

Wnt/ β -Catenin and Fgf Signaling Control Collective Cell Migration by Restricting Chemokine Receptor Expression

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SUMMARY

Collective cell migration is a hallmark of embryonic morphogenesis and cancer metastases. However, the molecular mechanisms regulating coordinated cell migration remain poorly understood. A genetic dissection of this problem is afforded by the migrating lateral line primordium of the zebrafish. We report that interactions between Wnt/ β -catenin and Fgf signaling maintain primordium polarity by differential regulation of gene expression in the leading versus the trailing zone. Wnt/ β -catenin signaling in leader cells informs coordinated migration via differential regulation of the two chemokine receptors, *cxcr4b* and *cxcr7b*. These findings uncover a molecular mechanism whereby a migrating tissue maintains stable, polarized gene expression domains despite periodic loss of whole groups of cells. Our findings also bear significance for cancer biology. Although the Fgf, Wnt/ β -catenin, and chemokine signaling pathways are well known to be involved in cancer progression, these studies provide *in vivo* evidence that these pathways are functionally linked.

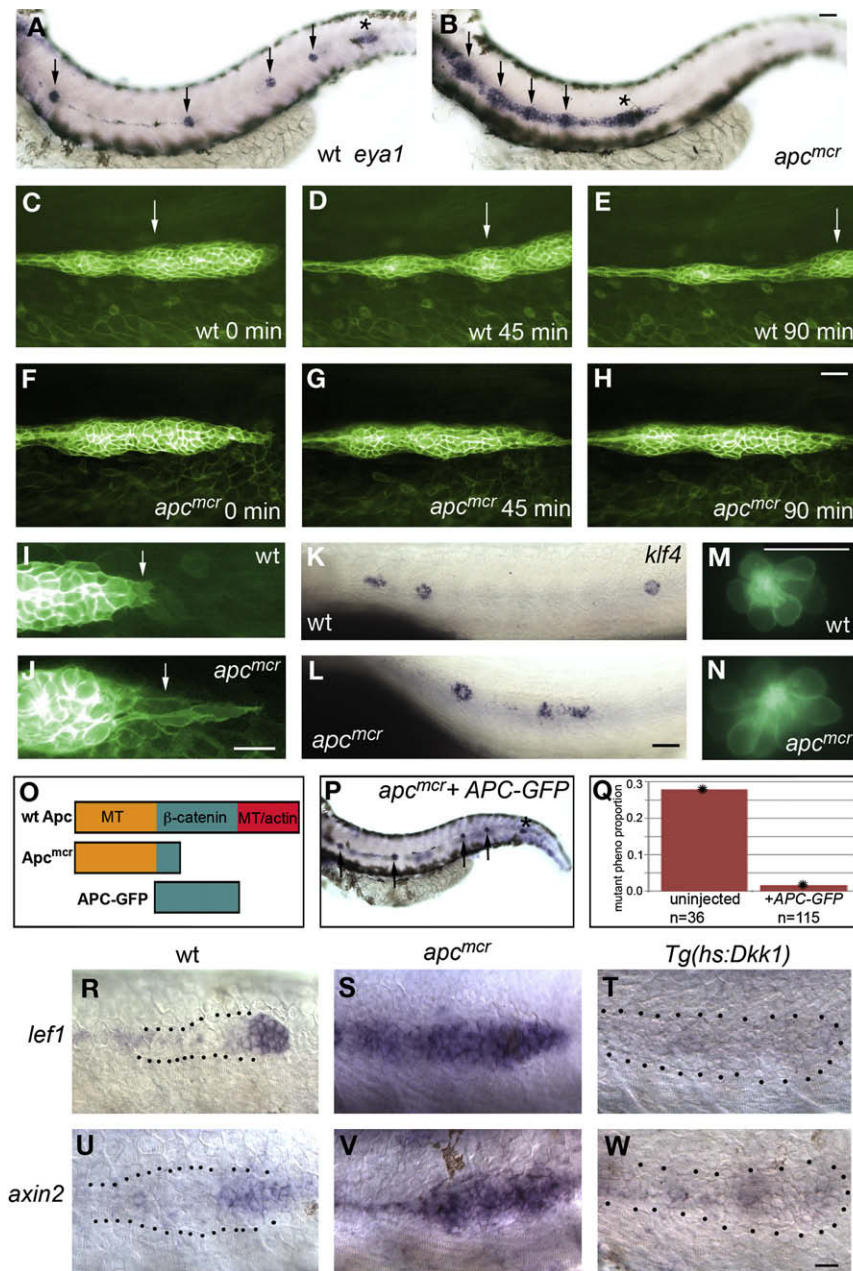
INTRODUCTION

Cell migration is a fundamental, tightly coordinated process during the embryonic and adult life of animals. Organogenesis, wound healing, and immune responses, for example, are characterized by a robust and exquisite orchestration of directed movements of cells toward specific locations. In some cases, cells migrate as individuals, e.g., neural crest cells and cells comprising our immune system (De Calisto et al., 2005; Redd et al., 2006). In other cases, cells migrate as groups adhering to each other via cell-cell adhesion molecules (Friedl, 2004; Rorth, 2007). Movement of groups of cells occurs, for example, during gastrulation, blood vessel formation, wound healing, and *Drosophila* border cell migration (Martin and Parkhurst, 2004; Montell, 2006; Rorth, 2007; Schmidt et al., 2007; Solnica-Krezel, 2006). Although much has been learned from *in vitro* studies about how individual cells migrate, the mechanisms integrating the migration and morphogenesis of groups of cells *in vivo* are

among the least understood processes in developmental biology. Important questions awaiting satisfactory mechanistic explanations include how cluster polarity is maintained and how tip cells communicate with cells in the back to ensure coordinated, directed migration. Elucidating the mechanisms regulating collective cell migration is not only crucial for our understanding of morphogenesis, but it is also highly relevant to cancer biology, as several human cancers, including breast and prostate cancer, invade tissues as groups of cells (Friedl et al., 2004; Hegerfeldt et al., 2002).

The relative simplicity and experimental accessibility of the zebrafish lateral line provide a robust model for elucidating mechanisms that regulate collective cell migration (Ghysen and Dambly-Chaudiere, 2004). The lateral line is a sensory system found in aquatic vertebrates that detects water movements. It consists of mechanosensory organs called neuromasts arranged in rows along the flanks of the animal (Metcalfe et al., 1985; Northcutt et al., 1995; Platt, 1896; Schulze, 1861; Stone, 1922). Mature neuromasts are composed of mechanosensory hair cells in the center, and supporting cells and mantle cells at the periphery. These sensory organs arise from a neurogenic placode that forms posterior to the otic placode and delaminates to become the migrating primary lateral line primordium. During migration, neuromast precursors (proneuromasts) are sequentially deposited from the trailing zone of the primordium approximately every 3–5 somites (Figure 1A; see Movies S1 and S2 available online) (Gompel et al., 2001; Metcalfe et al., 1985). It has been suggested that the directionality of this collective cell migration is not controlled by a gradient of an extrinsic guidance molecule, but rather by the polarized expression of the two chemokine receptors, *cxcr7b* and *cxcr4b*, within the primordium (Dambly-Chaudiere et al., 2007; Haas and Gilmour, 2006; Valentin et al., 2007). To date, however, the molecular mechanisms that establish and maintain this expression asymmetry during migration are not understood. Primordium polarity also underlies proneuromast formation and deposition from the trailing zone of the primordium. Cells in the leading third of the primordium are unpatterned, whereas trailing cells are organized into rosette-shaped proneuromasts and express proneural and neurogenic genes preceding hair cell differentiation (Itoh and Chitnis, 2001; Lecaudey et al., 2008; Nechiporuk and Raible, 2008).

Our studies have identified important cell-cell signaling events that occur between cells in the leading and trailing zones of a migrating cell cluster that function to maintain its polarity as it migrates. The network is based on localized activation of the



Wnt/ β -catenin pathway in the leading zone of the primordium. Subsequent interactions between the Wnt/ β -catenin and Fgf signaling pathways serve to restrict activation of these pathways into mutually exclusive domains. Restricted activation of Wnt/ β -catenin signaling controls the localized expression of *cxcr4b* and *cxcr7b* and coordinates cell migration with sensory organ deposition by restricting Fgf signaling to trailing cells.

RESULTS

Misregulation of the Wnt/ β -Catenin Signaling Pathway Causes Cell Migration Defects

To investigate a possible role of Wnt/ β -catenin signaling in lateral line development, we analyzed a recessive zebrafish mutation in

apc (*adenomatous polyposis coli*) (*apc^{mcr}*) (Hurlstone et al., 2003; Peifer and Polakis, 2000). APC is a scaffolding protein with several protein-binding domains (Nathke, 2005). It is best known for regulating the Wnt/ β -catenin signaling pathway, in which it is a necessary component of the complex that targets β -catenin for destruction in the absence of active Wnt signaling (Bienz, 2002; Rubinfeld et al., 1996). Approximately 85% of human colon cancer patients possess mutations in APC that lead to constitutive activation of the Wnt/ β -catenin signaling pathway in affected cells (Kinzler and Vogelstein, 1996; Reya and Clevers, 2005). In addition to its role in regulating the Wnt/ β -catenin pathway, in vitro studies of migrating cells have shown that association of the C terminus of APC with microtubules is necessary for normal migration (Kroboth et al., 2007). Similarly to the majority of

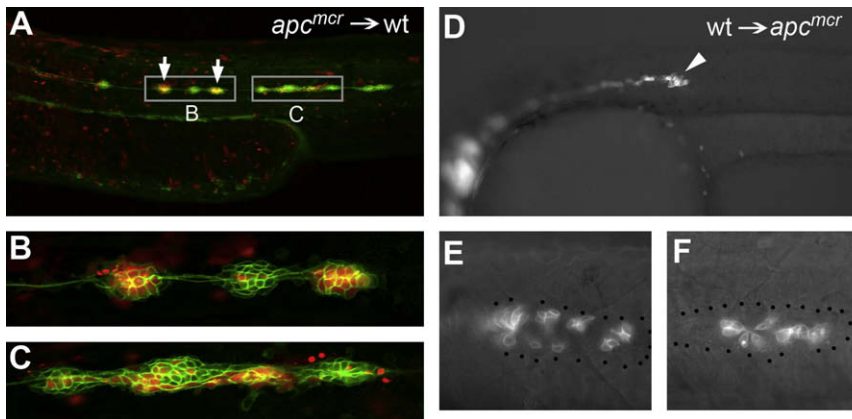


Figure 2. *apc^{mcr}* Mutant Cells Display Cell-Autonomous Defects and Exert a Non-Cell-Autonomous Effect on Neighboring WT Cells

(A) Mosaic embryo generated by transplanting red fluorescently labeled *apc^{mcr}* cells into *Tg(Cldnb:lynGFP)* embryos.

(B and C) Higher-magnification views of the boxed areas in (A). (B) Red mutant cells are deposited and form proneuromasts in ectopic positions (arrows). (C) *apc^{mcr}* cells induce morphogenesis defects in neighboring green WT cells.

(D) Overview of an embryo generated by transplanting fluorescently labeled WT cells into *apc^{mcr}* mutant embryos.

(E and F) Higher-magnification images of two additional WT to *apc^{mcr}* transplants.

mutations in human APC, the zebrafish *apc^{mcr}* mutation truncates the protein, deleting the central and C-terminal domains necessary for regulating Wnt/ β -catenin signaling and normal cytoskeletal association, respectively (Figure 1O) (Hurlstone et al., 2003).

We characterized lateral line primordium development in wild-type (WT) and *apc^{mcr}* mutant embryos by using static and dynamic assays. In situ hybridization with *eya1*, a lateral line marker, at 36 hours postfertilization (hpf) demonstrates that the mutant primordium does not reach the tail tip (Figures 1A and 1B). WT primordia travel at a constant speed and periodically deposit proneuromasts, whereas *apc^{mcr}* mutant primordia stall at ~27–29 hpf after leaving behind a disorganized band of cells (Figures 1A–1H; Movies S1–S4). Individual cells within the *apc^{mcr}* mutant primordium are motile but fail to undergo directed coordinated cell migration. Tip cells in the leading zone of WT primordia respond to guidance cues and are important for directional migration toward the tail tip (Figure 1I; Movie S5) (Haas and Gilmour, 2006). In stalling *apc^{mcr}* mutant primordia, tip cells attempt to migrate posteriorly and elongate in the process, but they are held back by the remaining primordia cells that tumble randomly (Figure 1J; Movie S6). These findings suggest that *apc^{mcr}* mutant primordia fail to migrate due to a loss of directed cell migration in trailing cells in the presence of normal tip cells. Irrespectively, proneuromasts form within the mutant primordium, and deposited cells differentiate to form all the cell types normally present in neuromasts, such as support and hair cells, as revealed by *klf4* and *brn3c* expression, respectively (Figures 1K–1N, Movies S2 and S4).

To determine which Apc functions are necessary for normal lateral line morphogenesis, we injected mRNA coding for the central zone of the human APC gene (*APC-GFP*, Figure 1O) into one-cell-stage embryos from a cross of *apc^{mcr}* heterozygotes (Miller and Moon, 1997). APC-GFP encodes domains necessary for the regulation of β -catenin, but it lacks domains needed for microtubule association (Barth et al., 2002; Zumbunn et al., 2001). Among 115 injected embryos, only 2 had the characteristic *apc^{mcr}* lateral line phenotype, as assayed by incorporation of DASPEI, a vital dye that labels mechanosensory hair cells, and by *eya1* in situ hybridization. All other injected *apc^{mcr}* homozygotes were indistinguishable from WT embryos (Figures 1P and 1Q). In contrast, uninjected embryos from the same cross contained the expected frequency of mutants

($n = 10/36$) (Figure 1Q). A total of 44 of the injected embryos were genotyped and, as expected, about one-quarter ($n = 10$) of these phenotypically normal embryos were homozygous for *apc^{mcr}*. This demonstrates that regulation of Wnt/ β -catenin signaling is crucial for normal lateral line morphogenesis.

Misregulation of Wnt/ β -catenin signaling in *apc^{mcr}* mutants is also apparent at the level of gene expression. In situ hybridization analysis revealed that expression of the Wnt/ β -catenin target genes *lef1* and *axin2* is restricted to the leading zone of the WT primordium (Figures 1R, 1U, 1T, and 1W). In contrast, 36 hpf *apc^{mcr}* mutants express these genes throughout the primordium (Figures 1S and 1V). To investigate why *apc^{mcr}* primordia migrate normally until 27–29 hpf, we performed *lef1* gene expression analyses and discovered that *lef1* is normally restricted until 27 hpf, but expands into trailing cells over the next 2 hours, coinciding with the onset of primordium stalling (Figure S1; Movies S2 and S4). Therefore, all subsequent analyses were performed at 32 hpf or older, when the primordium had stalled in 100% of the mutants ($n > 500$). The expression analyses and injection experiments suggest that primordium migration fails in *apc^{mcr}* mutant embryos due to ectopic activation of Wnt/ β -catenin signaling in the trailing zone of the primordium, and not because of a failure of the Apc protein to associate with microtubules.

***apc^{mcr}* Exhibits Cell-Autonomous Migration Defects but also Affects Neighboring WT Cells**

The gene expression analyses indicate that *apc^{mcr}* is required within the primordium. To investigate how *apc^{mcr}* mutant cells behave in an otherwise WT primordium, we transplanted red-labeled mutant cells into green *Tg(claudinb:gfp)* embryos during early gastrula stages and observed their behavior in mosaic primordia. In seven of nine mosaic embryos, *apc^{mcr}* mutant cells stopped migrating and were deposited ectopically (Figures 2A–2C, arrows) between regularly spaced WT proneuromasts, indicating that the *apc^{mcr}* mutation acts cell autonomously. However, ectopic clusters always contained green WT cells, demonstrating that *apc^{mcr}* mutant cells exert a non-cell-autonomous effect on neighboring WT cells, either via aberrant cell adhesion or aberrant cell signaling (Figures 2B and 2C) ($n = 7$). In the two embryos in which *apc^{mcr}* mutant cells did not affect cell deposition, mutant cells were deposited early during migration, before the *apc^{mcr}* phenotype arises (data not shown). WT cells transplanted into *apc^{mcr}* mutant embryos did not rescue the

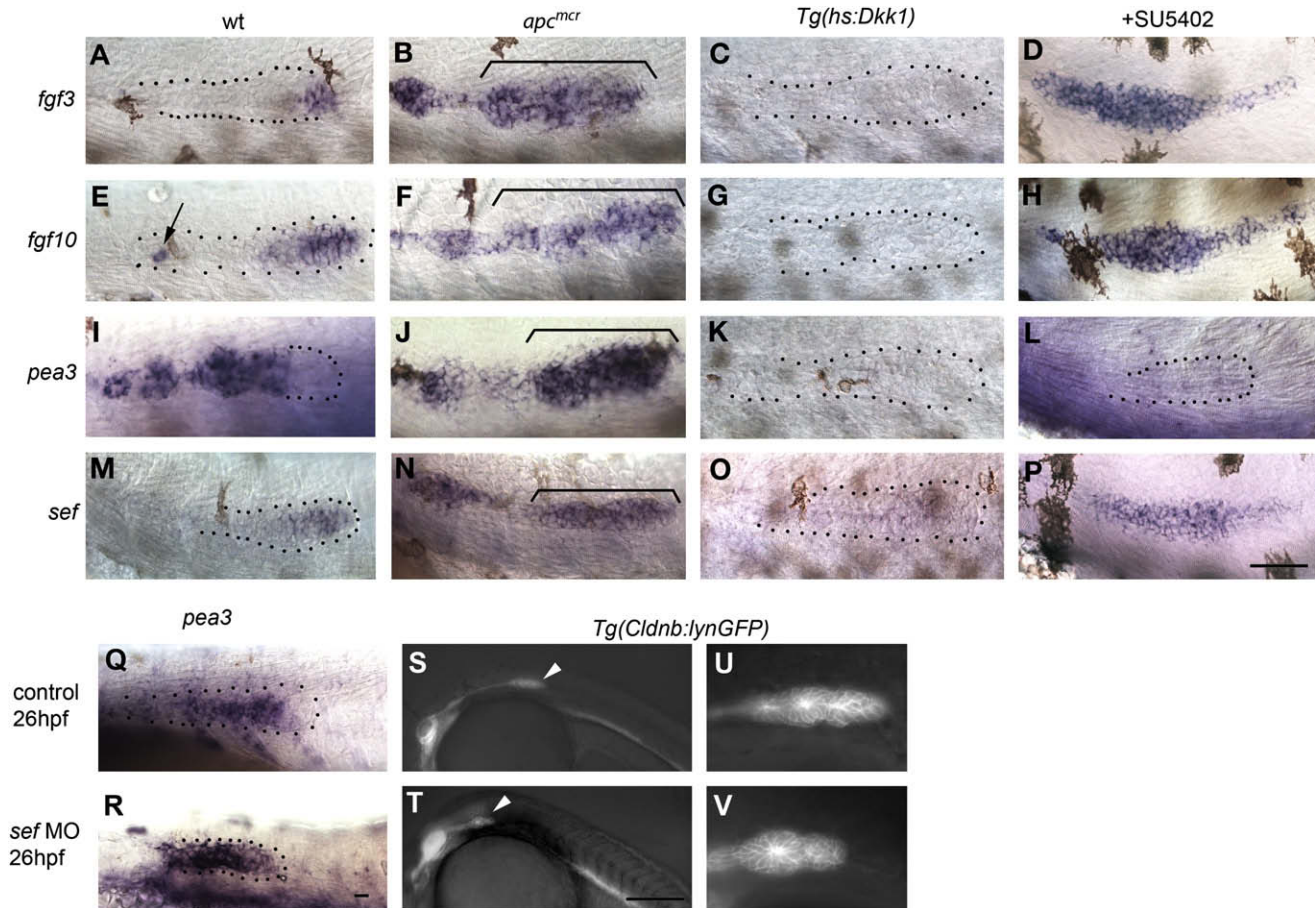


Figure 3. Wnt/ β -Catenin Signaling Regulates Fgf Signaling in the Migrating Primordium

(A–H) *fgf3* and *fgf10* are restricted to the leading zone of (A and E) WT primordia and are upregulated in (B and F) *apc^{mcr}* mutant primordia. (C and G) Their expression is lost in the absence of Wnt/ β -catenin signaling. (D and H) *fgf3* and *fgf10* are upregulated in the absence of Fgf signaling.

(I) *pea3* expression in WT primordia shows that Fgf signaling is only active in the trailing cells.

(J and K) *pea3* is expanded in 38 hpf (J) *apc^{mcr}* mutants and lost in (K) *Tg(hs:Dkk1)* embryos.

(L) *pea3* expression is abolished by SU5402 treatment.

(M) The Fgf pathway inhibitor *sef* is expressed in the leading zone of WT primordia.

(N and O) *sef* expression is expanded in (N) *apc^{mcr}* mutants and abolished in (O) *Tg(hs:Dkk1)* embryos.

(P) *sef* expression does not require Fgf signaling, as it is present in SU5402-treated primordia.

(Q–V) (S and T) Injection of *sef* morpholino (MO) disrupts primordium migration, (U and V) although the primordia orient correctly toward the posterior. (Q and R) *pea3* expression expands into the leading region of *sef* morphant primordia. WT, *apc^{mcr}*, and SU5402-treated embryos were fixed between 32 and 36 hpf, *Tg(hs:Dkk1)* embryos were heat shocked at 26 hpf and fixed at 32 hpf, and *sef* morphant embryos were fixed at 26 hpf.

Brackets in (B), (F), (G), and (N) indicate the primordium. Scale bars in (A)–(P) and (Q), (R), (U), and (V) are equal to 40 μ M. Scale bars for (S) and (T) equal 100 μ M.

phenotype (Figures 2D–2F, $n = 17$). At ~ 29 hpf, WT cells slowed down together with the surrounding *apc^{mcr}* cells. These data demonstrate that the *apc^{mcr}* phenotype is primordium autonomous and is not caused by defects in the muscle or skin of the mutants.

Wnt/ β -Catenin Signaling Regulates Fgf Signaling in the Migrating Primordium

Abrogation of the Fgf signaling pathway by application of the Fgf receptor inhibitor SU5402 immediately prior to primordium migration leads to primordium stalling, which is a phenotype that is strikingly similar to that observed in *apc^{mcr}* mutant embryos (Figure S2; Movie S7). We therefore asked whether Fgf and Wnt/ β -catenin signaling interact during primordium migra-

tion. *fgf3* and *fgf10* are the only *fgf* ligands detectable in the primordium and are expressed in the leading zone of the WT primordium (Lecaudey et al., 2008; Nechiporuk and Raible, 2008), overlapping with the Wnt/ β -catenin targets *lef1* and *axin2* (Figures 1R, 1U, 3A, and 3E). To determine whether Wnt/ β -catenin signaling regulates *fgf3* and *fgf10* expression in the leading zone of the primordium, we analyzed the expression patterns of these *fgf* ligands after changes in the level of Wnt/ β -catenin pathway activation. *fgf3* and *fgf10* expression is upregulated in the entire primordium of *apc^{mcr}* mutant embryos (Figures 3B and 3F). Conversely, reducing Wnt/ β -catenin signaling by overexpressing the inhibitor *dkk1* during primordium migration by heat shocking *Tg(hs:Dkk1)* transgenic embryos or by blocking β -catenin-induced transcription by heat shocking *Tg(hs: Δ TCF:GFP)* embryos

(data not shown) leads to strikingly decreased expression of *fgf3* and *fgf10* (Figures 3C and 3G) (Lewis et al., 2004; Stoick-Cooper et al., 2007). Importantly, Fgf signaling is not necessary for *fgf3* and *fgf10* expression in the primordium, as embryos treated with SU5402 show strong ectopic expression of these *fgf* ligands (Figures 3D and 3H). Therefore, *fgf3* and *fgf10* expression is regulated by Wnt/ β -catenin signaling in the leading zone of the primordium.

To determine where Fgf signaling is active, we investigated the expression of the transcription factor *pea3*, a known target of the Fgf pathway (Raible and Brand, 2001; Roehl and Nusslein-Volhard, 2001). Strikingly, *pea3* is excluded from the leading zone cells in migrating WT primordia, but is detected throughout the trailing end of the primordium (Figure 3I). These data indicate that Fgf ligands are able to act at a distance, and that signaling may be inhibited in the leading zone. In contrast, 38 hpf *apc^{mcr}* mutant embryos express *pea3* throughout the primordium and in deposited cells. *pea3* is lost upon induction of *dkk1*, mirroring changes in *fgf3* and *fgf10* expression (Figures 3J and 3K). *pea3* expression is lost in SU5402-treated primordia, despite the dramatic upregulation of *fgf* ligands, confirming efficient inhibition of Fgf receptor activation (Figures 3D, 3H, and 3L).

The striking absence of Fgf signaling activity from the leading cells that produce Fgf ligands in WT primordia could be caused by the presence of an inhibitor or the absence of Fgf receptors. Indeed, the only Fgf receptor present in the primordium, *fgfr1*, is expressed at very low levels in the leading zone of the WT primordium (Figures S3A and S3B) (Lecaudey et al., 2008; Nechiporuk and Raible, 2008). In *apc^{mcr}* mutant embryos, *fgfr1* is also initially low in the leading zone of the primordium but becomes upregulated over time (Figures S3C and S3D). This upregulation can be explained by our finding that *fgfr1* is an Fgf pathway target in the primordium, as it is lost upon treatment with SU5402 (Figure S3E). We hypothesize that stalling of the primordium exposes the leading zone to increasing levels of Fgf ligands over time, leading to upregulation of *fgfr1*. Another important mechanism to inhibit Fgf signaling is via the expression of the cytoplasmic membrane-associated Fgf signaling inhibitor *sef* (*il17rd*) (Furthauer et al., 2002; Tsang et al., 2002). Since *sef* is coexpressed with *lef1* and *axin2* in the leading region of the primordium (Figure 3M), we tested whether Wnt/ β -catenin signaling controls expression of this Fgf pathway inhibitor. Indeed, in addition to regulating *fgf* ligand expression, the Wnt/ β -catenin signaling pathway also regulates expression of *sef*, as this gene is ectopically expressed in trailing cells in *apc^{mcr}* mutant primordia and is downregulated when Wnt/ β -catenin signaling is depleted by induction of *dkk1* (Figures 3N and 3O). *sef* expression does not depend on Fgf signaling, as it is ectopically expressed in trailing and deposited cells, even though it is downregulated in other regions of the embryo in SU5402-treated embryos (Figure 3P; Figures S4A and S4C). Depletion of *sef* by morpholino injections causes expansion of the Fgf targets *pea3* and *dkk1* (see below) into the leading zone of the primordium, accompanied by a primordium migration defect (Figures 3Q–3V; Figure S5) (Asai et al., 2006). Therefore, even though we cannot rule out the existence of additional mechanisms, *sef* plays an important role in inhibiting Fgf pathway activation in the leading zone, likely in combination with low *fgfr1* expression.

sprouty 4 (*spry4*) is another well known Fgfr1 signal transduction inhibitor that is expressed in trailing cells of the WT primordium (Figure S6A) (Furthauer et al., 2001). In contrast to *sef*, *spry4* acts as a classical Fgf feedback attenuator, as it is lost upon SU5402 treatment, but is expanded in *apc^{mcr}* mutant primordia due to upregulation of Fgf signaling (Figures S6B and S6C). Thus, *spry4* is not involved in inhibiting Fgf signaling in the leading zone.

To confirm the selectivity of SU5402 drug treatments, we compared treated embryos to a genetic knockdown of Fgf signaling via heat shock induction of *Tg(hs:dn-fgfr1)*, which expresses a dominant-negative Fgfr1 (Lee et al., 2005) (Figures S7A–S7D). A 1 hr heat shock induction of *Tg(hs:dn-fgfr1)* is similarly effective in eliminating *pea3* and *dkk1* and expanding *sef*; however, transgenic embryos begin to die within a few hours after the treatment (Figures S7E–S7G). To avoid possible interference from dying cells with the gene expression analyses, we chose SU5402 to inhibit Fgf signaling.

Wnt/ β -Catenin-Mediated Fgf Signaling Is Required in Trailing Cells for Proneuromast Development

A recent study by Millimaki et al. (2007) showed that Fgf signaling is required for the induction and maintenance of zebrafish ear and lateral line hair cells. Fgf signaling also controls proneuromast development in the lateral line by inducing radial epithelialization of support cells and regulating expression of the proneural transcription factor *atoh1* in patches containing cells fated to give rise to hair cells and support cells (Figure 4B) (Lecaudey et al., 2008; Nechiporuk and Raible, 2008; Millimaki et al., 2007).

Proneuromast development was analyzed in different experimental conditions. During WT proneuromast formation, cells organize into rosettes that can be detected by cell shape changes and focal accumulation of Claudin-GFP (Figure 4A). In 100% of *Tg(hs:Dkk1)* transgenic and SU5402-treated embryos, no proneuromasts can be distinguished, as Fgf signaling is disrupted in both conditions (Figures 4C and 4E). As expected, no *atoh1* expression is found in these primordia (Figures 4D and 4F). In contrast, in *apc^{mcr}* mutant embryos, in which Fgf signaling is active, proneuromasts show normal cell type specification (Figures 1K–1N). Immediately after primordium stalling, the leading zone in *apc^{mcr}* mutant primordia is still unpatterned; however, ~5 hours later, a proneuromast begins to form in the leading zone (Figures 4G and 4H; Movie S4). As primordium stalling occurs before ectopic rosetto-genesis in *apc^{mcr}* primordia, rosetto-genesis in the leading zone is not the cause of the migration defect.

Rosettogenesis Does Not Influence Primordium Migration

Our analysis of Wnt/ β -catenin signaling revealed that primordia migrate in the absence of rosettes. Irrespective of when we abrogate Wnt/ β -catenin (and, secondarily, Fgf signaling) by inducing *dkk1* during migration, primordia continue to migrate to the tail tip in the absence of rosetto-genesis. Primordium migration is evidenced by the presence of the lateral line nerve (Figures 4J and 4L, arrows; Figure S8) and time-lapse imaging (Movie S8). We heat shocked embryos at 24 hpf, after migration has started and shortly before the deposition of the first trunk neuromast (Figure 4I), and at 28 hpf, shortly after deposition of the second proneuromast (L2, Figure 4K). After each heat shock,

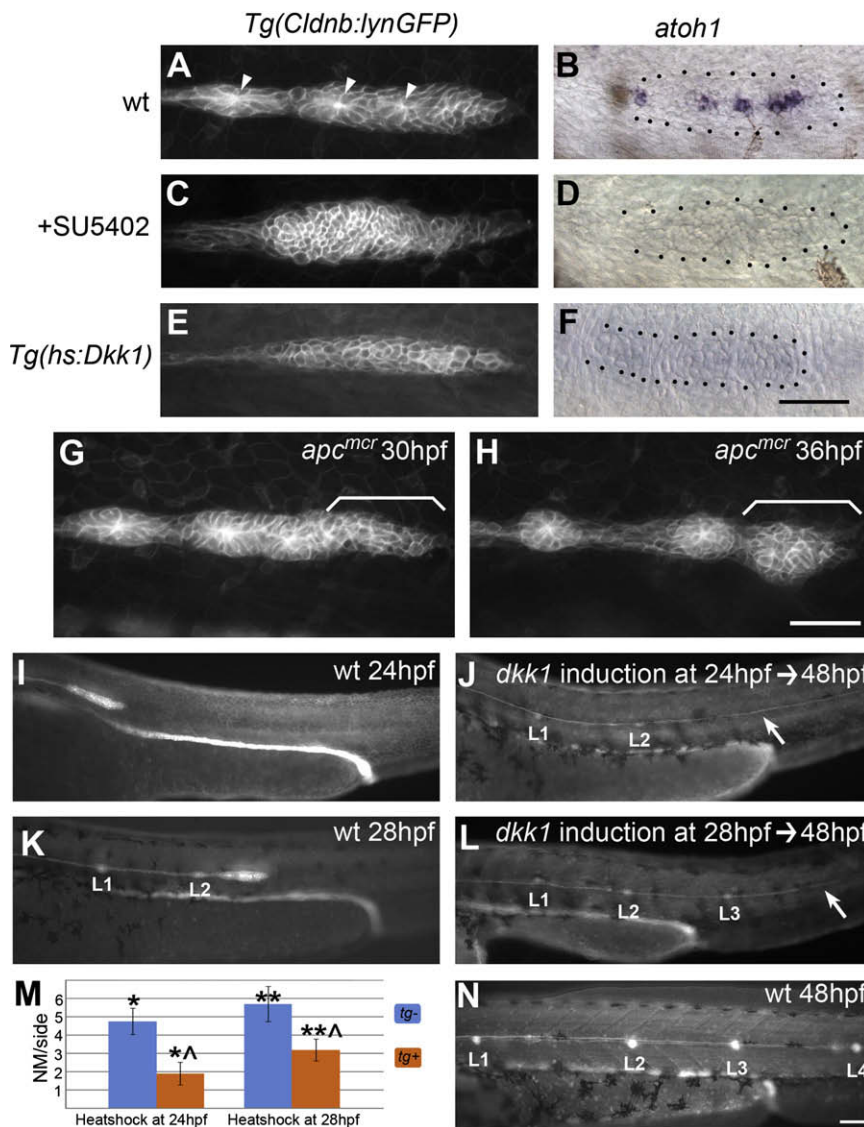


Figure 4. Wnt/ β -Catenin-Mediated Fgf Signaling Is Necessary for Neurogenesis and Rosette Formation

(A and B) (A) The trailing zone of WT primordia contains proneuromasts distinguishable by their rosette morphology, focal accumulation of Claudin-GFP (arrows), and expression of the proneural gene (B) *atoh1* at 32 hpf.

(C and D) (C) Loss of Fgf signaling by treatment with SU5402 between 20 and 38 hpf causes loss of rosette formation and (D) *atoh1* expression.

(E and F) (E) Similarly, abrogating expression of Fgf ligands by inhibiting Wnt/ β -catenin via heat shock induction of *Dkk1* at 20 hpf leads to a loss of rosettes and (F) *atoh1* expression at 28 hpf.

(G and H) Still images of [Movie S4](#). Ectopic rosette formation does not contribute to the *apc^{mcr}* migration phenotype, as stalled mutant primordia still have normally unpatterned leading zones (bracket in [G]). Ectopic rosette formation occurs in the leading zone at 5 hr after stalling (bracket in [H]).

(I–N) *dkk1* disrupts neuromast (NM) deposition without affecting migration. (I) At 24 hpf, the WT primordium is migrating, but no NM deposition has occurred. (J) *dkk1* induction at 24 hpf leads to the formation of a small L2 and complete loss of more posterior NMs without affecting migration, as evidenced by the presence of the lateral line nerve (white arrow). (K) By 28 hpf, the WT primordium has deposited two NMs, L1 and L2. (L) *dkk1* induction at 28 hpf ablates the NMs posterior to L3. (M) Quantification of NM numbers for *dkk1* induction at 24 hpf and 28 hpf. On average, the primordium is able to deposit one additional NM after *dkk1* induction (orange bars). Data are shown as means \pm SD (* $p < 0.001$, ** $p < 0.001$, $p < 0.001$ Student's *t* test). (N) At 48 hpf, the WT primordium has deposited all posterior NMs.

only one additional neuromast was deposited (Figures 4J–4M). The effects of loss of Wnt/ β -catenin signaling on rosetogenesis and migration were confirmed in *Tg(hs Δ TCF:GFP)* embryos (Figure S9). The finding that Wnt/ β -catenin signaling-depleted primordia migrate suggests that Wnt pathway activation in the leading zone is not required for migration, but that ectopic expression of Wnt/ β -catenin signaling in trailing cells impedes migration (Figure 7B).

Fgf Signaling Inhibits Wnt/ β -Catenin Signaling via *dkk1*

To further interrogate how Wnt/ β -catenin and Fgf signaling interact, we examined the effects of loss of Fgf signaling on Wnt/ β -catenin pathway activation in migrating primordia. Pharmacological abrogation of the Fgf pathway during migration leads to ectopically expressed *lef1* and *axin2* in the trailing domain of the primordium (Figures 1R, 1U, 5A, and 5B), which is similar to the phenotype observed in *apc^{mcr}* mutants (Figures 1S and 1V). These data indicate that Fgf signaling inhibits the Wnt/ β -catenin pathway in the trailing zone of the migrating pri-

primordium. However, the ectopic activation of Fgfs in the *apc^{mcr}* mutant trailing zone, where β -catenin is constitutively active, is not able to suppress Wnt/ β -catenin target genes. (Figures 1S, 1V, 3B, and 3F). Therefore, the Fgf-induced inhibitory factor must be acting upstream of β -catenin. A likely candidate is *dkk1*, a diffusible inhibitor of the Wnt/ β -catenin pathway (Niehrs, 2006). Indeed, *dkk1* is strongly expressed in a zone directly adjacent to the area in which Wnt/ β -catenin signaling is active and within the *pea3* expression domain, marking the zone of Fgf pathway activation (Figure 5C). However, it is excluded from the most trailing cells. In contrast, *dkk1* is expressed throughout the lateral line of *apc^{mcr}* mutants (Figure 5D).

Although *dkk1* is classically thought to be a direct target of the Wnt/ β -catenin pathway (Niehrs, 2006), its expression is abolished from migrating WT and *apc^{mcr}* mutant primordia after Fgf pathway abrogation by SU5402 treatment or heat shock induction of *Tg(hs:dn-fgfr1)* (Figures 5E and 5F; data not shown). This demonstrates that *dkk1* expression depends on Fgf signaling in the primordium. The proximity of *dkk1*-expressing cells to

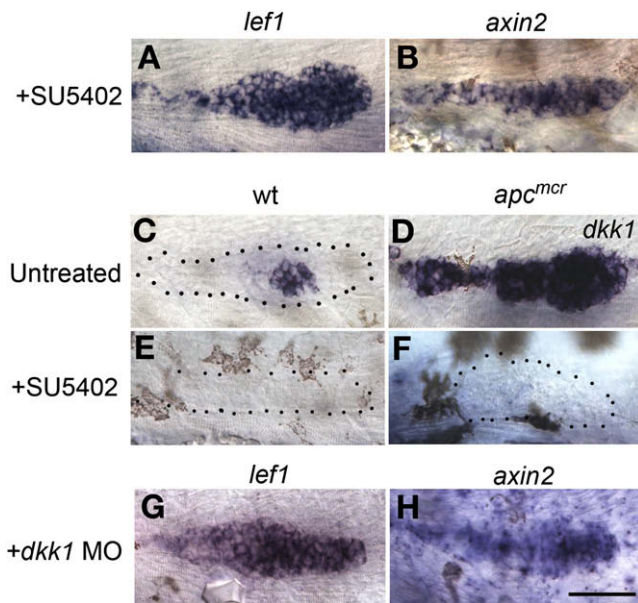


Figure 5. Fgf Signaling Inhibits Wnt/ β -Catenin Signaling via Induction of *dkk1*

(A and B) SU5402 treatment leads to ectopic induction of the Wnt/ β -catenin signaling targets *lef1* and *axin2* in 36 hpf embryos.

(C) In WT embryos, *dkk1* is expressed adjacent to the unpatterned tip of the primordium.

(D–F) (D) *dkk1* is a Fgf target, as it is highly upregulated in *apc^{mcr}* mutant primordia and is absent in (E) Fgf signaling-depleted WT, as well as in (F) Fgf signaling-depleted *apc^{mcr}* mutant primordia.

(G and H) Morpholino knockdown of *dkk1* causes expansion of *lef1* and *axin2*, similar to loss of Fgf signaling. The scale bar is equal to 40 μ m.

the leading zone suggests that *dkk1* might also be controlled by Wnt signals; however, this is not the case, as *dkk1* expression is completely lost in *apc^{mcr}* mutant embryos treated with SU5402 (Figure 5F). Thus, expansion of *dkk1* expression in *apc^{mcr}* mutant embryos is due to ectopic Fgf pathway activation and is only indirectly due to expansion of Wnt/ β -catenin signaling. As expected, knockdown of *dkk1* by morpholino injection leads to expansion of Wnt/ β -catenin target genes *lef1* and *axin2* in the primordium and causes primordium stalling (Figures 5G and 5H; data not shown) (Seiliez et al., 2006). Dkk1 is thought to be a diffusible inhibitor; however, in the primordium, it appears that Dkk1 does not diffuse very far, as *dkk1*- and *lef1*-expressing cells are situated adjacent to one another (Figure S10). We have excluded that Dkk1 activity might be inhibited in leading zone cells, as heat shock induction of *dkk1* is able to repress Wnt target activation (Figures 1T and 1W).

Taken together, these data imply a mechanism whereby Wnt/ β -catenin pathway activation spatially restricts itself through Fgf-mediated induction of *dkk1*. This negative feedback loop between Wnt/ β -catenin signaling and Fgf signaling ensures their mutually exclusive activation and maintains primordium polarity.

Localized Wnt/ β -Catenin Signaling Is Necessary for Asymmetric Expression of Chemokine Receptors

Chemokine signaling is also crucial for primordium migration. Coordinated directional migration requires asymmetric expression of the chemokine receptors, *cxcr4b* and *cxcr7b*, in the pri-

midium and expression of the ligand *sdf1a* (*cxcl12a*) in a narrow stripe along the horizontal myoseptum (Dambly-Chaudiere et al., 2007; David et al., 2002; Haas and Gilmour, 2006; Li et al., 2004; Valentin et al., 2007). *sdf1a* is normally expressed in *apc^{mcr}* embryos, consistent with the tissue-autonomous effect of *apc^{mcr}* revealed by transplantation experiments (Figures 2A–2C; data not shown).

In WT primordia, *cxcr4b* is most highly expressed in the leading zone of the primordium and is downregulated in trailing cells (Figure 6A) (Valentin et al., 2007). Conversely, *cxcr7b* is excluded from the leading zone but is strongly expressed in the trailing half of the primordium and in deposited cells (Figure 6B) (Dambly-Chaudiere et al., 2007; Valentin et al., 2007). In *apc^{mcr}* mutant embryos, *cxcr4b* is ectopically expressed in trailing cells and *cxcr7b* expression is completely absent (Figures 6C and 6D). This initially led us to believe that either the Wnt/ β -catenin or the Fgf signaling pathway initiates *cxcr4b* expression. However, Fgf signaling is not active in the leading zone of WT primordia, and, more importantly, pharmacological Fgf pathway inhibition with SU5402 also causes ectopic expression of *cxcr4b* and loss of *cxcr7b* (Figures 6E and 6F). Therefore, Fgf signaling does not regulate chemokine receptor expression in WT primordia. Instead, the misregulation of chemokine receptors in SU5402-treated embryos is a secondary effect of ectopic activation of Wnt/ β -catenin signaling. Interestingly, shutting down Wnt/ β -catenin signaling by heat shock induction of *dkk1* does not affect *cxcr4b* expression, revealing that another factor activates *cxcr4b* expression in the leading zone (Figure 6G; data not shown). Since *cxcr4b* is ectopically expressed in *apc^{mcr}* mutant primordia, we conclude that the Wnt/ β -catenin pathway is capable of inhibiting a repressor of *cxcr4b* in the most trailing cells. *cxcr7b*, however, expands into the leading zone when Wnt/ β -catenin signaling is inhibited (Figure 6H). Therefore, Wnt/ β -catenin signaling in the leading zone of the WT primordium localizes chemokine receptor expression by inhibiting expression of *cxcr7b*. Conversely, *cxcr4b* expression is inhibited in the trailing zone due to the absence of Wnt/ β -catenin signaling.

DISCUSSION

Our results demonstrate that the migrating primordium is stably patterned by interactions between the Wnt/ β -catenin and Fgf signaling pathways. The Wnt/ β -catenin pathway is activated only in leading zone cells of the primordium, where it stimulates the production of Fgf ligands and their inhibitor, *sef* (Figure 7A; red domain, yellow Fgf splotch). This results in activation of the Fgf pathway only in trailing cells (Figures 7A and 7B, green domain). Fgf pathway activation in trailing cells leads, in turn, to the production of *dkk1*, which restricts activation of the Wnt/ β -catenin pathway to the leading zone. Future experiments will address why *dkk1* is not expressed in all trailing cells.

Restriction of the Fgf and Wnt/ β -catenin pathways is necessary for asymmetric expression of the chemokine receptors, *cxcr4b* and *cxcr7b* (Figure 7B). In contrast, *apc^{mcr}* mutant primordia are not polarized, and they display uniform activation of the Wnt/ β -catenin and Fgf pathways throughout all cells (Figure 7B; Figure S11A). Ectopic activation of these pathways causes upregulation of the inhibitors *sef* and *dkk1* throughout the primordium. Despite the upregulation of *sef*, Fgf signaling

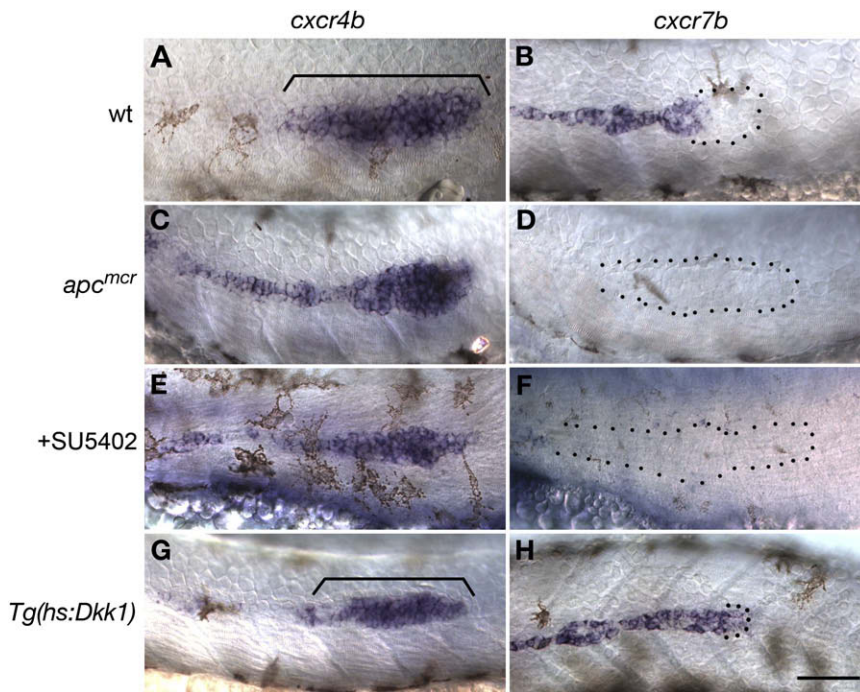


Figure 6. Localized Wnt/β-Catenin Signaling Is Necessary for Asymmetric Expression of Chemokine Receptors

(A and B) In 36 hpf WT embryos, (A) *cxcr4b* is restricted to the leading zone, and (B) *cxcr7b* is restricted to the trailing zone of the migrating primordium.

(C–F) Expanded Wnt/β-catenin signaling in *apc^{mcr}* mutants and SU5402-treated embryos leads to (C and E) expansion of *cxcr4b* and (D and F) loss of *cxcr7b*.

(G) *cxcr4b* expression is not affected by a loss of Wnt/β-catenin signaling.

(H) Loss of Wnt/β-catenin signaling leads to expansion of *cxcr7b* into the leading zone of the primordium. The scale bar is equal to 40 μM.

is still active, as revealed by the expression of the Fgf target, *pea3* (Figures 3J and 7B). It seems likely that Sef abrogates Fgf signaling only in the presence of low levels of Fgfr1, as observed in WT primordia. Upregulation of *dkk1* has no consequence on Wnt/β-catenin signaling in *apc^{mcr}* embryos, as the Wnt pathway is constitutively activated by the mutation regardless of signaling events at the membrane. Interestingly, the pathway antagonists *sef* and *dkk1* are commonly thought to be feedback induced by the Fgf and Wnt/β-catenin pathways, respectively (Chamorro et al., 2005; Furthauer et al., 2002). One exception is the Fgf inhibitor *dusp6*, which is Wnt/β-catenin dependent during axis formation in zebrafish development (Tsang et al., 2004). We have discovered that in the lateral line primordium, *sef* expression is induced and maintained entirely by the Wnt/β-catenin pathway, whereas the Wnt/β-catenin inhibitor *dkk1* is regulated by the Fgf signaling pathway. This ensures that these two pathways are active in exclusive domains. These findings may warrant the reinvestigation of the hierarchy of these interactions in other developing organ systems.

Regulation of Chemokine Receptors by the Wnt/β-Catenin Pathway

The combination of gene expression changes and resulting phenotypes in the different experimental paradigms revealed that Wnt/β-catenin signaling in the leading zone restricts expression of *cxcr7b* to trailing cells (Figure 7). Ectopic activation of the Wnt/β-catenin pathway in *apc^{mcr}* or Fgf-depleted embryos causes loss of *cxcr7b* accompanied by ectopic expression of *cxcr4b* in the trailing-most cells (Figures S11A and S11B). This result might suggest that Wnt/β-catenin signaling activates *cxcr4b*; however, inhibiting the Wnt/β-catenin pathway does not lead to a loss of *cxcr4b*, indicating that activation of *cxcr4b* expression in the leading zone occurs via an Fgf- and Wnt/β-catenin-independent mechanism (Figure 7). Furthermore, the observa-

tion that Wnt/β-catenin signaling is sufficient, but not necessary, for *cxcr4b* expression implies that ectopic Wnt/β-catenin signaling in trailing cells inhibits a transcriptional repressor of *cxcr4b* (“R” in Figure 7A). This repressor is independent of Fgf signaling, as *cxcr4b* is also repressed in trailing cells of SU5402-treated embryos. Our data suggest that the underlying cause of the migration defect in *apc^{mcr}* mutant and SU5402-treated embryos is the lack of *cxcr7b* and expansion of *cxcr4b* expression in trailing cells. Support for this conclusion also stems from time-lapse analyses of *cxcr7b* morpholino-injected embryos in which trailing cells tumble, but tip cells still extend normally toward the tail (Valentin et al., 2007).

Several models could explain how localized chemokine receptor expression controls directional migration. Based on experimental and genetic manipulations in which primordia migrated in either direction along the horizontal myoseptum, Sdf1a does not appear to be expressed in a gradient (Haas and Gilmour, 2006; Smith et al., 1994; Stone, 1923). Therefore, it was suggested that polarized expression of *cxcr4b* and *cxcr7b* is likely responsible for setting up an Sdf1a gradient within the primordium (Dambly-Chaudiere et al., 2007; Valentin et al., 2007). Since Sdf1a binds both Cxcr7b and Cxcr4b, the two receptors either have to bind Sdf1a with different affinities or initiate different intracellular signaling pathways in order to modulate the Sdf1a signal along the a-p axis of the primordium.

A recent study has elucidated a mechanism by which Sdf1 signaling is controlled by Cxcr4b and Cxcr7b during zebrafish primordial germ cell (PGC) migration (Boldajpour et al., 2008). Cxcr7b expressed in somatic tissue does not itself signal but acts as an Sdf1 sink, thus creating an Sdf1 gradient along which PGCs migrate. If Cxcr7b functions as an Sdf1a sink in the primordium, it could be necessary for establishing an Sdf1a gradient across the migrating primordium, as suggested by Dambly-Chaudiere et al. (2007). Possibly, the sequestration of Sdf1a by *cxcr7b*-expressing trailing cells, coupled with the sloping expression of *cxcr4b*, enables individual cells within the primordium to orient toward the tail. In this model, *apc^{mcr}* and SU5402-treated primordia fail to migrate because all cells possess the same chemokine receptor expression, and the ability to generate

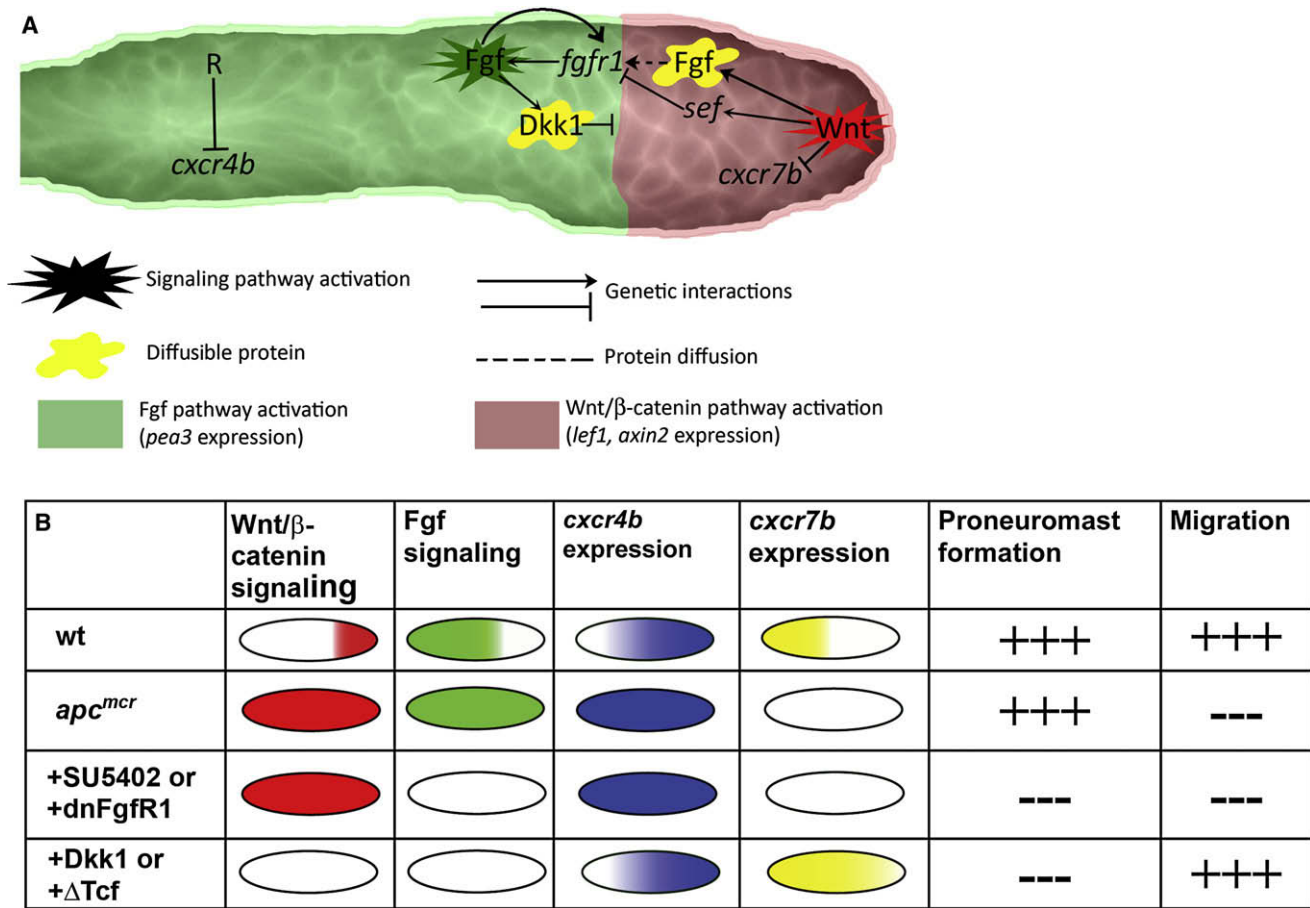


Figure 7. Schematic Models of Genetic Interactions between Signaling Pathways and Gene Expression Patterns in WT and Manipulated Primordia

(A) In WT primordia, Wnt/β-catenin pathway activation in the leading zone leads to Fgf pathway activation in the trailing zone. Exclusivity of these domains is maintained by the induction of *dkk1* by Fgf signaling in trailing cells and induction of *sef* by Wnt/β-catenin signaling in leading cells. *cxcr7b* expression in leading cells is inhibited by Wnt/β-catenin signaling, and *cxcr4b* expression is restricted from the trailing zone via the activity of an uncharacterized repressor (R) that is inhibited if Wnt/β-catenin signaling is active throughout the primordium.

(B) Summary of gene expression patterns and associated phenotypes in the different experimental manipulations.

an Sdf1a protein gradient across any individual cell is lost. It is intriguing that tip cells continue to attempt directional migration long after trailing cells have begun tumbling in *apc^{mcr}* and SU5402-treated embryos. We believe that *apc^{mcr}* cells behaviorally and genetically resemble WT cells, as they express high levels of *cxcr4b* and lack *cxcr7*. As tip cells in *apc^{mcr}* mutant embryos cannot pull trailing cells, this demonstrates that tip cells are not the only force-generating cells in the primordium, which is supported by live observations of individually labeled trailing cells (Haas and Gilmour, 2006).

Our data are consistent with Cxcr7b acting as an Sdf1a sink, and we have demonstrated that Wnt pathway activation is necessary for restricting Cxcr7b to trailing cells, where it could act to fine tune the Sdf1a gradient. Alternatively, Cxcr7b could be activating an intracellular signaling pathway in trailing cells that is triggered by a secondary guidance signal produced by the tip cells to coordinate directed migration. This model was suggested by Haas and Gilmour (2006) and is based on their finding that a few WT tip cells can rescue the migration of *cxcr4b*-nega-

tive trailing cells. Future detailed genetic and biochemical analysis of the characteristics and binding partners of *cxcr7b* will determine which model or combination of models is correct.

The Wnt/β-Catenin/Fgf Feedback Loop Coordinates Primordium Migration with Proneuromast Formation

Two studies investigating how loss of Fgf signaling affects proneuromast formation (rosetogenesis) found a tight correlation between rosetogenesis and migration. They concluded that normally formed rosettes are a prerequisite for primordium migration (Lecaudey et al., 2008; Nechiporuk and Raible, 2008). However, our experiments demonstrate that the loss of Wnt/β-catenin signaling, secondarily causing the loss of Fgf signaling in *Tg(hs:Dkk1)* and *Tg(hsΔTCF:GFP)* embryos, does not cause primordium migration defects, even though it completely eliminates neuromast formation and deposition (Figures 4 and 7B; Figures S8 and S9; Movie S8) Therefore, neuromast formation and primordium migration are not interdependent. Our analyses revealed that the difference between SU5402-treated

embryos and *Tg(hs:Dkk1)* embryos is that in SU5402-treated embryos Wnt/ β -catenin signaling is still active, whereas in *Tg(hs:Dkk1)* embryos both Fgf and Wnt/ β -catenin signaling are abolished. Thus, the primary cause of migration defects in SU5402-treated embryos is not the lack of rosettes, but the concurrent ectopic activation of Wnt/ β -catenin signaling in trailing cells, causing the loss of *cxcr7b* and ectopic *cxcr4b* expression (Figure 7B). Migration in *Tg(hs:Dkk1)* embryos is not affected, as *cxcr7b* is present in trailing cells and *cxcr4b* is unaffected. Comparing the effects of Wnt/ β -catenin signaling manipulations on primordium behavior reveals that migration can occur in the absence of Wnt/ β -catenin signaling, but that it is disrupted in the presence of ectopic Wnt/ β -catenin signaling in trailing cells.

Importantly, the comparison between *apc^{mcr}*, SU5402-treated *Tg(hs:Dkk1)* embryos, and heat-shocked *Tg(hs:Dkk1)* embryos revealed that the Wnt/ β -catenin/Fgf feedback loop in the primordium coordinates both migration and proneuromast formation by setting up primordium polarity via restriction of Wnt/ β -catenin signaling to the leading zone, and by restriction of Fgf signaling to the trailing zone, where Fgf signaling initiates neurogenesis.

Nature of β -Catenin Signaling Pathway Activation

The identity of the ligand(s) that activates the β -catenin pathway in this system remains elusive. The best-known activators of the β -catenin signaling pathway are secreted Wnt ligands; however, this pathway can also be activated by other factors, such as ligands binding to G protein-coupled receptors (Shevtsov et al., 2006). Since the Sdf1a belongs to this class of proteins, this signaling pathway presented an interesting candidate for involvement in the regulation of β -catenin in the primordium. However, morpholino knockdown of Sdf1a does not cause any changes in the expression of the β -catenin target gene *axin2* (Figures S12A and S12B). Importantly, depleting Dkk1 protein, which normally binds and inactivates the LRP family of Wnt receptors, causes upregulation of *axin2* and *lef1* (Figures 5G and 5H). Likewise, inhibiting Wnt signaling by heat shock induction of *dkk1* leads to the loss of these two genes (Figures 1T and 1W). Combined, these data reveal that the β -catenin signaling pathway must be activated by one or several canonical Wnt ligands. Wnt ligands could be locally produced within the leading zone of the primordium, they could be ubiquitously expressed in the environment, or they could be dynamically expressed surrounding the migrating leading zone of the primordium.

Collective Cell Migration in Cancer

This work may have important implications for the spread of cancer cells. Several types of cancers invade surrounding tissue as collectives rather than as individual cells (Friedl, 2004; Friedl et al., 1995, 2004). The process of collective cell migration in cancer is still not well understood, but several studies indicate that clusters of cancer cells might be polarized similarly to the lateral line primordium (Hegerfeldt et al., 2002; Wolf et al., 2007). It is likely that, just like in the lateral line primordium, opposing signaling pathways interact to set up this polarity.

CXCR4-SDF1 signaling is known to play a crucial role in the spread of many types of cancer, and the work described here provides functional connections between this signaling cassette and the Wnt/ β -catenin and Fgf signaling pathways, both of which

are implicated in various steps of tumorigenesis (Polakis, 2000; Kwabi-Addo et al., 2004).

Since upregulation of Wnt signaling is commonly associated with tissue invasion and metastasis, it appears counterintuitive that *apc* mutant cells in the lateral line fail to migrate. However, APC mutant cells in the colon exhibit migration defects and fail to migrate from the base of the crypt toward the tip of the villus, where they would normally be shaved off into the lumen of the gut (Radtko and Clevers, 2005). CXCR4 is expressed in normal and transformed colon cells, but whether this compromised migration of APC mutant cells is caused by misregulation of chemokine signaling remains to be investigated (Jordan et al., 1999; Kim et al., 2005).

Irrespective of whether the signaling pathways employed to set up tissue polarity are identical between lateral line primordia and cancers, our findings provide an important conceptual framework for elucidating how interacting signaling pathways might control directional migration of clusters of cancer cells.

Self Organization within the Migrating Primordium

The migrating primordium is a highly dynamic tissue. Cells are dividing along the whole axis of the primordium, and clusters of cells constituting ~10%–20% of the migrating tissue are dropped off from the trailing zone at regular intervals (Laguerre et al., 2005). Therefore, the identity of the trailing and leading zones must be continuously reinforced. In addition, the primordium encounters tissues with strikingly different positional identities during its head-to-tail migration. Because of this dynamism, a self-organized, tissue-autonomous signaling network is required to pattern the primordium. The model proposed here provides a mechanism whereby the primordium can be stably patterned, even though the relative location of individual cells is constantly changing via intercalary cell divisions and proneuromast deposition. The biological logic of the system, if not the molecules, may be found in other examples of morphogenesis involving highly dynamic organ anlagen.

EXPERIMENTAL PROCEDURES

Fish Strains

Time-lapse recordings were made by using *Tg(Cldnb:lynGFP)* embryos, gift from D. Gilmour (Haas and Gilmour, 2006). Hair cells were visualized by using *Tg(Brn3c:GAP43-GFP)^{s356t}* embryos (Xiao et al., 2005). *apc^{mcr}* mutants were a gift from H. Clevers (Hurlstone et al., 2003). *Tg(hs:Dkk1)* embryos were employed to inhibit Wnt/ β -catenin signaling activation (Stoick-Cooper et al., 2007). *Tg(hs Δ TCF:GFP)* embryos were used to confirm Wnt/ β -catenin reduction phenotypes (Lewis et al., 2004). *Tg(hsp70l:dnfgr1-EGFP)pd1* embryos were used to confirm the SU5402 selectivity treatments (Lee et al., 2005).

In Situ Hybridization

Hybridization and staining were performed as described (Kopinke et al., 2006). In situ probes used: *sef*, *fgf3* (Kudoh et al., 2001), *cxcr4b*, *cxcr7b* (Dambly-Chaudiere et al., 2007), *lef1* (Dorsky et al., 2003), *eya1* (Sahly et al., 1999), *pea3* (Munchberg et al., 1999), *kif4 (bik1f)* (Kudoh et al., 2001), *fgf10* (Ng et al., 2002), *axin2* (gift from R. Dorsky), and *dkk1* (gift from C. Houart). Embryos were mounted in 100% glycerol. Images were taken with an Axiocam camera mounted on a Zeiss Axioskop 2 plus microscope.

SU5402 Treatments

SU5402 (Calbiochem; gift from M. Brand) (Mohammadi et al., 1997) was diluted to 25 μ M in E3 medium containing 1% DMSO. Dechorionated embryos

were incubated from 18–20 hpf to 36–38 hpf. The effectiveness of Fgf signaling inhibition was confirmed by loss of *pea3* expression. Treated embryos were washed several times in E3 prior to fixation. Soaking embryos in 1% DMSO only did not cause a phenotype.

Hs Induction of *dkk1*, *Δtcf*, and *dnFgfR1*

Heterozygous fish were crossed to WT animals. Offspring were incubated at 42°C for 1 hr at various stages and were fixed several hours later depending on the experiment. A total of 50% of the embryos did not carry the transgene and served as a control. Effective inhibition of Wnt/β-catenin signaling was confirmed by loss of *axin2* and *lef1* expression from the primordia of heat-shocked *dkk1* and *Δtcf* embryos. Effective inhibition of FgfR1 was confirmed by loss of *pea3* expression.

Morpholino Injections

MO-*dkk1*, with a sequence of 5'-GAGAGCATGGCGATGTGCATCATGT-3', was used (Open Biosystems) (Seilliez et al., 2006). A 2 mM solution (1 nl) was injected. This dose of morpholino yielded a spectrum of phenotypes identical to those reported, including reduction or loss of anterior sensory organs. MO-*il17rd*, with a sequence of 5'-CGCAAGTCTCCGTGACCCAGCCATT-3', was used (Open Biosystems) (Asai et al., 2006). A 3 mM solution (1 nl) was injected. MO2-*cxc112a*, with a sequence of 5'-ATCACTTTGAGATCCATGTTTGA-3', was used (Open Biosystems) (David et al., 2002). A 2 mM solution (1 nl) was used. This dose recapitulated the published phenotype. Morpholinos were diluted in 0.1 M KCl and 5% phenol red in water.

Apc Rescue Experiment

apc^{mcr} rescue was performed as described (Hurlstone et al., 2003; Miller and Moon, 1997). mRNA was synthesized from a construct containing a central fragment of the human APC gene (aa 1020–2032) (Hurlstone et al., 2003; Miller and Moon, 1997). Injected 72 hpf embryos were assayed for hair cell phenotypes by soaking them in 2 mg/ml DASPEI ([2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide]; Invitrogen) for 10 min (Whitfield et al., 1996). The significance of the rescue effect was tested by using a test of difference in population proportions. To assay the rescue in individual mutants, individual blastomeres of 8- to 16-cell-stage embryos were injected. This resulted in the production of *apc^{mcr}* homozygotes with unilateral rescue assayed by *eya1* in situ.

Transplantation Experiments

Tg(Cldnb:lynGFP) or *Tg(Cldnb:lynGFP);apc^{mcr}* donor embryos were injected with 5% Alexa568 and 3% lysine-fixable biotinylated-dextran (Invitrogen) at the one- to two-cell stage (diluted in 0.2 mM KCl). Cells were transplanted into the presumptive placode region (Kozlowski et al., 1997) of *Tg(Cldnb:lynGFP)* or *Tg(Cldnb:lynGFP);apc^{mcr}* between 30% and 50% epiboly. Host embryos were screened for lateral line clones and were fixed at 36–40 hpf. For *apc^{mcr}* to WT transplantations, donor embryos were raised to 36–40 hpf for genotyping.

SUPPLEMENTAL DATA

Supplemental Data include twelve figures, eight movies, and supplemental Experimental Procedures and can be found with this article online at <http://www.developmentalcell.com/cgi/content/full/15/5/749/DC1/>.

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REFERENCES

- Asai, Y., Chan, D.K., Starr, C.J., Kappler, J.A., Kollmar, R., and Hudspeth, A.J. (2006). Mutation of the atrophin2 gene in the zebrafish disrupts signaling by fibroblast growth factor during development of the inner ear. *Proc. Natl. Acad. Sci. USA* *103*, 9069–9074.
- Barth, A.I., Siemers, K.A., and Nelson, W.J. (2002). Dissecting interactions between EB1, microtubules and APC in cortical clusters at the plasma membrane. *J. Cell Sci.* *115*, 1583–1590.
- Bienz, M. (2002). The subcellular destinations of APC proteins. *Nat. Rev. Mol. Cell Biol.* *3*, 328–338.
- Boldajipour, B., Mahabaleswar, H., Kardash, E., Reichman-Fried, M., Blaser, H., Minina, S., Wilson, D., Xu, Q., and Raz, E. (2008). Control of chemokine-guided cell migration by ligand sequestration. *Cell* *132*, 463–473.
- Chamorro, M.N., Schwartz, D.R., Vonica, A., Brivanlou, A.H., Cho, K.R., and Varnus, H.E. (2005). FGF-20 and DKK1 are transcriptional targets of β-catenin and FGF-20 is implicated in cancer and development. *EMBO J.* *24*, 73–84.
- Dambly-Chaudiere, C., Cubedo, N., and Ghysen, A. (2007). 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.* *7*, 23.
- David, N.B., Sapede, D., Saint-Etienne, L., Thisse, C., Thisse, B., Dambly-Chaudiere, C., Rosa, F.M., and Ghysen, A. (2002). 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. USA* *99*, 16297–16302.
- De Calisto, J., Araya, C., Marchant, L., Riaz, C.F., and Mayor, R. (2005). Essential role of non-canonical Wnt signalling in neural crest migration. *Development* *132*, 2587–2597.
- Dorsky, R.I., Itoh, M., Moon, R.T., and Chitnis, A. (2003). Two *tcf3* genes cooperate to pattern the zebrafish brain. *Development* *130*, 1937–1947.
- Friedl, P. (2004). Preshpecification and plasticity: shifting mechanisms of cell migration. *Curr. Opin. Cell Biol.* *16*, 14–23.
- Friedl, P., Noble, P.B., Walton, P.A., Laird, D.W., Chauvin, P.J., Tabah, R.J., Black, M., and Zanker, K.S. (1995). Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants in vitro. *Cancer Res.* *55*, 4557–4560.
- Friedl, P., Hegerfeldt, Y., and Tusch, M. (2004). Collective cell migration in morphogenesis and cancer. *Int. J. Dev. Biol.* *48*, 441–449.
- Furthauer, M., Reifers, F., Brand, M., Thisse, B., and Thisse, C. (2001). *sprouty4* acts in vivo as a feedback-induced antagonist of FGF signaling in zebrafish. *Development* *128*, 2175–2186.
- Furthauer, M., Lin, W., Ang, S.L., Thisse, B., and Thisse, C. (2002). Sef is a feedback-induced antagonist of Ras/MAPK-mediated FGF signalling. *Nat. Cell Biol.* *4*, 170–174.
- Ghysen, A., and Dambly-Chaudiere, C. (2004). Development of the zebrafish lateral line. *Curr. Opin. Neurobiol.* *14*, 67–73.
- Gompel, N., Cubedo, N., Thisse, C., Thisse, B., Dambly-Chaudiere, C., and Ghysen, A. (2001). Pattern formation in the lateral line of zebrafish. *Mech. Dev.* *105*, 69–77.
- Haas, P., and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev. Cell* *10*, 673–680.
- Hegerfeldt, Y., Tusch, M., Brocker, E.B., and Friedl, P. (2002). Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, β1-integrin function, and migration strategies. *Cancer Res.* *62*, 2125–2130.
- Hurlstone, A.F., Haramis, A.P., Wienholds, E., Begthel, H., Korving, J., Van Eeden, F., Cuppen, E., Zivkovic, D., Plasterk, R.H., and Clevers, H. (2003). The Wnt/β-catenin pathway regulates cardiac valve formation. *Nature* *425*, 633–637.
- Itoh, M., and Chitnis, A.B. (2001). Expression of proneural and neurogenic genes in the zebrafish lateral line primordium correlates with selection of hair cell fate in neuromasts. *Mech. Dev.* *102*, 263–266.

- Jordan, N.J., Kolios, G., Abbot, S.E., Sinai, M.A., Thompson, D.A., Petraki, K., and Westwick, J. (1999). Expression of functional CXCR4 chemokine receptors on human colonic epithelial cells. *J. Clin. Invest.* *104*, 1061–1069.
- Kim, J., Takeuchi, H., Lam, S.T., Turner, R.R., Wang, H.J., Kuo, C., Foshag, L., Bilchik, A.J., and Hoon, D.S. (2005). Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. *J. Clin. Oncol.* *23*, 2744–2753.
- Kinzler, K.W., and Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. *Cell* *87*, 159–170.
- Kopinke, D., Sasine, J., Swift, J., Stephens, W.Z., and Piotrowski, T. (2006). Retinoic acid is required for endodermal pouch morphogenesis and not for pharyngeal endoderm specification. *Dev. Dyn.* *235*, 2695–2709.
- Kozłowski, D.J., Murakami, T., Ho, R.K., and Weinberg, E.S. (1997). Regional cell movement and tissue patterning in the zebrafish embryo revealed by fate mapping with caged fluorescein. *Biochem. Cell Biol.* *75*, 551–562.
- Kroboth, K., Newton, I.P., Kita, K., Dikovskaya, D., Zumbunn, J., Waterman-Storer, C.M., and Nathke, I.S. (2007). Lack of adenomatous polyposis coli protein correlates with a decrease in cell migration and overall changes in microtubule stability. *Mol. Biol. Cell* *18*, 910–918.
- Kudoh, T., Tsang, M., Hukriede, N.A., Chen, X., Dedekian, M., Clarke, C.J., Kiang, A., Schultz, S., Epstein, J.A., Toyama, R., et al. (2001). A gene expression screen in zebrafish embryogenesis. *Genome Res.* *11*, 1979–1987.
- Kwabi-Addo, B., Ozen, M., and Ittmann, M. (2004). The role of fibroblast growth factors and their receptors in prostate cancer. *Endocr. Relat. Cancer* *11*, 709–724.
- Laguerre, L., Soubiran, F., Ghysen, A., König, N., and Dambly-Chaudière, C. (2005). Cell proliferation in the developing lateral line system of zebrafish embryos. *Dev. Dyn.* *233*, 466–472.
- Lecaudey, V., Cakan-Akdogan, G., Norton, W.H., and Gilmour, D. (2008). Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* *135*, 2695–2705.
- Lee, Y., Grill, S., Sanchez, A., Murphy-Ryan, M., and Poss, K.D. (2005). Fgf signaling instructs position-dependent growth rate during zebrafish fin regeneration. *Development* *132*, 5173–5183.
- Lewis, J.L., Bonner, J., Modrell, M., Ragland, J.W., Moon, R.T., Dorsky, R.I., and Raible, D.W. (2004). Reiterated Wnt signaling during zebrafish neural crest development. *Development* *131*, 1299–1308.
- Li, Q., Shirabe, K., and Kuwada, J.Y. (2004). Chemokine signaling regulates sensory cell migration in zebrafish. *Dev. Biol.* *269*, 123–136.
- Martin, P., and Parkhurst, S.M. (2004). Parallels between tissue repair and embryo morphogenesis. *Development* *131*, 3021–3034.
- Metcalfe, W.K., Kimmel, C.B., and Schabtach, E. (1985). Anatomy of the posterior lateral line system in young larvae of the zebrafish. *J. Comp. Neurol.* *233*, 377–389.
- Miller, J.R., and Moon, R.T. (1997). Analysis of the signaling activities of localization mutants of β -catenin during axis specification in *Xenopus*. *J. Cell Biol.* *139*, 229–243.
- Millimaki, B.B., Sweet, E.M., Dhason, M.S., and Riley, B.B. (2007). Zebrafish *atoh1* genes: classic proneural activity in the inner ear and regulation by Fgf and Notch. *Development* *134*, 295–305.
- Mohammadi, M., McMahon, G., Sun, L., Tang, C., Hirth, P., Yeh, B.K., Hubbard, S.R., and Schlessinger, J. (1997). Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. *Science* *276*, 955–960.
- Montell, D.J. (2006). The social lives of migrating cells in *Drosophila*. *Curr. Opin. Genet. Dev.* *16*, 374–383.
- Munchberg, S.R., Ober, E.A., and Steinbeisser, H. (1999). Expression of the Ets transcription factors *erm* and *pea3* in early zebrafish development. *Mech. Dev.* *88*, 233–236.
- Nathke, I. (2005). Relationship between the role of the adenomatous polyposis coli protein in colon cancer and its contribution to cytoskeletal regulation. *Biochem. Soc. Trans.* *33*, 694–697.
- Nechiporuk, A., and Raible, D.W. (2008). FGF-dependent mechanosensory organ patterning in zebrafish. *Science* *320*, 1774–1777.
- Ng, J.K., Kawakami, Y., Buscher, D., Raya, A., Itoh, T., Koth, C.M., Rodriguez Esteban, C., Rodriguez-Leon, J., Garrity, D.M., Fishman, M.C., et al. (2002). The limb identity gene *Tbx5* promotes limb initiation by interacting with *Wnt2b* and *Fgf10*. *Development* *129*, 5161–5170.
- Niehrs, C. (2006). Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* *25*, 7469–7481.
- Northcutt, R.G., Brandle, K., and Fritzsche, B. (1995). Electroreceptors and mechanosensory lateral line organs arise from single placodes in axolotls. *Dev. Biol.* *168*, 358–373.
- Peifer, M., and Polakis, P. (2000). Wnt signaling in oncogenesis and embryogenesis—a look outside the nucleus. *Science* *287*, 1606–1609.
- Platt, J.B. (1896). Ontogenetic differentiations of the ectoderm in neurus. *Quart. J. Microsc. Vac.* *38*, 486–547.
- Polakis, P. (2000). Wnt signaling and cancer. *Genes Dev.* *14*, 1837–1851.
- Radtke, F., and Clevers, H. (2005). Self-renewal and cancer of the gut: two sides of a coin. *Science* *307*, 1904–1909.
- Raible, F., and Brand, M. (2001). Tight transcriptional control of the ETS domain factors *Erm* and *Pea3* by Fgf signaling during early zebrafish development. *Mech. Dev.* *107*, 105–117.
- Redd, M.J., Kelly, G., Dunn, G., Way, M., and Martin, P. (2006). Imaging macrophage chemotaxis in vivo: studies of microtubule function in zebrafish wound inflammation. *Cell Motil. Cytoskeleton* *63*, 415–422.
- Reya, T., and Clevers, H. (2005). Wnt signalling in stem cells and cancer. *Nature* *434*, 843–850.
- Roehl, H., and Nusslein-Volhard, C. (2001). Zebrafish *pea3* and *erm* are general targets of FGF8 signaling. *Curr. Biol.* *11*, 503–507.
- Rorth, P. (2007). Collective guidance of collective cell migration. *Trends Cell Biol.* *17*, 575–579.
- Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S., and Polakis, P. (1996). Binding of GSK3 β to the APC- β -catenin complex and regulation of complex assembly. *Science* *272*, 1023–1026.
- Sahly, I., Andermann, P., and Petit, C. (1999). The zebrafish *eya1* gene and its expression pattern during embryogenesis. *Dev. Genes Evol.* *209*, 399–410.
- Schmidt, M., Paes, K., De Maziere, A., Smyczek, T., Yang, S., Gray, A., French, D., Kasman, I., Klumperman, J., Rice, D.S., et al. (2007). EGFL7 regulates the collective migration of endothelial cells by restricting their spatial distribution. *Development* *134*, 2913–2923.
- Schulze, F.E. (1861). Ueber die Nervenendigung in den sogenannten Schleimkanaelen der Fische und ueber entsprechende Organe der durch Kiemen atmenden Amphibien. *Arch. Anat. Physiol.* *3*, 759–769.
- Selliez, I., Thisse, B., and Thisse, C. (2006). FoxA3 and gooseoid promote anterior neural fate through inhibition of Wnt8a activity before the onset of gastrulation. *Dev. Biol.* *290*, 152–163.
- Shevtsov, S.P., Haq, S., and Force, T. (2006). Activation of β -catenin signaling pathways by classical G-protein-coupled receptors: mechanisms and consequences in cycling and non-cycling cells. *Cell Cycle* *5*, 2295–2300.
- Smith, K.J., Levy, D.B., Maupin, P., Pollard, T.D., Vogelstein, B., and Kinzler, K.W. (1994). Wild-type but not mutant APC associates with the microtubule cytoskeleton. *Cancer Res.* *54*, 3672–3675.
- Solnica-Krezel, L. (2006). Gastrulation in zebrafish—all just about adhesion? *Curr. Opin. Genet. Dev.* *16*, 433–441.
- Stoick-Cooper, C.L., Weidinger, G., Riehle, K.J., Hubbert, C., Major, M.B., Fausto, N., and Moon, R.T. (2007). Distinct Wnt signaling pathways have opposing roles in appendage regeneration. *Development* *134*, 479–489.
- Stone, L.S. (1922). Experiments on the development of the cranial ganglia and the lateral line sense organs in *Amblystoma punctatum*. *J. Exp. Zool.* *35*, 421–496.
- Stone, L.S. (1923). On rotation of the lateral line sense organs in *Amblystoma punctatum*. *Abst. Proc. Am. Assoc. Anat. Rec.* *25*, 114.
- Tsang, M., Friesel, R., Kudoh, T., and Dawid, I.B. (2002). Identification of Sef, a novel modulator of FGF signalling. *Nat. Cell Biol.* *4*, 165–169.

Tsang, M., Maegawa, S., Kiang, A., Habas, R., Weinberg, E., and Dawid, I.B. (2004). A role for MKP3 in axial patterning of the zebrafish embryo. *Development* *131*, 2769–2779.

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

Whitfield, T.T., Granato, M., van Eeden, F.J., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.P., Jiang, Y.J., et al. (1996). Mutations affecting development of the zebrafish inner ear and lateral line. *Development* *123*, 241–254.

Wolf, K., Wu, Y.I., Liu, Y., Geiger, J., Tam, E., Overall, C., Stack, M.S., and Friedl, P. (2007). Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. *Nat. Cell Biol.* *9*, 893–904.

Xiao, T., Roeser, T., Staub, W., and Baier, H. (2005). A GFP-based genetic screen reveals mutations that disrupt the architecture of the zebrafish retinotectal projection. *Development* *132*, 2955–2967.

Zumbrunn, J., Kinoshita, K., Hyman, A.A., and Nathke, I.S. (2001). Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 β phosphorylation. *Curr. Biol.* *11*, 44–49.