF system is ultimately dependent on distant assistance from the Wnt system.

It remains unclear, however, why FGF signaling dependent *dkk1* expression is restricted to the most immature proto-neuromasts at the leading end. One possibility is that while its expression near the leading end is dependent on FGF signaling, it is also dependent on Wnt signaling, which is only high enough at the leading end. If so, *dkk1* expression may be restricted to a leading domain with some overlap of Wnt and FGF signaling. As *dkk1* expression is restricted to the leading proto-neuromasts it is likely that additional, as yet undefined mechanisms, contribute to inhibition of Wnt signaling in more trailing domains, where *dkk1* is not expressed.

FGF signaling facilitates morphogenesis of center-oriented epithelial rosettes

The Wnt-dependent FGF signaling system plays a key role in coordinating both the morphogenesis of epithelial rosettes and specification of a sensory hair cell progenitor at the center of the nascent neuromast (Figures 3 and 4). It initiates changes in the morphology of the cells making them lose their mesenchymal morphology and adopt a more columnar epithelial morphology (Figure 3) (Lecaudey et al., 2008; Nechiporuk and Raible, 2008). It also promotes association of actin with apical junctions, which promotes apical constriction and eventually reorganization of cells into center-oriented epithelial rosettes (Figure 3B) (Hava et al., 2009). How FGF promotes actin association with junctional complexes remains unclear in the proto-neuromast.

Actin can associate with apical junctions to promote apical constriction by two distinct mechanisms, depending on the distribution of apical junctional complexes. When mature junctional complexes are uniformly distributed around the cell, a continuous actin-myosin belt formed in association with apical junctions can contract like a purse string to promote apical constriction. This mode of apical constriction is thought to operate in the vertebrate neural epithelium during neurulation (Baker and Schroeder, 1967). On the other hand, when less mature focal adhesions exist between neighboring cells, an actin-myosin meshwork distributed over the entire apical surface can contract in a ratchet-like manner pulling together focal adhesions to promote apical constriction and epithelial rosette formation (Martin et al., 2009). This mechanism results in pulsatile anisotropic contraction of apical junctions, and rosette formation by this mechanism is influenced by mechanical tensions that develop in the tissue as cells move and dynamically remodel junctions with neighboring cells. This mode of apical constriction is seen during *Drosophila* gastrulation. It is not clear which of these mechanisms operate when FGF promotes apical constriction in proto-neuromasts. Nevertheless, consistent with a ratchet-like mechanism operating during apical constriction in this context, FGF signaling is only essential for the initial establishment of epithelial rosettes within forming proto-neuromasts, as inhibition of FGF signaling with SU5402 only prevents the formation of epithelial rosettes in new and recently formed proto-neuromasts; it does not dismantle epithelial rosettes found at the trailing end of the migrating pLLP or in deposited neuromasts (Lecaudey et al., 2008; Nechiporuk and Raible, 2008).

FGF signaling facilitates specification of a central sensory hair cell progenitor

FGF signaling not only promotes formation of epithelial rosettes in proto-neuromasts, it also initiates expression of *atoh1a*, which encodes a basic helix-loop-helix transcription factor that gives proto-neuromast cells the potential to become sensory hair cell progenitors (Figure 4) (Nechiporuk and Raible, 2008). In addition, FGF signaling initiates expression of *deltaA* (Matsuda and Chitnis, 2010), which encodes a Notch ligand. DeltaA activates Notch in neighboring cells, inhibiting them from expressing *atoh1a*. Both *atoh1a* and *deltaA* are

initially expressed at the highest levels in central cells of the prospective neuromast, reflecting the pattern of FGF signaling initiated in the forming proto-neuromast. *Atoh1a* then promotes expression of *deltaD* (Matsuda and Chitnis, 2010), which encodes another Notch ligand, and together, DeltaD and DeltaA activate Notch in neighboring cells, ensuring that they do not express *atoh1a*. This process of lateral inhibition restricts expression of *atoh1a* to the central cell, which is specified as a sensory hair cell progenitor, while the neighbors, prevented from expressing *atoh1a*, are allowed to become support cells.

The *atoh1a*-expressing sensory hair cell precursor becomes a new source of FGF in maturing proto-neuromasts

Once *atoh1a* expression is established in the central proto-neuromast cell, this cell, which becomes specified as a hair cell progenitor, becomes a new source of FGF10 in maturing proto-neuromasts (Figure 5) (Matsuda and Chitnis, 2010). *Atoh1a* promotes expression of *fgf10*, while it inhibits expression of its receptor, *fgfr1* (Matsuda and Chitnis, 2010). Hence, while the central hair cell progenitor becomes a new focal source of FGF signals, it also becomes less responsive to FGF signals. As Notch activation inhibits *atoh1a* expression in the neighboring cells, these cells, specified as support cells, continue to express the FGF receptor and remain competent to respond to FGF signals. FGF10 from the central hair cell progenitor activates FGF signaling in neighboring support cells and this helps to maintain their expression of both *fgfr1* and *notch3*. As the central *atoh1a*-expressing cell shuts *fgfr1* off and becomes less responsive to FGF signaling, maintenance of *atoh1a* expression becomes less dependent on FGF signaling and more dependent on autoregulation (Figure 5C). *Atoh1a* begins to drive expression of another *atoh1* homologue, *atoh1b*. *Atoh1b*, in turn, drives *atoh1a* expression, and cross-activation helps maintain *atoh1a* expression (Matsuda and Chitnis, 2010). *Atoh1a* only drives *atoh1b* expression in the most mature proto-neuromast at the trailing end of the pLLP. This continues to be true in *mind bomb* mutants, where there is a profound loss of Notch signaling and a much higher level of *atoh1a* expression. This suggests that the ability of *atoh1a* to drive *atoh1b* expression is not just limited by the amount of *atoh1a* expression, and its expression is determined by additional as yet unidentified factors.

The ability of *Atoh1a* to drive *fgf10* and *deltaD* expression in the central sensory hair progenitor is a key step in the formation of a mature proto-neuromast. By expressing both *fgf10* and *deltaD* the hair cell progenitor is able to maintain both FGF and Notch signaling in the neighboring support cells. As a source of FGF10, the central sensory hair cell progenitor has the potential to induce expression of *atoh1a* and formation of more hair cell progenitors in its neighbors. At the same time their induction remains under the tight control of Notch signaling, which inhibits expression of *atoh1a* and prevents production of more hair cell progenitors until they are needed.

Unresolved issues related to the role of Notch signaling in selecting a central hair cell progenitor

Lateral inhibition mediated by Notch signaling amplifies small differences in the potential of cells to express *atoh1a* and restricts its expression to a central cell in the proto-neuromast cell cluster. Since the central cells initially express the most DeltaA and DeltaD, they are most effective at activating Notch in their neighbors. This makes the surrounding cells express less *atoh1a* and therefore less DeltaD. This, in turn, makes surrounding cells less effective at activating Notch in central cells. As a consequence the central cell retains *atoh1a* expression or expresses progressively more *atoh1a*, while the neighboring cells express progressively less. Eventually *atoh1a* is restricted to the central cell, while it is lost from the neighbors.

If *atoh1a* and *deltaA* expression had been uniformly initiated in the proto-neuromast, lateral inhibition would have made the central cell least likely to retain *atoh1a* expression, as it would have Notch activated by all its neighbors. On the other hand, a cell at the edge of the cluster would receive no inhibition from neighbors outside the cluster, and would be more likely to be selected first. It is therefore important to understand how the centrally biased expression of *atoh1a* and *deltaA* is established. It is likely that centrally biased expression of *atoh1* and *deltaA* in the proto-neuromast is determined by the restricted pattern of FGF signaling initiated in the forming proto-neuromast (Figure 4B). However, it remains unclear how FGF ligand, secreted in response to Wnt signaling in leading cells, establishes the initial restricted center-biased pattern of FGF signaling.

The role of *atoh1a* and Notch signaling in determining sensory hair cell fate is strikingly similar in the lateral line and in the vertebrate ear. However, in the mouse and chick ear an additional early role for Jagged-Notch signaling has been suggested in initiating *atoh1* expression (Kiernan et al., 2006; Neves et al., 2010). In this context it is thought that *sox2* expression, induced in an earlier step by FGF signaling, defines a pro-sensory domain within which cells have the potential to become either hair cells or support cells. Within this domain Wnt signaling initiates Jagged1 (Jag1) expression. Jag1 then activates Notch in its neighbors to promote more Jag1 expression. Mutual Jag1-Notch signaling between adjacent cells prevents differentiation and helps maintain *sox2* expression. In this manner Jag1-Notch signaling helps maintain a *sox2*-expressing pro-sensory domain within which cells have the potential to express *atoh1*. Delta-Notch signaling initiated by *atoh1*-expressing cells then mediates lateral inhibition to determine which subset of cells maintain *atoh1* expression to become hair cells, and which ones lose *atoh1* expression to become support cells (Neves et al., 2010). Two Jagged homologues, Jagged1b and Jagged2 are expressed in the pLLP (Gwak et al., 2010), however, it is not known if Jagged-Notch signaling contributes to determining the initial pattern of *atoh1a* expression in the lateral line system. It should be noted that in Mib1 mutants, characterized by broad loss of Notch signaling, there did not appear to be any problem initiating *atoh1a* expression (Matsuda and Chitnis, 2010). This raises questions about whether Notch has a similar essential role in initiating *atoh1a* expression in the pLLP system.

Another issue that remains unresolved is which Notch target genes mediate effects of distinct Notch ligands and Notch receptors in the pLLP and whether the genes activated by Delta-Notch signaling are distinct from those activated by Jagged-Notch signaling. Though it is clear that lateral inhibition mediated by Notch signaling inhibits *atoh1a* expression and restricts its expression to a central cell in maturing proto-neuromasts, it is not known which Hairy Enhancer of split Related (HER) genes, or other genes expressed in response to Notch signaling in the pLLP, are essential for inhibiting *atoh1a* expression.

Restriction of *atoh1a* expression is essential for effective pLLP morphogenesis

Though *atoh1a* and Notch signaling are primarily involved in determining the specification of central sensory hair cell progenitors, failure of Notch signaling allows unregulated expansion of cells expressing *atoh1* and this has cascading effects on many aspects of pLLP morphogenesis (Matsuda and Chitnis, 2010). As the proto-neuromast matures, FGF10, secreted by the central *atoh1a*-expressing cell has the potential to activate FGF signaling and induce *atoh1a* expression in neighboring cells. This is normally prevented by lateral inhibition mediated by Notch signaling. When Notch signaling fails, the neighboring cells begin expressing *atoh1a*. These cells then become a new source of FGF10 and they induce more neighbors to express *atoh1a*. This cycle repeats until the majority of the cells in the pLLP start expressing *atoh1a*. When cells express *atoh1a* they reduce their expression of *fgfr1*. As a consequence the expanded expression of *atoh1a* is accompanied by progressive loss of *fgfr1* expression. This severely attenuates and disorganizes FGF signaling in the

pLLP, and as FGF signaling is progressively lost, eventually there is a failure of many FGF-dependent processes, including morphogenesis of epithelial rosettes in the proto-neuromasts.

Support cells may provide an adhesive link between hair cells and surrounding non-sensory cells in the pLLP

Loss of Notch signaling not only prevents effective morphogenesis of epithelial rosettes, it eventually results in fragmentation of the migrating pLLP (Matsuda and Chitnis, 2010). This rather surprising effect is also related to unregulated expansion of the *atoh1a*-expressing cells and the accompanying loss of support cells that appear to have an essential role in holding inner hair cells and surrounding outer non-sensory cells together (Figure 6). Support cells express both *n-cadherin* and *e-cadherin*. This allows them to form effective adhesive junctions with both inner hair cells that only express *n-cadherin* and surrounding non-sensory cells that only express *e-cadherin*. It remains unclear what determines restricted expression of *n-cadherin* in a small cluster of cells that includes the hair cell progenitor and surrounding support cells in mature proto-neuromasts and deposited neuromasts. However, *Atoh1a* prevents *e-cadherin* expression in the central hair cell progenitor, which normally leaves *e-cadherin* expression limited to support cells and their surrounding non-sensory cells that do not express *atoh1a*. When Notch signaling fails, *atoh1a* expression expands from central cells into surrounding cells that would normally express both *e-cadherin* and *n-cadherin*, and they eventually shut off *e-cadherin* expression. Once *e-cadherin* expression is lost from these cells, they express only *n-cadherin*. It is thought that this prevents the expanded *atoh1a*-expressing population of cells, now specified as hair cell progenitors, from developing effective adhesive junctions with surrounding non-sensory cells expressing only *e-cadherin*. This might contribute to the eventual fragmentation of the pLLP following loss of Notch signaling. The role for support cells in serving as an adhesive link between outer non-sensory cells and inner hair cells suggested in the pLLP system is similar to a role previously proposed for support cells in the zebrafish ear (Haddon et al., 1999).

n-cadherin and *e-cadherin* are expressed in complementary domains of the pLLP

Not only are *n-cadherin* and *e-cadherin* expressed in partially complementary domains of the mature proto-neuromasts and deposited neuromasts, these cadherins are also more generally expressed in complementary patterns in the pLLP (Figure 6B) (Matsuda and Chitnis, 2010). Expression of *n-cadherin* is prominent in the leading domain, where it is broadly expressed in the cohesive column of migrating pLLP cells. At the trailing end, where cells are preparing for deposition, its expression then becomes restricted to the central hair cell progenitor and its surrounding support cells. In contrast, *e-cadherin* is expressed at relatively low levels in the migrating pLLP cells and its expression becomes more prominent in trailing proto-neuromast cells that are preparing for deposition as they form stable epithelial rosettes. The differential expression of *n-cadherin* and *e-cadherin* transcripts in relatively motile versus non-motile domains, respectively, of the pLLP is reminiscent of changes in many types of cancer, where cells normally express *e-cadherin*, and initiation of *n-cadherin* expression is associated with acquisition of migratory behavior in metastatic cells (Hazan et al., 2004). N-cadherin also facilitates collective migration in *Xenopus* neural crest cells (Theveneau et al., 2010). In this context, N-cadherin-dependent adhesions inhibit Rac activity and the formation of cell protrusions on the sides of a cell where junctional complexes are transiently made with neighboring cells. At the same time, absence of N-cadherin-dependent adhesions facilitates activation of Rac1 and formation of cell protrusions at the free edge of leading cells. These effects improve directed collective migration of *Xenopus* neural crest cells. In light of these observations, it is possible that broad *n-cadherin* expression in the leading domain contributes to cohesive interactions in the column of cells of the pLLP undergoing collective migration, and the switch to more

prominent *e-cadherin* expression in the trailing end facilitates formation of stable epithelial rosettes as the cells stop moving.

A significant limitation of the hypotheses describing potential roles of *n-cadherin* and *e-cadherin* the zebrafish pLLP system is that they are based on patterns of transcript expression. Ultimately, the validation of these hypotheses will depend on the development of strategies to visualize dynamic changes in the localization and trafficking of cadherin proteins. At this stage it remains unclear how changes in the morphology and migration of the pLLP in *n-cadherin* mutants might relate to its proposed functions.

The mechanisms that determine prominent expression of *n-cadherin* and *e-cadherin* transcripts in leading and trailing domains of the pLLP, respectively, remain poorly defined. Though Wnt signaling dominates in the leading migratory domain and FGF signaling becomes more prominent in the trailing domain, there is no simple regulatory relationship between these signaling pathways and the differential expression of *n-cadherin* and *e-cadherin*. However, recent studies have shown that Wnt signaling can influence trafficking of N-cadherin in a manner that allows neural crest to migrate more effectively in zebrafish embryos (Piloto and Schilling, 2010). So even if there is no simple regulatory relationship between Wnt signaling and *n-cadherin* transcript expression, there is a possibility that Wnt signaling can influence its function by determining expression of factors that regulate N-cadherin trafficking. It will be informative to examine how Wnt signaling influences N-cadherin trafficking in the pLLP system.

Chemokine signaling determines migration of the pLLP

The pLLP is guided along the horizontal myoseptum from the otic vesicle to the tip of the tail by the chemokine signaling system (Figure 7). This system is widely used by diverse tissues including germ cells, leukocytes, blood vessels, neurons and metastatic cancer cells (Raman et al., 2011). In most of these contexts the migratory path followed by cells is defined by the transient and dynamic expression of chemokine ligands like CXCL12a or CXCL12b (also called Stromal Derived Factor 1a or Stromal Derived Factor 1b) that serve as chemotactic signals leading cells along distinct paths. CXCL12a can interact with receptors like CXCR4b to initiate G protein coupled signaling, which triggers a transient influx of calcium ions (Bleul et al., 1996). This calcium influx can then determine directed protrusive activity of a cell or determine cell shape and adhesion to direct migration of different types of cells using distinct mechanisms (Blaser et al., 2006). CXCL12a can also interact with receptors like CXCR7b, which is not thought to be as effective at initiating G protein coupled signaling (Graham, 2009). Instead, interaction of CXCL12a with CXCR7b is thought to promote β -arrestin-dependent MAPK ERK1/2 signaling (Rajagopal et al., 2010). The lesson learnt from analysis of chemokine signaling dependent cell migration in diverse tissue contexts is that its ligands and receptors have evolved to work together and direct migration of cells following very diverse strategies. So how might it operate in the pLLP system?

The path followed by the pLLP is defined by the transient expression of the chemokine, SDF1a or CXCL12a, in superficial muscle pioneer cells of the horizontal myoseptum (David et al., 2002; Li et al., 2004; Haas and Gilmour, 2006). Its expression is initiated at about 20 hours post fertilization (hpf), just before pLLP migration begins and is eventually lost after the pLLP completes migration. The appearance of a new oblique domain of *cxc112a* expression in association with somite 1 at about 29 hpf also defines the dorsal route that will eventually be taken by the primordium of the dorsal branch of the posterior lateral line at about 38 hpf (David et al., 2002). In addition, ectopic expression of *cxc112a* is able to redirect pLLP migration (Li et al., 2004) and loss of *cxc112a* expression results in failure of migration along the horizontal myoseptum (Valentin et al., 2007). Finally, as Hedgehog

signaling from the axial midline is required for specification of horizontal myoseptum cells that express *cxc112a*, loss of hedgehog signaling also results in failure of pLLP migration. Together these observations show that CXCL12a has an essential role in directing migration of the pLLP.

Though the expression of *cxc112a* in the horizontal myoseptum determines the path that will be followed by the pLLP, it is unlikely to determine which direction the pLLP migrates, as the levels of *cxc112a* transcript, rostral and caudal to pLLP, are relatively uniform during migration. In *fused somite/tbx24 (fss)* mutants, in which *cxc112a* expression is selectively retained in rostral somites and lost in the caudal somites, the pLLP migrates to the caudal end of the stripe of *cxc112a* expression, it then makes a “U turn”, and the leading cells then direct migration along the horizontal myoseptum in the rostral direction (Haas and Gilmour, 2006). This demonstrates that there is no inherent directional information in the stripe of *cxc112a* expression, and the pLLP can migrate along it in both a rostral and caudal direction. In addition, as the original leading end continues to direct migration, this demonstrates that there is inherent polarity in the migrating pLLP system. This polarization is determined by the differential expression of two chemokine receptors, CXCR4b, whose transcripts dominate at the leading end, and CXCR7b, whose transcripts dominate at the trailing end of the pLLP (Figure 7A). Though expression of *cxc4b* and *cxc7b* transcripts is largely complementary in leading and trailing domains of the pLLP, respectively, there is some overlapping expression of the receptors in cells at the middle of the pLLP (Valentin et al., 2007).

Loss of *cxc4b* function results in failure of directed migration, though the pLLP does not lose all mobility and instead typically shows undirected tumbling behavior (Haas and Gilmour, 2006). Transplantation of just a few wild-type cells into a *cxc4b* mutant pLLP can rescue migratory behavior when the wild-type cells find their way to the leading free edge of the pLLP, but not when they are in the interior of the system. Following transplantation, tumbling of the pLLP randomly moves the transplanted wild-type cells around, and some find their way to the free edge of the pLLP in *cxc4b* mutants. Once at the edge they can respond to CXCL12a with directed protrusive activity. This keeps transplanted wild-type cells at the leading edge, allows them to steer the *cxc4b* mutant pLLP and restore directed migration (Haas and Gilmour, 2006). This suggests that, although expression of *cxc4b* gives cells the potential to respond to chemokine signals and direct migration, this potential cannot be realized unless the cell is at a free edge of the pLLP. Loss of *cxc7b* function also results in failure of directed migration. Without *cxc7b* the pLLP slows down and prematurely stalls. Again, transplantation of a few wild-type cells can restore migration of the pLLP, except in this context migration is rescued only if the wild-type cells find their way to the trailing end of the pLLP (Valentin et al., 2007).

How does polarized expression of CXCR4b and CXCR7b determine directed migration of the pLLP? One favored hypothesis is based on a role for CXCR7b as a scavenger of the CXCL12a ligand (Figure 7A). While CXCL12a interacts with CXCR4b to trigger G protein coupled signal transduction and protrusive cell activity that promotes cell migration in leading cells, interaction of CXCL12a with trailing cells expressing CXCR7b is thought to promote rapid internalization and degradation of CXCL12a (Luker et al., 2010). As CXCR7b-expressing cells deplete CXCL12a from the trailing end, it is thought they generate a local gradient of CXCL12a, with low levels at the trailing end (Dambly-Chaudiere et al., 2007). In this context, only the *cxc4b*-expressing cells at the leading edge of the pLLP see high enough levels of CXCL12a to respond with sustained protrusive activity that guides pLLP migration. A role for CXCR7b as a scavenger of CXCL12a has been suggested in a number of other tissue contexts, including germ cell migration, where expression of CXCR7b in cells outside the migratory path is thought to scavenge CXCL12a,

restricting its availability to the desired migratory path (Boldajipour et al., 2008; Naumann et al., 2010). However, a local gradient of CXCL12a has not yet been demonstrated in association with the pLLP and there remains some controversy in the field about whether the primary role of CXCR7b is to establish a local gradient of CXCL12a availability. Independent of its role in establishing a local gradient of CXCL12a, if CXCR7b is unable to effectively engage the G protein coupled signaling system to promote protrusive activity then CXCL12a-CXCR7b interactions at the trailing end are not likely to direct migration in the opposite direction.

Recent studies in the mouse describe how co-expression of CXCR4b and CXCR7b contributes to effective migration of interneuron cells in the cortex and why migration is impaired when function of either is lost (Sanchez-Alcaniz et al., 2011; Wang et al., 2011). These studies emphasize that CXCL12a-CXCR4b interactions result in sustained G protein coupled signaling that promotes cell migration, but this is followed by internalization and degradation of the CXCR4b receptor (Tarasova et al., 1998). On the other hand, interaction of CXCL12a with CXCR7 results in rapid internalization of the CXCL12a-CXCR7b complex and degradation of CXCL12a, while CXCR7b is recycled back to the cell surface (Luker et al., 2010). In this context it is thought that CXCR7b limits the amount of CXCL12a available to interact with CXCR4b. Limiting CXCL12a prevents saturating interactions between CXCL12a and CXCR4b, which in the absence of CXCR7b, cause so much internalization and degradation of the CXCL12a-CXCR4b complex that it exceeds the cell's ability to replace CXCR4b, and this, it is suggested, ultimately causes failure of interneuron migration (Sanchez-Alcaniz et al., 2011).

In a similar manner, CXCR7b could prevent exposure of CXCR4b cells to levels of CXCL12a that would ultimately saturate the receptors causing depletion of CXCR4b from the cell surface in the pLLP. Such a role could provide a potential explanation for some of the unique morphological changes seen following loss of *cxcr7b* function, including exaggerated protrusive activity, stretching and eventual stalling of the pLLP with rounded morphology (Valentin et al., 2007). The reduced ability to clear CXCL12a could allow the chemokine to accumulate and initially stimulate exaggerated protrusive activity. As CXCR7b expressing cells no longer make the trailing end non-responsive, the protrusive activity would be expected to extend to the trailing end of the pLLP, and the ectopic protrusive activity could contribute to stretching of the pLLP. Finally, following a period of exaggerated protrusive activity, saturating interactions with CXCL12a could result in internalization and degradation of the CXCR4b receptor, leaving the pLLP less responsive with a rounded morphology. Clarification of whether this is true will require development of tools for examining the trafficking of chemokine ligands and receptors and examining if exaggerated expression of CXCL12a does indeed result in depletion of the CXCR4b receptor. The CXCR7b receptor could of course have additional roles, some of which might be related to its ability, reported in other tissues, to engage with β -arrestin and drive MAPK ERK1/2 signaling (Rajagopal et al., 2010). In this context it is important to note that a recent study shows that the CXCR7/CXCR4 heterodimer constitutively recruits β -arrestin to enhance cell migration (Decaillot et al., 2011).

Establishing polarized *cxcr4b* and *cxcr7b* expression

The mechanisms that establish polarized *cxcr4b* and *cxcr7b* expression in the pLLP are not completely understood. However, studies by Aman and Piotrowski suggest that their complementary expression is linked to the development of polarized Wnt-FGF signaling in the pLLP (Figure 7B) (Aman and Piotrowski, 2008). Their study shows that broad exaggerated Wnt signaling in *apc* mutants results in expanded *cxcr4b* expression and reduced *cxcr7b* expression in the pLLP. As the initial broad domain of Wnt signaling in the

pLLP correlates with the early broad pattern of *cxc4b* expression it is possible that Wnt signaling helps initiate *cxc4b* expression in the pLLP.

While exaggerated Wnt signaling can expand *cxc4b* expression, heat shock induced Dkk1 expression, which inhibits Wnt signaling does not result in a loss of *cxc4b* expression (Aman and Piotrowski, 2008). This suggests that once *cxc4b* expression is initiated Wnt signaling is not essential for maintaining *cxc4b* expression. Instead, autoregulation may help maintain *cxc4b* expression. When either *cxc112a* or *cxc4b* function is lost *cxc4b* expression is reduced and it becomes restricted to a much smaller leading domain, where limited expression may continue to be maintained by Wnt signaling (Dambly-Chaudiere et al., 2007; Gamba et al., 2010). Reduction of the *cxc4b* expression domain, following loss of CXCL12A-CXCR4B signaling is accompanied by the complementary expansion of the *cxc7b* expression domain, showing that chemokine signaling via CXCR4b is also required to suppress *cxc7b* expression in the *cxc4b* expression domain.

While CXCR4b suppresses expression of *cxc7b* expression the leading domain, CXCR7b does not inhibit *cxc4b* expression in the trailing domain. Instead, it is Estrogen Receptor 1 (ESR1) signaling that inhibits *cxc4b* expression (Gamba et al., 2010). Transcripts for ESR1 are expressed throughout the pLLP and it is not known what limits ESR1 activation to the trailing domain, where it inhibits *cxc4b* expression.

The mechanisms that promote *cxc7b* expression in the trailing domain remain obscure. The initiation of *cxc7b* expression correlates with the appearance of a domain free of *cxc4b* at the trailing end of the pLLP, as migration commences. This is consistent with initiation of *cxc7b* expression being dependent on elimination of factors that would otherwise inhibit its expression at the trailing end. As Wnt signaling promotes *cxc4b* expression, the loss of trailing *cxc4b* expression could be linked to the initial polarization of Wnt-FGF signaling system, which creates a domain free of Wnt signaling at the trailing end of the pLLP. However, loss of Wnt signaling may not be enough to reduce *cxc4b*, because once initiated, *cxc4b* expression can be maintained by CXCL12a dependent autoregulation. The loss of trailing *cxc4b* expression is likely to be linked to establishment of ESR1 signaling, which inhibits *cxc4b* expression, however, little is known about the mechanisms that regulate its activation in the trailing domain.

Trailing cells actively follow leading cells in the pLLP

The polarized expression of *cxc4b* and *cxc7b* has an essential role in directing the migration of mesenchymal cells at the leading edge of the pLLP. These “pioneer” cells steer collective migration of the pLLP, while cells in more trailing domains, which have a more epithelial morphology, actively migrate in a direction determined by the leading cells (Figure 7C). While the apical ends of the trailing cells constrict to form epithelial rosettes, the basal ends of the cells extend cryptic lamellipodia to actively migrate in a direction thought to be determined by the pull of cells in front of them (Haas and Gilmour, 2006). Such cryptic lamellipodia were first demonstrated in models of epithelial wound healing, where the movement of cells in epithelial sheets toward each other to fill a gap created by the wound also involves collective migration (Farooqui and Fenteany, 2005). In this context too, “pioneer” cells at the wound edge direct migration of cells behind them. As the cells near the wound edge migrate, their apical adhesive interactions exert a “pull” or mechanical tension on cells behind them. It has been suggested that this mechanical tension stabilizes basal protrusions that direct migration of the cryptic lamellipodia (Lecaudey and Gilmour, 2006). The link between mechanical tension and directed migration is thought to involve an aspect of ERK1/2 MAPK signaling that requires Src Family Kinases (Matsubayashi et al., 2004). The mechanisms by which mechanical tension guides migration is also being studied in other model systems for collective migration including endothelial cells in tissue culture,

border cells in the *Drosophila* system, and metastatic cancer cells (Lecaudey and Gilmour, 2006; Friedl and Gilmour, 2009). What is learnt from these systems is likely to define avenues for further investigation of the mechanisms that direct the active migration of trailing cells in the pLLP. Ultimately, understanding these mechanisms will also be essential for a better understanding of how trailing cells eventually slow down and are deposited.

Mechanisms that determine the pattern of neuromast deposition

The pLLP periodically deposits the most mature proto-neuromast from its trailing end as it migrates from the otic vesicle to the tip of the tail. This periodic behavior could have been determined by cues extrinsic or intrinsic to the pLLP. One hypothesis was that the periodic organization of somites, over which the pLLP migrates, might provide extrinsic cues for periodic deposition of neuromasts. However, Aman et al have shown that this is unlikely, as mutations that alter the periodic pattern of somites do not alter the pattern of neuromast deposition (Aman et al., 2011). Their study shows that the pattern of neuromast deposition is determined by changes within the pLLP. However, unlike mechanisms that determine segmentation of somites, the rate of neuromast deposition is not determined by a mechanism that in any obvious way establishes oscillations within the pLLP. Instead they suggest it is determined by the rate of cell proliferation within the pLLP (Aman et al., 2011).

How might cell proliferation determine the pattern of neuromast deposition? The pattern of *cxcr4b* and *cxcr7b* expression in the leading and trailing domains of the pLLP defines domains with very distinct behaviors. Aman and Piotrowski suggest that the leading *cxcr7b*-free domain, where *cxcr4b* expression dominates, is relatively constant in size and it represents a domain where cells migrate effectively as a cohesive column (Aman et al., 2011). While the size of the *cxcr7b*-free domain remains relatively stable during pLLP migration, the *cxcr7b* expression domain waxes and wanes. Following deposition of a proto-neuromast, the domain of *cxcr7b* expression is reduced to a minimum. However, as cells proliferate and more cells are added to the system, the pLLP lengthens. As the pLLP lengthens, some as yet undefined self-regulating mechanism keeps the length of the cohesive leading *cxcr7b*-free domain constant. Hence progressive increase in the length of the pLLP beyond the regulated *cxcr7b*-free domain is accompanied by the emergence of additional trailing cells that express *cxcr7b*. The cells expressing *cxcr7b* begin to slow down. Once the *cxcr7b* expressing domain expands to include an entire proto-neuromast, cells that have been incorporated into an epithelial rosette are deposited as neuromasts, while the remaining cells are deposited as inter-neuromast cells. Eventually these cells stop moving, the *cxcr7b*-expressing cells are deposited, and the cycle repeats (Figure 8). In the context of this repeating cycle, cell proliferation is an important variable that determines how quickly the pLLP increases in size and how fast cells emerge from a leading *cxcr7b*-free domain, into a trailing *cxcr7b* expression domain where they slow down and stop moving.

Proliferation is regulated in the pLLP by both Wnt and FGF signaling, however, proliferation in the leading domain is primarily determined by Wnt signaling (Aman et al., 2011). The model proposed by Aman and Piotrowski predicts that loss of Wnt signaling-dependent proliferation might result in a decrease in the neuromast deposition rate. Three recent studies have shown that Wnt dependent proliferation in the pLLP is determined by *lef1* (Gamba et al., 2010; McGraw et al., 2011; Valdivia et al., 2011) however, these studies show that loss of *lef1* does not reduce the rate of neuromast deposition. While all three studies show that reduced proliferation eventually makes the pLLP run out of cells and stall prematurely, as discussed below, they come to distinct conclusions about what happens when *lef1* function is lost and how it affects the pattern of neuromast deposition.

Studies from the Ghysen lab first showed that *lef1* determines proliferation in the pLLP (Gamba et al., 2010). However, their studies emphasize the role of *lef1* in determining the

pattern of proliferation early in development prior to migration, when *lef1* is broadly expressed in the pLLP. Their studies shows that in *lef1* morphants reduced proliferation results in a smaller pLLP that migrates and deposits neuromasts in a pattern that is initially indistinguishable from control embryos. However, reduced proliferation makes the *lef1* morphant pLLP eventually run out of cells and it stalls. As they saw no effect on the pattern of neuromast deposition prior to stalling, they concluded that the timing of neuromast deposition is independent of primodium size and the pattern of *lef1*-dependent proliferation.

Studies by McGraw et al (McGraw et al., 2011) and Valdivia et al (Valdivia et al., 2011) have now re-examined *lef1* morphants and shown that their phenotypes are similar to those seen in two different *lef1* mutants identified by these groups. These studies extend the observations of the Gamba et al and emphasize different aspects of the role of *lef1* in determining the pattern of proliferation and its effects on the timing of neuromast deposition. Both studies concur that reduced proliferation at the leading end results in a rapidly shrinking pLLP that runs out of cells as it continues to deposit neuromasts. The study by McGraw et al goes on to suggest that the population of leading cells is rapidly depleted in the absence of *lef1* because dividing cells are not retained as progenitors at the leading end, instead they move out of the leading end and are more likely to be incorporated into forming neuromasts that are eventually deposited.

In contrast to the studies by Gamba et al, the study by Valdivia et al shows that reduced proliferation in the leading zone following loss of *lef1* not only makes the leading zone shrink in size, it also results in successive neuromasts being deposited at progressively closer intervals before the pLLP finally runs out of cells. It remains unclear why a change in spacing of deposited neuromasts was not seen in the study by Gamba et al. Nevertheless, Valdivia et al go on to show that when proliferation was generally reduced throughout the pLLP, neuromasts were deposited at longer intervals, consistent with what was originally described by Aman et al. Valdivia et al have elaborated the model initially proposed by Aman et al. to account for the shortening or lengthening, respectively, of the interval between deposited neuromasts, which follows reduced proliferation that is restricted to the leading zone or generally affects the entire pLLP. They suggest that the length of the cohesive column of migrating cells in the pLLP may be determined by as yet unidentified factors secreted by the leading zone cells. As *lef1*-dependent proliferation normally maintains the size of the leading zone, this homeostatic mechanism normally maintains the size of the cohesive column and movement of cells out of this cohesive zone into a trailing domain depends on the general rate of proliferation in the pLLP, as suggested by Aman et al. However, as loss of *lef1* results in a specific deficit of leading zone cells that are the source of factors that determine the effective length of the cohesive column, the cohesive column progressively shrinks and neuromasts are deposited following progressively shorter intervals. The study by Valdivia et al goes on to suggest that the premature termination of the pLLP migration and its break up into terminal neuromasts mirrors a change that normally takes place as the pLLP reaches the tip of the tail. Though the size of *lef1* expression domain is initially maintained in wild-type embryos, it eventually becomes progressively smaller as it reaches the end of its migration. The progressive reduction and eventual disappearance of the *lef1* domain is accompanied by the shrinking of the leading zone and the eventual break up of the pLLP into terminal neuromasts. Valdivia et al suggest that when *lef1* function is lost, loss of cells in the leading domain leads to premature shrinking of the leading zone, which is accompanied by premature break up of the pLLP into terminal neuromasts (Valdivia et al., 2011).

The models described above provide an excellent evolving framework for understanding how changes in the pattern of cell proliferation can influence the pattern of neuromast deposition. However, they also raise a number of interesting unanswered questions. For

example, it is not clear what secreted factors dependent on leading zone cells determine the size of the column of cohesive migrating cells. How are these factors related to the domain of *n-cadherin* expression and/or other cell surface proteins that might determine which cells can effectively migrate while retaining adhesive interactions with their neighbors? What changes in the trailing *cxcr7b* expression domain facilitate the slowing down of cells and their eventual separation from the remaining pLLP cells that continue migration as a cohesive column? Finally, it remains unclear what mechanism initially maintains the size of *lef1* domain and what factors extrinsic or intrinsic to the pLLP contribute to the shrinking and eventual disappearance of *lef1* domain as the pLLP reaches the tip of the tail and ends its migration.

The anatomy and organization of the “hair” in hair cells

The hair cells get their name from the approximately 100 cylindrical processes that together form a “hair bundle” which projects from the apex of the cell (Hudspeth, 1989). The processes are of progressively greater length and organized in height order so that their tips have a bevelled profile like that of hypodermic needle (Figure 9). The tallest, the kinocilium, is a true cilium, has a basal body with an eccentric location at the apex of the hair cell, and its microtubules can be labelled with an acetylated tubulin antibody. The adjacent progressively shorter processes are actin-rich stereocilia, and they can be visualized with phalloidin. The tip of each stereocilium is attached to the taller one next to it. When the bundle of stereocilia bend in the direction of the tall kinocilium the tension in the links between stereocilia increases. This makes transduction channels on the surface open more frequently, resulting in a depolarization of the hair cell. In contrast, when the bundle bends away from the kinocilium, tension in the links reduces, the transduction channels open less frequently, and the hair cell becomes hyperpolarized. As a consequence the eccentric location of the kinocilium and asymmetric morphology of the hair bundle defines the directional sensitivity of the hair cell. How this remarkable mechanosensory system works is described in an excellent review by Hudspeth (Hudspeth, 1989).

Each hair cell has a planar polarity defined by the asymmetric localization of the kinocilium, as it defines the axis along which a staggered array of stereocilia is aligned. In addition, when a hair cell progenitor divides to form two adjacent hair cells their kinocilia adopt adjacent eccentric positions so that the hair bundles are oriented along the same axis but in opposing directions (Figure 9A). Hence the sibling hairs cells eventually adopt opposing directional sensitivity. The proper asymmetric localization of kinocilia within a hair cell requires the function of planar polarity genes like *vangl2* (Lopez-Schier and Hudspeth, 2006). On the other hand, the axis of orientation of kinocilia is directly related to the direction of migration of the primordia prior to neuromast deposition (Lopez-Schier et al., 2004). This suggests that the axis of kinocilium orientation may be determined by tensions and/or other interactions that develop between cells in maturing proto-neuromasts during migration of the pLLP, however, details of this mechanism remain obscure.

While the direction of pLLP migration determines the axis of kinocilium orientation prior to proto-neuromast deposition, additional mechanisms help to maintain this directional information in mature neuromasts. Following one hour of exposure to the aminoglycoside, neomycin, all of the hair cells are lost, but the support cells are not damaged. Over the next 48 hours there is a remarkable recovery of hair cells with directional sensitivity of the neuromasts being restored. The mechanisms that restore planar polarity of regenerated hair cells remain unclear. Nevertheless, as in the initial development of the neuromasts, single hair cell progenitors are sequentially specified during regeneration. They then divide to form adjacent hair cells with the same axis of kinocilium orientation, but with opposite polarity (Lopez-Schier and Hudspeth, 2006). As a hair cell progenitor divides to form two hair cells, the next hair cell progenitor is selected from surrounding support cells. Though the original

location of the support cell may vary, its determination as the next hair cell progenitor becomes established when it assumes a stereotypical position along an axis that divides the neuromast into two equal halves and is orthogonal to the original axis of kinocilium polarization (Wibowo et al., 2011). For example, in the case of regenerating hair cells in the trunk and tail that have an anterior–posterior axis, these cells are specified at either dorsal or ventral poles of the neuromast. Why cells are selected in these positions remains unclear, however, restriction of hair cell progenitor fate to a single cell, as described earlier, remains dependent on effective Notch signaling.

Time lapse videos show that the plane of hair cell progenitor division is not a critical determinant of eventual planar polarization of the kinocilium, and that the progeny can undergo considerable reorientation following division (Wibowo et al., 2011). While siblings acquire opposing planar polarity, their eventual orientation may be determined by interactions with surrounding support cells. However, for planar polarity of adjacent cells to be properly coordinated, sequential specification of single hair cell progenitors is essential. When Notch signaling fails and too many cells are simultaneously specified, support cells are lost and the planar polarity of cells within the regenerating neuromast becomes uncoordinated.

Innervation of the neuromasts

As neuromasts are deposited their sensory hair cells are innervated by axons extended by the lateral line ganglion that follow the migrating pLLP (Metcalf, 1985; Gilmour et al., 2004). Recent studies show that the pLLP expresses GDNF and lateral line ganglia neurons express RET receptors that allow them to respond to these chemotactic signals released by the migrating primordium (Schuster et al., 2010). Details of how the sensory hair cells are eventually innervated by afferent neurons, which carry information back to the brain, and efferent neurons, carrying both inhibitory and excitatory signals to the sensory hair cells from the brain, will not be discussed in this review. However, the mechanisms that determine the somatotopic relationship between sensory hair cells and neurons that innervate them remains an area of active investigation. It represents an important dimension of the self-organization of the lateral line system (Alexandre and Ghysen, 1999; Gompel et al., 2001; Pujol-Marti et al., 2010).

Summary and Conclusions

This review has emphasized how the pLLP in zebrafish serves as a model for understanding the self-organization of a sensory organ system and for understanding how interactions between multiple signaling pathways coordinate cell fate and morphogenesis. It also illustrates that there are striking similarities in the mechanisms operating in the pLLP and in metastatic cancer cells, where collective migration is directed by chemokine signaling, proliferation is determined by Wnt signaling and a switch between expression of E-cadherin and N-cadherin can determine a switch from stationary to migratory behavior of cells. The pLLP also illustrates how a polarized Wnt and FGF system maintains a proliferating population of progenitors at the leading end and coordinates their progressive maturation into neuromasts, while maintaining the size of a pLLP that is continuously losing cells through the deposition of neuromasts. The neuromasts also serve as models for understanding growth and regeneration of the sensory system. Ultimately, understanding the self-organization of the lateral line system is likely to result in a deeper understanding of the self-organization and emergent behavior not only of the lateral line system but also other biological systems described above, with which the lateral line systems shares so many features.

References

- 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]
- Aman A, Nguyen M, Piotrowski T. Wnt/beta-catenin dependent cell proliferation underlies segmented lateral line morphogenesis. *Dev Biol*. 2011; 349:470–482. [PubMed: 20974120]
- 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]
- Baker PC, Schroeder TE. Cytoplasmic filaments and morphogenetic movement in the amphibian neural tube. *Developmental Biology*. 1967; 15:432–450. [PubMed: 6032487]
- Balak KJ, Corwin JT, Jones JE. Regenerated hair cells can originate from supporting cell progeny: evidence from phototoxicity and laser ablation experiments in the lateral line system. *J Neurosci*. 1990; 10:2502–2512. [PubMed: 2388077]
- 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]
- Blaser H, Reichman-Fried M, Castanon I, Dumstrei K, Marlow FL, Kawakami K, Solnica-Krezel L, Heisenberg CP, Raz E. Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. *Dev Cell*. 2006; 11:613–627. [PubMed: 17084355]
- Bleckmann H. Peripheral and central processing of lateral line information. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2008; 194:145–158. [PubMed: 18228078]
- Bluel CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature*. 1996; 382:829–833. [PubMed: 8752280]
- Boldajipour B, Mahabaleshwar H, Kardash E, Reichman-Fried M, Blaser H, Minina S, Wilson D, Xu Q, Raz E. Control of chemokine-guided cell migration by ligand sequestration. *Cell*. 2008; 132:463–473. [PubMed: 18267076]
- Braun CB, Coombs S. The overlapping roles of the inner ear and lateral line: the active space of dipole source detection. *Philos Trans R Soc Lond B Biol Sci*. 2000; 355:1115–1119. [PubMed: 11079381]
- Coffin AB, Ou H, Owens KN, Santos F, Simon JA, Rubel EW, Raible DW. Chemical screening for hair cell loss and protection in the zebrafish lateral line. *Zebrafish*. 2010; 7:3–11. [PubMed: 20192852]
- Coombs S, Finneran JJ, Conley RA. Hydrodynamic image formation by the peripheral lateral line system of the Lake Michigan mottled sculpin, *Cottus bairdi*. *Philos Trans R Soc Lond B Biol Sci*. 2000; 355:1111–1114. [PubMed: 11079380]
- Coombs, S.; Görner, P.; Münz, H.; Coombs, S.; Görner, P.; Münz, Hs. *The mechanosensory lateral line: neurobiology and evolution*. New York: Springer; 1989.
- Coombs, S.; van Netten, SM. *The hydrodynamics and structural mechanics of the Lateral line system*. In: Shadwick, RE.; Lauder, GV.; Shadwick, RE.; Lauder, GVs, editors. *Fish Biomechanics*. New York: Elsevier; 2006. p. 103-139.
- Dambly-Chaudiere 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]
- David NB, Sapede D, Saint-Etienne L, Thisse C, Thisse B, Dambly-Chaudiere 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]
- Decaillot FM, Kazmi MA, Lin Y, Ray-Saha S, Sakmar TP, Sachdev P. CXCR7/CXCR4 heterodimer constitutively recruits {beta}-arrestin to enhance cell migration. *J Biol Chem*. 2011
- Dijkgraaf, S. A Short Personal Review of the History of Lateral Line research. In: Coombs, S.; Gorner, P.; Munz, H.; Coombs, S.; Gorner, P.; Munz, Hs, editors. *The Mechanosensory Lateral Line - Neurobiology and Evolution*. New York: Springer-Verlag; 1989. p. 7-14.

- Dorsky RI, Sheldahl LC, Moon RT. A transgenic Lef1/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. *Dev Biol.* 2002; 241:229–237. [PubMed: 11784107]
- Dufourcq P, Roussigne M, Blader P, Rosa F, Peyrieras N, Vríz S. Mechano-sensory organ regeneration in adults: the zebrafish lateral line as a model. *Mol Cell Neurosci.* 2006; 33:180–187. [PubMed: 16949838]
- Farooqui R, Fenteany G. Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. *J Cell Sci.* 2005; 118:51–63. [PubMed: 15585576]
- Faucherre A, Pujol-Marti J, Kawakami K, Lopez-Schier H. Afferent neurons of the zebrafish lateral line are strict selectors of hair-cell orientation. *PLoS One.* 2009; 4:e4477. [PubMed: 19223970]
- Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. *Nat Rev Mol Cell Biol.* 2009; 10:445–457. [PubMed: 19546857]
- Gamba L, Cubedo N, Ghysen A, Lutfalla G, Dambly-Chaudiere 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]
- 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.* 2010; 239:3163–3171. [PubMed: 20981829]
- Ghysen A, Dambly-Chaudiere C. The lateral line microcosmos. *Genes Dev.* 2007; 21:2118–2130. [PubMed: 17785522]
- Gilmour D, Knaut H, Maischein HM, Nusslein-Volhard C. Towing of sensory axons by their migrating target cells in vivo. *Nat Neurosci.* 2004; 7:491–492. [PubMed: 15097993]
- Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature.* 1998; 391:357–362. [PubMed: 9450748]
- Gompel N, Cubedo N, Thisse C, Thisse B, Dambly-Chaudiere C, Ghysen A. Pattern formation in the lateral line of zebrafish. *Mech Dev.* 2001; 105:69–77. [PubMed: 11429283]
- Gompel N, Dambly-Chaudiere C, Ghysen A. Neuronal differences prefigure somatotomy in the zebrafish lateral line. *Development.* 2001; 128:387–393. [PubMed: 11152637]
- Graham GJ. D6 and the atypical chemokine receptor family: Novel regulators of immune and inflammatory processes. *European Journal of Immunology.* 2009; 39:342–351. [PubMed: 19130487]
- 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]
- Gwak JW, Kong HJ, Bae YK, Kim MJ, Lee J, Park JH, Yeo SY. Proliferating neural progenitors in the developing CNS of zebrafish require Jagged2 and Jagged1b. *Mol Cells.* 2010; 30:155–159. [PubMed: 20680491]
- Haas P, Gilmour D. Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev Cell.* 2006; 10:673–680. [PubMed: 16678780]
- Haddon C, Mowbray C, Whitfield T, Jones D, Gschmeissner S, Lewis J. Hair cells without supporting cells: further studies in the ear of the zebrafish mind bomb mutant. *J Neurocytol.* 1999; 28:837–850. [PubMed: 10900088]
- 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.* 2003; 4:219–234. [PubMed: 12943374]
- Hashimoto H, Itoh M, Yamanaka Y, Yamashita S, Shimizu T, Solnica-Krezel L, Hibi M, Hirano T. Zebrafish Dkk1 functions in forebrain specification and axial mesendoderm formation. *Dev Biol.* 2000; 217:138–152. [PubMed: 10625541]
- 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]
- Hazan RB, Qiao R, Keren R, Badano I, Suyama K. Cadherin switch in tumor progression. *Ann N Y Acad Sci.* 2004; 1014:155–163. [PubMed: 15153430]

- Hernandez 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]
- Hudspeth AJ. How the ear's works work. *Nature.* 1989; 341:397–404. [PubMed: 2677742]
- 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]
- Kiernan AE, Xu J, Gridley T. The Notch ligand JAG1 is required for sensory progenitor development in the mammalian inner ear. *PLoS Genet.* 2006; 2:e4. [PubMed: 16410827]
- Knaut H, Blader P, Strahle U, Schier AF. Assembly of trigeminal sensory ganglia by chemokine signaling. *Neuron.* 2005; 47:653–666. [PubMed: 16129396]
- Lecaudey V, Cakan-Akdogan G, Norton WH, Gilmour D. Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development.* 2008; 135:2695–2705. [PubMed: 18599504]
- Lecaudey V, Gilmour D. Organizing moving groups during morphogenesis. *Curr Opin Cell Biol.* 2006; 18:102–107. [PubMed: 16352429]
- Lee Y, Grill S, Sanchez A, Murphy-Ryan M, Poss KD. Fgf signaling instructs position-dependent growth rate during zebrafish fin regeneration. *Development.* 2005; 132:5173–5183. [PubMed: 16251209]
- Lewis JL, Bonner J, Modrell M, Ragland JW, Moon RT, Dorsky RI, Raible DW. Reiterated Wnt signaling during zebrafish neural crest development. *Development.* 2004; 131:1299–1308. [PubMed: 14973296]
- Li Q, Shirabe K, Kuwada JY. Chemokine signaling regulates sensory cell migration in zebrafish. *Dev Biol.* 2004; 269:123–136. [PubMed: 15081362]
- Liao JC. A review of fish swimming mechanics and behaviour in altered flows. *Philos Trans R Soc Lond B Biol Sci.* 2007; 362:1973–1993. [PubMed: 17472925]
- Lopez-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]
- Lopez-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]
- Luker KE, Steele JM, Mihalko LA, Ray P, Luker GD. Constitutive and chemokine-dependent internalization and recycling of CXCR7 in breast cancer cells to degrade chemokine ligands. *Oncogene.* 2010; 29:4599–4610. [PubMed: 20531309]
- Marques SR, Lee Y, Poss KD, Yelon D. Reiterative roles for FGF signaling in the establishment of size and proportion of the zebrafish heart. *Dev Biol.* 2008; 321:397–406. [PubMed: 18639539]
- Martin AC, Kaschube M, Wieschaus EF. Pulsed contractions of an actin-myosin network drive apical constriction. *Nature.* 2009; 457:495–499. [PubMed: 19029882]
- Matsubayashi Y, Ebisuya M, Honjoh S, Nishida E. ERK Activation Propagates in Epithelial Cell Sheets and Regulates Their Migration during Wound Healing. *Current Biology.* 2004; 14:731–735. [PubMed: 15084290]
- Matsuda M, Chitnis AB. *Atoh1a* expression must be restricted by Notch signaling for effective morphogenesis of the posterior lateral line primordium in zebrafish. *Development.* 2010; 137:3477–3487. [PubMed: 20876657]
- McDermott BM Jr, Asai Y, Baucom JM, Jani SD, Castellanos Y, Gomez G, McClintock JM, Starr CJ, Hudspeth AJ. Transgenic labeling of hair cells in the zebrafish acousticolateralis system. *Gene Expr Patterns.* 2010; 10:113–118. [PubMed: 20085825]
- 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. *Development.* 2011; 138:3921–3930. [PubMed: 21862556]
- 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]

- Metcalfe WK. Sensory neuron growth cones comigrate with posterior lateral line primordial cells in zebrafish. *J Comp Neurol.* 1985; 238:218–224. [PubMed: 4044912]
- 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]
- Mogdans J, Wojtenek W, Hanke W. The puzzle of hydrodynamic information processing: how are complex water motions analyzed by the lateral line? *Eur J Morphol.* 1999; 37:195–199. [PubMed: 10342456]
- Molina GA, Watkins SC, Tsang M. Generation of FGF reporter transgenic zebrafish and their utility in chemical screens. *BMC Dev Biol.* 2007; 7:62. [PubMed: 17553162]
- Montgomery JC, Baker CF, Carton AG. The lateral line can mediate rheotaxis in fish. *Nature.* 1997; 389:960–963.
- Naumann U, Cameroni E, Pruenster M, Mahabaleshwar H, Raz E, Zerwes HG, Rot A, Thelen M. CXCR7 functions as a scavenger for CXCL12 and CXCL11. *PLoS One.* 2010; 5:e9175. [PubMed: 20161793]
- Nechiporuk A, Raible DW. FGF-dependent mechanosensory organ patterning in zebrafish. *Science.* 2008; 320:1774–1777. [PubMed: 18583612]
- Neves J, Parada C, Chamizo M, Giraldez F. Jagged 1 regulates the restriction of Sox2 expression in the developing chicken inner ear: a mechanism for sensory organ specification. *Development.* 2010; 138:735–744. [PubMed: 21266409]
- Nunez VA, Sarrazin AF, Cubedo N, Allende ML, Dambly-Chaudiere C, Ghysen A. Postembryonic development of the posterior lateral line in the zebrafish. *Evol Dev.* 2009; 11:391–404. [PubMed: 19601973]
- Parinov S, Kondrichin I, Korzh V, Emelyanov A. Tol2 transposon-mediated enhancer trap to identify developmentally regulated zebrafish genes in vivo. *Dev Dyn.* 2004; 231:449–459. [PubMed: 15366023]
- Parsons MJ, Pisharath H, Yusuff S, Moore JC, Siekmann AF, Lawson N, Leach SD. Notch-responsive cells initiate the secondary transition in larval zebrafish pancreas. *Mech Dev.* 2009; 126:898–912. [PubMed: 19595765]
- Piloto S, Schilling TF. Ovo1 links Wnt signaling with N-cadherin localization during neural crest migration. *Development.* 2010; 137:1981–1990. [PubMed: 20463035]
- Pujol-Marti J, Baudoin JP, Faucherre A, Kawakami K, Lopez-Schier H. Progressive neurogenesis defines lateralis somatotopy. *Dev Dyn.* 2010; 239:1919–1930. [PubMed: 20549716]
- Rajagopal S, Kim J, Ahn S, Craig S, Lam CM, Gerard NP, Gerard C, Lefkowitz RJ. Beta-arrestin- but not G protein-mediated signaling by the “decoy” receptor CXCR7. *Proc Natl Acad Sci U S A.* 2010; 107:628–632. [PubMed: 20018651]
- Raman D, Sobolik-Delmaire T, Richmond A. Chemokines in health and disease. *Experimental Cell Research.* 2011; 317:575–589. [PubMed: 21223965]
- Rouse GW, Pickles JO. Paired development of hair cells in neuromasts of the teleost lateral line. *Proceedings of the Royal Society B: Biological Sciences.* 1991; 246:123–128.
- Sanchez-Alcaniz JA, Haeghe S, Mueller W, Pla R, Mackay F, Schulz S, Lopez-Bendito G, Stumm R, Marin O. Cxcr7 controls neuronal migration by regulating chemokine responsiveness. *Neuron.* 2011; 69:77–90. [PubMed: 21220100]
- Santos F, MacDonald G, Rubel EW, Raible DW. Lateral line hair cell maturation is a determinant of aminoglycoside susceptibility in zebrafish (*Danio rerio*). *Hear Res.* 2006; 213:25–33. [PubMed: 16459035]
- Sarrazin AF, Villablanca EJ, Nunez VA, Sandoval PC, Ghysen A, Allende ML. Proneural gene requirement for hair cell differentiation in the zebrafish lateral line. *Dev Biol.* 2006; 295:534–545. [PubMed: 16678150]
- Scheer N, Groth A, Hans S, Campos-Ortega JA. An instructive function for Notch in promoting gliogenesis in the zebrafish retina. *Development.* 2001; 128:1099–1107. [PubMed: 11245575]
- Schuster K, Dambly-Chaudiere 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]

- Stoick-Cooper CL, Weidinger G, Riehle KJ, Hubbert C, Major MB, Fausto N, Moon RT. Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development*. 2007; 134:479–489. [PubMed: 17185322]
- Tarasova NI, Stauber RH, Michejda CJ. Spontaneous and ligand-induced trafficking of CXCL12 chemokine receptor 4. *J Biol Chem*. 1998; 273:15883–15886. [PubMed: 9632631]
- Theveneau E, Marchant L, Kuriyama S, Gull M, Moepps B, Parsons M, Mayor R. Collective chemotaxis requires contact-dependent cell polarity. *Dev Cell*. 2010; 19:39–53. [PubMed: 20643349]
- Tsang M, Friesel R, Kudoh T, Dawid IB. Identification of Sef, a novel modulator of FGF signalling. *Nat Cell Biol*. 2002; 4:165–169. [PubMed: 11802164]
- Valdivia LE, Young RM, Hawkins TA, Stickney HL, Cavodeassi F, Schwarz Q, Pullin LM, Villegas R, Moro E, Argenton F, Allende ML, Wilson SW. Lef1-dependent Wnt/ β -catenin signalling drives the proliferative engine that maintains tissue homeostasis during lateral line development. *Development*. 2011; 138:3931–3941. [PubMed: 21862557]
- Valentin G, Haas P, Gilmour D. The chemokine SDF1 α coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. *Curr Biol*. 2007; 17:1026–1031. [PubMed: 17570670]
- Van Trump WJ, McHenry MJ. The morphology and mechanical sensitivity of lateral line receptors in zebrafish larvae (*Danio rerio*). *J Exp Biol*. 2008; 211:2105–2115. [PubMed: 18552300]
- 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]
- Wang Y, Li G, Stanco A, Long JE, Crawford D, Potter GB, Pleasure SJ, Behrens T, Rubenstein JL. CXCR4 and CXCR7 have distinct functions in regulating interneuron migration. *Neuron*. 2011; 69:61–76. [PubMed: 21220099]
- Wibowo I, Pinto-Teixeira F, Satou C, Higashijima S, Lopez-Schier H. Compartmentalized Notch signaling sustains epithelial mirror symmetry. *Development*. 2011; 138:1143–1152. [PubMed: 21343366]
- Yang Y, Chen J, Engel J, Pandya S, Chen N, Tucker C, Coombs S, Jones DL, Liu C. Distant touch hydrodynamic imaging with an artificial lateral line. *Proc Natl Acad Sci U S A*. 2006; 103:18891–18895. [PubMed: 17132735]
- Yeo SY, Kim M, Kim HS, Huh TL, Chitnis AB. Fluorescent protein expression driven by her4 regulatory elements reveals the spatiotemporal pattern of Notch signaling in the nervous system of zebrafish embryos. *Dev Biol*. 2007; 301:555–567. [PubMed: 17134690]

Figure 1.

Periodic deposition of neuromasts by the posterior Lateral Line Primordium (pLLP) A. Schematic of migration of the pLLP from the otic vesicle to the tip of the tail as it periodically deposits primary neuromasts L1 to L8. B. Images from a time lapse movie made using confocal microscopy of pLLP migration using a *CldnB::LynGFP* transgenic embryo showing progressive stages of neuromast deposition. Arrow points to depositing neuromast. C. Superimposition of a bright field and fluorescent image of a zebrafish larva at 5 days with neuromasts labelled with Yo-Pro-1. Arrows point to primary neuromasts L1 to L8 on the left side of the embryo, the image shows secondary neuromasts (*) and a few neuromasts from the right side of the embryo (not labelled).

Figure 2.

Polarized Wnt and FGF signaling in the pLLP. **A.** The leading end of the pLLP is dominated by Wnt signaling while FGF signaling dominates at the trailing end. Cells assume a mesenchymal morphology at the leading end, where Wnt signaling dominates, while FGF signaling facilitates adoption of epithelial morphology and formation of epithelial rosettes, in trailing domains. **B.** Two color whole mount *in situ* hybridization showing Wnt signaling-dependent *lef1* expression (red brown) and FGF signaling-dependent *pea3* expression (purple) in the trailing domain, with some area of overlap in between. **C.** A Wnt-dependent source of FGF3 and FGF10 at the leading end establishes a domain of FGF signaling in an adjacent domain. While Wnt signaling drives expression of Sef, which locally inhibits FGF signaling, FGF signaling drives expression of Dkk1, which inhibits Wnt signaling.

Figure 3.

Wnt-dependent FGF signaling facilitates morphogenesis of an epithelial rosette in a newly formed proto-neuromast. A. A Wnt-dependent source of FGFs (red cells) establishes a domain of FGF signaling (green cells) in an adjacent domain. B. FGF signaling in trailing cells facilitates adoption of epithelial morphology and apical constriction, which contributes to epithelial rosette formation.

Figure 4.

Wnt-dependent FGF signaling initiates center biased *atoh1a* expression in a newly formed proto-neuromast (A). B. FGF-dependent *pea3* expression reveals restricted center-biased FGF signaling at the leading end of the pLLP (arrow). This initiates center biased *atoh1a* and *deltaA* expression. Atoh1a initiates *deltaD* expression and together DeltaA and DeltaD activate Notch in neighboring cells, which inhibits *atoh1a* expression. C. Lateral inhibition, mediated by Notch signaling keeps *atoh1a* restricted to a central cell. This cell becomes a hair cell progenitor, while its neighbors, in which *atoh1a* is suppressed become support cells. When Notch signaling fails *atoh1a* expression spreads to adjacent cells and hair cell progenitors (blue) expand in number at the cost of support cells (orange).

Figure 5.

The central *atoh1a*-expressing cell becomes a new source of FGF10 in maturing proto-neuromasts. A. Atoh1a drives expression of *fgf10* and inhibits expression of *fgfr1*. B. As a source of FGF10, the *atoh1a* expressing cell maintains FGF signaling at the trailing end of the pLLP. However, loss of *fgfr1* expression makes this cell less responsive to FGF signaling. C. In the most mature trailing proto-neuromast Atoh1a drives expression of *atoh1b*, Atoh1b drives *atoh1a* expression. In this manner cross-activation helps maintain *atoh1a* expression in trailing neuromasts, where its expression becomes less dependent on FGF signaling. As the central cell becomes a source of DeltaD and FGF10 it maintains Notch and FGF signaling in neighboring cells. As long as Notch activation is maintained in the neighboring cells they do not express *atoh1a* in response to FGF signaling.

Figure 6.

A speculative model of how support cells expressing both *e-cadherin* and *n-cadherin* contribute to cohesive integrity of the pLL system. A. (a) Comparative expression of *atoh1a* (black), *e-cadherin* (green), *n-cadherin* (red) in neuromast cells with distinct fate in a wild-type (WT) and *mib1* mutant (No Notch) embryo. (b) A side view illustrates how expression of both *e-cadherin* (green) and *n-cadherin* in support cells (yellow) would allow them to develop adhesive junctions (arrows) with both central *n-cadherin* expressing hair cell progenitors (red) and surrounding non-sensory cells expressing only *e-cadherin* (green). (c) In the absence of Notch signaling hair cell progenitors expand at the expense of support cells, (d) resulting in a loss of cohesive interactions. B. (a) Schematic of the relatively complementary expression *n-cadherin* (red) and *e-cadherin* (green) in the migrating the pLLP. Prospective support cells in the trailing domain (yellow) express both *e-* and *n-cadherin*. (b) Loss of prospective support cells could contribute to fragmentation of the pLLP.

Figure 7.

Polarized expression of CXCR4b and CXCR7b in the pLLP. A. Degradation of CXCL12a by CXCR7b may contribute to establishment of a transient local gradient of CXCL12a. Failure of CXCR7b activation in trailing cells to promote protrusive migratory activity ensures a polarized response to CXCL12a expressed in a relatively pattern in the migratory path. B. Schematic of potential interactions that determine polarized chemokine receptor expression. C. Trailing epithelial cells contribute to collective migration by extending crptic basal lamellipodia.

The neuromast deposition cycle

Figure 8.

An evolving model of the neuromast deposition cycle. (a–d) Progressive changes in the pLLP during a deposition cycle. The leading cohesive column of migrating *cxcr7*-free cells is shown in green. Trailing *cxcr7*-expressing cells slowing down for deposition are shown in red. The size bar shows length of the migrating column of green cells remains relatively constant as proliferation adds to the total length of the migrating pLLP. In b, the dotted lines show formation of a new proneuomast as the pLLP lengthens. Prospective and deposited neuromasts are serially labelled 1–4 in the schematic.

Figure 9.

The anatomy and organization of sensory hair cells. **A.** The central hair cell progenitor (blue) divides to form two hair cells. Microtubule-containing kinocilia form at eccentric adjacent positions in daughter cells, along an axis defined by the direction of migration of the pLLP prior to neuromast depsoition. Development of the kinocilium is accompanied by formation of rows of progressively shorter actin containing stereocilia. **B.** Schematic of a side view of a hair cell. The tip of each process is attached to the adjacent taller process by a linking filament. Tension in these links influences the probability of cation channels opening. Hence bending toward the kinocilium increases probability of channel opening while bending away contributes to lower probability of opening. **C.** During regeneration a hair cell progenitor divides to form daughter with opposing polarity. (i) A new hair cell progenitor is then specified toward either dorsal or ventral poles of the neuromast. (ii) In this manner adjacent columns of hair cells with opposing planar plarity are progressively established. Eventually the ellipsoid shape of the neuromast is lost and it become more rounded as hair cells move away from the equator to establish mirror symmetric populations with opposing planar polarity.