Published in final edited form as:

Curr Opin Genet Dev. 2013 August ; 23(4): 438–444. doi:10.1016/j.gde.2013.05.003.

Planar Cell Polarity in vertebrate limb morphogenesis

Bo Gao and Yingzi Yang

National Human Genome Research Institute, Bethesda, MD 20892

Abstract

Studies of the vertebrate limb development have contributed significantly to understanding the fundamental mechanisms underlying growth, patterning and morphogenesis of a complex multicellular organism. In the limb, well-defined signaling centers interact to coordinate limb growth and patterning along the three axes. Recent analyses of live imaging and mathematical modeling have provided evidence that polarized cell behaviors governed by morphogen gradients play an important role in shaping the limb bud. Furthermore, the Wnt/Planar Cell Polarity (PCP) pathway that controls uniformly polarized cellular behaviors in a field of cells has emerged to be critical for directional morphogenesis in the developing limb. Directional information coded in the morphogen gradient may be interpreted by responding cells through regulating the activities of PCP components in a Wnt morphogen dose-dependent manner.

Introduction

The vertebrate developing limb is one of the models that have been investigated extensively to understand how a complex three-dimensional structure form from initially homogeneous cells. The embryonic precursor of a limb, the limb bud, is composed of a mesenchyme core covered by surface ectoderm. Limb buds grow along three axes: proximal-distal (P-D, from shoulder to digit tip), anterior-posterior (A-P, from thumb to little finger) and dorsal-ventral (D-V, from back of hand to palm). As limbs exhibit characteristic morphologies and contain well defined signaling centers that each primarily controls limb development along one of the three axes, the developing limb bud provides a unique model system to identify the underlying mechanisms by which growth and patterning are controlled and coordinated by cell-cell signaling. Soon after the limb bud forms, a thickened ectodermal structure, the apical ectodermal ridge (AER), arises at its distal tip. *Fibroblast growth factor* (*Fgf*) family members expressed in the AER pattern the P-D axis by antagonizing retinoic acid (RA) signaling from the proximal limb [1,2]. Removing the AER or *Fgfs* expressed in the AER shortens the P-D axis resulting in limb truncation [3,4]. The dorsal ectoderm-derived *Wnt7a* specifies the D-V axis by inducing the expression of *Lmx1b* in the dorsal limb mesenchyme [5]. The signaling center patterning the A-P axis is the zone of polarizing activity (ZPA). The ZPA is a group of mesenchymal cells located at the posterior limb bud margin that patterns the A-P axis by secreting Sonic hedgehog (Shh). These signaling centers interact to coordinate three-dimensional limb bud growth and patterning [6,7]. However, in the regulation of growth (cell proliferation and survival) and patterning (cell fate determination in a temporal and spatial order), much less is understood about how directional information

© 2013 Published by Elsevier Ltd.

Corresponding author: Yingzi Yang (yingzi@mail.nih.gov), Tel: 301-402-2034, Fax: 301-402-2170.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

is provided such that directional limb growth and patterning (i.e., preferential limb elongation along the P-D axis) is achieved.

Early studies in 1977–1980 by Holmes and Trelstad demonstrated that limb mesenchymal cells involved in cartilage morphogenesis are both structurally and functionally polarized by measuring the axes of Golgi-nucleus [8–10]. However, the underlying mechanisms were unknown before the molecular and genetic tools became available. In order to achieve the uniform polarity, limb mesenchymal cells must employ a mechanism that can establish and coordinate polarized cell behaviors with respect to their developmental axes. Recent studies have found that the Wnt/Planar Cell Polarity (PCP) signaling pathway, which has emerged to be critical for many fundamental developmental and physiological processes, plays a key role in limb P-D elongation. PCP originally refers to the collective polarization of cells within the epithelial plane and has been further extended to mesenchymal cells. In vertebrates, Wnt morphogen has been implicated in regulating PCP in mesenchymal cells. Here, we review recent advances on how polarized cell behaviors are regulated in developing limb and highlight the role of Wnt/PCP in this process.

Polarized cell behaviors in limb development

In development, organs and tissues form with characteristic architecture, which requires asymmetrical or polarized cellular behaviors. A prominent example of these is preferential elongation of the limb along the P-D axis. In theory, such a directional process can be regulated by several distinct mechanisms. For instance, for a long time, it was thought that P-D limb elongation is the net result of anisotropic growth driven by a graded proliferation rate along the P-D axis with higher proliferation in the distal mesenchyme [11]. Therefore, it had been proposed that Fgfs secreted from the AER set up this mitogenic gradient by inducing downstream mitogen-activated protein kinase (MAPK) signal cascade [12–14]. However, both the notion of “growth-based morphogenesis” and the role of Fgfs in regulating graded proliferation have been challenged recently [3,15–17]. Mathematical modeling using data acquired from dynamic imaging of the developing limb bud and measuring the spatial distribution of cell proliferation strongly suggested that directional cell behaviors, rather than graded proliferation, are major driving forces for the formation of a distally extending limb bud [18]. In addition, removal of *Fgf4* and *Fgf8*, which are major Fgfs in the AER [3], leads to much reduced limb bud elongation without significant effect on cell proliferation or survival in the distal limb mesenchyme, suggesting that Fgfs have additional roles in the limb bud [3]. Indeed, an early study in the chick limb bud demonstrated that Fgf4 could function as a chemoattractant to re-orient mesenchymal cells toward an ectopic source of Fgf4, suggesting that cells may migrate distally towards the AER where Fgfs are highly expressed [19].

Two recent studies closely examined polarized cell behaviors at different stages of the developing limb buds [20,21]. By live imaging and lineage tracing in mouse, and chick limbs or zebrafish fins, Wyngaarden et al investigated early stage of limb bud initiation and found that oriented cell migration and division contribute to limb bud formation from the lateral plate. Importantly, they found that only beads soaked in Wnt5a, not Fgf8, Shh or RA, attract lateral plate mesoderm (LPM) cells to move toward the beads [20]. Similar observation has been made in the mouse developing palate, in which both Wnt5a and Fgf10 act as potent chemoattractants for palate mesenchymal cell migration [22]. However, Gros et al found Wnt5a, not Fgf8, is able to orient cell movement and division by transplanting Fgf8-soaked beads or ectopically expressing *Wnt5a* in the early formed chick limb bud. Fgf8 promotes cells to move with greater velocity, but random direction. Therefore, they proposed a model of coordinated but distinct effects of Wnt5a and Fgf8 in shaping the limb bud, in which a Wnt5a gradient chemoattracts mesenchymal cells toward the distal limb

bud, while Fgf8 signaling establishes a gradient of cell velocity along the P-D axis [21]. Similar to the elongating limb bud, whole body elongation along the A-P axis (from head to tail) also requires Wnt5a and Fgfs. While the exact mechanism by which Wnt5a controls A-P body axis extension is unclear, Fgfs have been demonstrated to drive body axis elongation by setting up a posterior-to-anterior gradient of cell motility with no directionality when taking extracellular matrix as a stationary reference [23]. Taken together, it is clear that Fgf and Wnt5a signaling play key roles in regulating polarized cell behaviors, including directional cell migration and division during limb morphogenesis. However, fundamental questions such as the molecular mechanism whereby Wnt5a and Fgf regulate polarized cell behaviors still remain to be answered.

An emerging mechanism critical for directional morphogenesis is Planar Cell Polarity (PCP), which was originally identified as a type of uniform polarity of a large number of epithelial cells within the plane orthogonal to their apical-basal axis. Now PCP has been found in mesenchymal cells as well [24]. More importantly, Wnt5a has been implicated in regulating PCP in vertebrates [25,26].

A branch of Wnt signaling: Planar Cell Polarity (PCP)

PCP has been most extensively studied in *Drosophila*, in which it typically controls the proximal-to-distal orientation of bristles in the wing and crystal-like organization of ommatidia in the compound eye [27,28]. In vertebrates, PCP is essential to regulate A-P body axis elongation through a process called convergent extension in which mesenchymal cells intercalate with each other and move toward the midline such that the cells converge along the mediolateral (M-L) axis forcing the body axis to extend along the A-P axis [29,30]. Interestingly, PCP can control oriented cell division in this process [31]. In mammals, the roles of PCP have been further expanded and PCP is a fundamental mechanism in development. It is required for the regulation of neural tube closure, orientation of sensory hair cells in the inner ear, determination of left-right asymmetry, axon guidance, organogenesis and wound healing [24,29,32]. The significance of PCP in development and other biological processes is further demonstrated by the increasing number of PCP mutations identified in various human diseases in recent years [33]. PCP is highly conserved molecularly from fly to mammals. The aforementioned developmental processes in different organisms are largely controlled by the same set of core PCP proteins, which include a seven-pass transmembrane protein Frizzled (Fz), its associated cytoplasmic protein Dishevelled (Dvl) and Diego (Dgo), a four-pass transmembrane protein Van Gogh (Vang), a cytoplasmic protein Prickle (Pk) that interacts with Vang, and a seven-pass transmembrane cadherin-like protein Flamingo (Fmi). Prior to the establishment of PCP, these proteins are distributed randomly on the cell membrane. Once cells display uniform planar polarity across the tissue, Fz, Dsh and Dgo accumulate on one side of the cell, while Vang and Pk preferentially locate to the opposite side. Fmi co-localizes with both groups of proteins (Figure 1) [29,32]. Asymmetric localization of these proteins is considered to be a molecular readout of established PCP. Mutations in any one of these proteins leads to disruption of PCP.

A distinct character of PCP is that a large group of cells are uniformly polarized in the same direction with respect to the body axis. Obviously, a global cue is a strong candidate to convey directional information. Wnt morphogen gradients have been proposed to provide such a cue, largely because the Frizzled receptors are core PCP proteins [34,35]. Similar to the zebrafish *Vang* mutant *trilobite* (*tri*, *Vangl2*), the zebrafish *pipetail* (*ppt*, *Wnt5b*) and *silberblick* (*slb*, *Wnt11*) mutants both exhibit a broadened and shortened A-P body axis, indicating that convergent extension movement, a process regulated by PCP, requires Wnt signaling [25,36,37]. Furthermore, it has been demonstrated that Wnt5a and its receptor

Ror2 genetically interact with a core PCP protein, Vangl2 (Vang-like 2) in mammals [26,38]. Although current studies in *Drosophila* have not yet revealed a role of Wnts in PCP [34], the term of “Wnt/PCP” has been widely used in vertebrates.

Among the 19 different Wnts in mammals, the majority of them transduce their signals by stabilizing β -catenin in the canonical or Wnt/ β -catenin pathway. This pathway is fundamentally important and evolutionarily conserved in controlling embryonic development and adult physiology [39]. Wnt5a and Wnt11 do not stabilize β -catenin in most cases *in vivo*. They have been proposed to transduce their signals through multiple distinct pathways instead, which include inhibition of canonical Wnt signaling, Wnt/Calcium pathway and Wnt/PCP pathway [40]. Since context-dependent combination of Wnt receptors determines the activity of Wnts and pathway(s) Wnts transduce their signals, other Wnts have also been implicated in regulating PCP [41].

Frizzled receptors mediate Wnt signaling and they are also core PCP proteins. However, it is still largely unknown how Wnt signal is interpreted by a group of responding cells to initiate and establish PCP. Most mouse *Frizzled* mutants exhibit PCP defects [42–44], indicating that regulation of PCP by Frizzled receptors are dose-dependent and different Frizzled receptors share functional redundancy. Therefore, it would be a difficult task to dissect the highly redundant function of Frizzled in PCP and their other functions in the canonical Wnt pathway. In contrast, recent advances in understanding PCP regulation of vertebrate limb development have shown that Vang like (Vangl) proteins that have fewer family members and are dedicated to the PCP pathway allow us to address the signaling mechanism of PCP more easily.

Wnt/PCP in limb development

PCP has been implicated to play important roles in the developing limb by manipulating PCP components [45]. However, the first definitive demonstration of PCP is asymmetrical Vangl2 localization in the newly formed chondrocytes along the P-D limb axis (Figure 2) [38]. As cartilage elongation is the major driving force of limb elongation, it is not surprising that asymmetrical Vangl2 localization is most prominent in chondrocytes. As both *Wnt5a* and *Ror2* null mouse mutants exhibit loss of Vangl2 asymmetrical localization and shorter and broader cartilage phenotype, Wnt/PCP pathway is demonstrated to play an essential role in controlling cartilage P-D elongation in limb development [38,46–48]. Indeed, the dominant negative *loop-tail* mutation of *Vangl2* (*Vangl2^{Lp}*) leads to digit and cartilage defects resembling the phenotypes of human BDB (Brachydactyly type B) and RS (Robinow syndrome) patients [38,49], which are caused by *Wnt5a* and/or *Ror2* mutations [50–52]. If *Vangl1*, the other mammalian *Vang* homologue, is also deleted in the *Vangl2* null mutant, the limb defects are even more severe [53]. Furthermore, both *Wnt5a* and *Ror2* genetically interact with *Vangl2* as *Vangl2 Looptail (Lp)* mutant enhances the severity of the *Wnt5a* or *Ror2* mutant embryo, indicating they may function in the same pathway [26,38]. Interestingly, Wnt5a induces Ror2-Vangl2 receptor complex formation and *Ror2^{-/-}*; *Vangl2^{-/-}* double mutant phenocopies *Wnt5a^{-/-}* in the limb with failure of digits outgrowth and long bone elongation, further supporting the key role of Wnt5a/Ror2/PCP signaling pathway in limb morphogenesis [38].

How and when PCP is first established in the developing limb are fundamentally important questions. Functionally, Wnt5a acts early during both limb bud initiation and early limb bud growth by regulating asymmetrical cell behaviors [20,21]. It is likely that PCP signal is already induced in the early stages, but may have not been stabilized to allow asymmetrical localization of Vangl2. If the appearance of biased Vangl2 localization is the first sign of stable and strong PCP establishment, PCP is most strong in chondrocytes initially formed

between E11.5 and E12.5, as Vangl2 proteins distribute almost randomly throughout the mesenchymal cell membrane by E11.5 [38]. At E12.5, Vangl2 asymmetrical localization can be clearly observed in Sox9-positive chondrocytes, not in non-differentiated mesenchymal cells, likely because stabilization of PCP proteins requires close cell-cell contact, which is weaker in the loosed mesenchymal cells but stronger in the condensed mesenchymal cells that are differentiating into chondrocytes [38]. However, the role of PCP in polarized cell behaviors in earlier limb bud before E11.5 cannot be excluded for several reasons. First, Wnt5a has been shown to be critical in cell migration and division in earlier limb bud [20,21].

Whether PCP signaling affects these processes has not been tested directly in the early limb bud. Second, technically, subtle biased PCP protein localization, which may occur earlier, is difficult to detect. Third and most importantly, formation of asymmetrically localized core PCP proteins is a readout of PCP, but not the only indicator of active PCP signaling.

PCP proteins function to coordinate a field of cells and orient them uniformly through regulating intracellular cytoskeleton arrangement and intercellular interaction between neighboring cells. Such cell-cell interaction is reinforced and amplified by positive feedback loops, leading to the asymmetric localization of core PCP proteins, hence PCP establishment. Therefore, core proteins must conduct their functions prior to visible asymmetrical localization of core PCP proteins. In this regard, it is important to determine whether PCP is still required for limb development after establishment of asymmetrical localization of core PCP proteins.

Longitudinal growth of long bone mainly occurs through continual elongation of chondrocyte columns and subsequent chondrocyte hypertrophy, a process called endochondral ossification [54]. Columnar chondrocytes divide laterally, then daughter cells intercalate back into the original column of the parental cell, leading to longitudinal elongation of growth plate. Such a convergent extension-like process may be regulated by PCP [45]. Li et al. found that retrovirally expressed *Vangl2* or dominant-negative *Frizzled7* in the chick growth plate affects the division plane of proliferative chondrocytes, suggesting a role of PCP in columnar organization of chondrocytes during endochondral ossification [45]. Interestingly, Randall et al has shown that they could promote columnar organization of digested growth plate chondrocyte pellets *in vitro* by activating Wnt/PCP pathway components. Best columns formed with a combination of *Ror2*, *Frizzled7* and *Wnt5a* [55]. However, it is also possible that the severe long bone phenotype of *Wnt5a*^{-/-}, *Ror2*^{-/-}, or *Vangl1*^{-/-}; *Vangl2*^{-/-} might result from defective cartilaginous anlage formation caused by impaired PCP in earlier limbs, not its direct function in the growth plate. Therefore, rigorous genetic experiments are required to test these hypotheses.

As we mention above, uniform planar polarity across a large field of cells requires a global cue with respect to the body axis. In the developing limb, Wnt5a is the only Wnt morphogen that forms a gradient from the distal to proximal limb [56] and regulates PCP [38]. Thus, it is possible that mesenchymal cells in the developing limb bud gain directional information by interpreting the Wnt5a dosage change so that cartilage extends distally in the direction of the Wnt5a gradient. Biochemically, this maybe achieved through dose-dependent Vangl2 phosphorylation induced by Wnt5a and Ror2, suggesting an instructive role of Wnt5a in regulating PCP [38,49]. However, a permissive role of Wnt5a signaling in establishing PCP cannot be excluded prior to further rigorous genetic investigations. Taken together, Wnt5a/PCP signaling plays a key role in limb morphogenesis. Its exact functional mechanism at different developmental stages warrants further studies (Figure 3).

4. Conclusion

While the *Wnt5a* null mutant exhibited severe P-D elongation defects, the P-D axis is still established to some extent. In the early limb bud prior to cartilage formation, distal limb mesenchymal cells that are located adjacent to the AER still moved toward the overlying ectoderm albeit at reduced velocity and efficiency. In addition, the division orientation of these distal cells is normal [21]. This observation has been explained by AER-derived Fgfs signaling, which controls the velocity of randomly moving cells. Cells influenced by Fgfs move faster and eventually move closer to the AER through mass action [21]. A similar model had been also proposed in posterior elongation of the tail bud [23]. Such evidence raises an important question: Is Wnt5a the only morphogen providing an instructive cue to PCP in limb development? As planar polarity of limb mesenchymal cells is always perpendicular to the AER, the AER or Fgfs might interact with PCP, either directly as an alternative global cue or indirectly through maintaining the normal P-D limb patterning. Nonetheless, Wnt5a seems to be absolutely required for PCP establishment in developing limbs because *Wnt5a* null mutants have no sign of planar polarity [38]. Thus, the future challenge of PCP in limb morphogenesis is to dissect its functional mechanisms at different stages of development (Figure 3), distinguish the role of Wnt5a as an instructive cue or a permissive signal in PCP, and understand the crosstalk between Wnt5a and other signaling pathways in regulating PCP. In conclusion, recent advances in polarized cell behaviors have shed new light on how limb morphogenesis is achieved at the cellular and molecular level and also provided mechanistic insight into other developmental processes like tail bud elongation and craniofacial morphogenesis that also requires PCP.

Acknowledgments

The authors are supported by the Intramural Research Program of the National Human Genome Institute of the National Institutes of Health.

References and Recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Cooper KL, Hu JK, ten Berge D, Fernandez-Teran M, Ros MA, Tabin CJ. Initiation of proximal-distal patterning in the vertebrate limb by signals and growth. *Science*. 2011; 332:1083–1086. [PubMed: 21617075]
2. Rosello-Diez A, Ros MA, Torres M. Diffusible signals, not autonomous mechanisms, determine the main proximodistal limb subdivision. *Science*. 2011; 332:1086–1088. [PubMed: 21617076]
3. Sun X, Mariani FV, Martin GR. Functions of FGF signalling from the apical ectodermal ridge in limb development. *Nature*. 2002; 418:501–508. [PubMed: 12152071]
4. Saunders JW Jr. The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. *The Journal of experimental zoology*. 1948; 108:363–403. [PubMed: 18882505]
5. Riddle RD, Ensini M, Nelson C, Tsuchida T, Jessell TM, Tabin C. Induction of the LIM homeobox gene *Lmx1* by WNT7a establishes dorsoventral pattern in the vertebrate limb. *Cell*. 1995; 83:631–640. [PubMed: 7585966]
6. Towers M, Wolpert L, Tickle C. Gradients of signalling in the developing limb. *Current opinion in cell biology*. 2012; 24:181–187. [PubMed: 22169676]
7. Zeller R, Lopez-Rios J, Zuniga A. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. *Nature reviews Genetics*. 2009; 10:845–858.

8. Holmes LB, Trelstad RL. Patterns of cell polarity in the developing mouse limb. *Developmental biology*. 1977; 59:164–173. [PubMed: 892225]
9. Trelstad RL. Mesenchymal cell polarity and morphogenesis of chick cartilage. *Developmental biology*. 1977; 59:153–163. [PubMed: 561010]
10. Holmes LB, Trelstad RL. Cell polarity in precartilag mouse limb mesenchyme cells. *Developmental biology*. 1980; 78:511–520. [PubMed: 7409312]
11. Ede DA, Law JT. Computer simulation of vertebrate limb morphogenesis. *Nature*. 1969; 221:244–248. [PubMed: 5812579]
12. Hornbruch A, Wolpert L. Cell division in the early growth and morphogenesis of the chick limb. *Nature*. 1970; 226:764–766. [PubMed: 5443258]
13. Niswander L, Tickle C, Vogel A, Booth I, Martin GR. FGF-4 replaces the apical ectodermal ridge and directs outgrowth and patterning of the limb. *Cell*. 1993; 75:579–587. [PubMed: 8221896]
14. Reiter RS, Solorsh M. Mitogenic property of the apical ectodermal ridge. *Developmental biology*. 1982; 93:28–35. [PubMed: 7128937]
15. Fernandez-Teran MA, Hinchliffe JR, Ros MA. Birth and death of cells in limb development: a mapping study. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2006; 235:2521–2537. [PubMed: 16881063]
16. Lu P, Yu Y, Perdue Y, Werb Z. The apical ectodermal ridge is a timer for generating distal limb progenitors. *Development*. 2008; 135:1395–1405. [PubMed: 18359901]
17. Yu K, Ornitz DM. FGF signaling regulates mesenchymal differentiation and skeletal patterning along the limb bud proximodistal axis. *Development*. 2008; 135:483–491. [PubMed: 18094024]
- **18. Boehm B, Westerberg H, Lesnicar-Pucko G, Raja S, Rautschka M, Cotterell J, Swoger J, Sharpe J. The role of spatially controlled cell proliferation in limb bud morphogenesis. *PLoS biology*. 2010; 8:e1000420. Three-dimensional analysis of developing limb buds combining mathematical modeling and quantitative data of shape change and proliferation rate; Demonstrates directional cell behaviors rather than graded proliferation rate drive the limb bud outgrowth. [PubMed: 20644711]
19. Li S, Muneoka K. Cell migration and chick limb development: chemotactic action of FGF-4 and the AER. *Developmental biology*. 1999; 211:335–347. [PubMed: 10395792]
- **20. Wyngaarden LA, Vogeli KM, Ciruna BG, Wells M, Hadjantonakis AK, Hopyan S. Oriented cell motility and division underlie early limb bud morphogenesis. *Development*. 2010; 137:2551–2558. By live imaging initiation of mouse, chick limb buds and zebrafish fins, they showed Wnt5a is required for oriented cell migration and division. [PubMed: 20554720]
- **21. Gros J, Hu JK, Vinegoni C, Feruglio PF, Weissleder R, Tabin CJ. WNT5A/JNK and FGF/ MAPK pathways regulate the cellular events shaping the vertebrate limb bud. *Current biology : CB*. 2010; 20:1993–2002. Focusing on limb buds after initiation in mouse and chick, they found Wnt5a and Fgf signaling contributes to oriented cell behaviors and cell velocity, respectively. [PubMed: 21055947]
22. He F, Xiong W, Yu X, Espinoza-Lewis R, Liu C, Gu S, Nishita M, Suzuki K, Yamada G, Minami Y, et al. Wnt5a regulates directional cell migration and cell proliferation via Ror2-mediated noncanonical pathway in mammalian palate development. *Development*. 2008; 135:3871–3879. [PubMed: 18948417]
- **23. Benazeraf B, Francois P, Baker RE, Denans N, Little CD, Pourquie O. A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature*. 2010; 466:248–252. Live imaging presomitic mesoderm during the elongation of body A-P axis shows Fgf signaling establishes a gradient of cell velocity, a similar role of Fgf signaling discovered in limb bud in ref 21. [PubMed: 20613841]
24. Yang Y. Planar cell polarity during development. Preface. *Current topics in developmental biology*. 2012; 101:xi–xiii. [PubMed: 23140634]
25. Rauch GJ, Hammerschmidt M, Blader P, Schauerte HE, Strahle U, Ingham PW, McMahon AP, Haffter P. Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harbor symposia on quantitative biology*. 1997; 62:227–234.

26. Qian D, Jones C, Rzadzinska A, Mark S, Zhang X, Steel KP, Dai X, Chen P. Wnt5a functions in planar cell polarity regulation in mice. *Developmental biology*. 2007; 306:121–133. [PubMed: 17433286]
27. Vladar EK, Antic D, Axelrod JD. Planar cell polarity signaling: the developing cell's compass. *Cold Spring Harbor perspectives in biology*. 2009; 1:a002964. [PubMed: 20066108]
28. McNeill H. Planar cell polarity: keeping hairs straight is not so simple. *Cold Spring Harbor perspectives in biology*. 2010; 2:a003376. [PubMed: 20182624]
29. Gray RS, Roszko I, Solnica-Krezel L. Planar cell polarity: coordinating morphogenetic cell behaviors with embryonic polarity. *Developmental cell*. 2011; 21:120–133. [PubMed: 21763613]
30. Wang Y, Nathans J. Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development*. 2007; 134:647–658. [PubMed: 17259302]
31. Gong Y, Mo C, Fraser SE. Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. *Nature*. 2004; 430:689–693. [PubMed: 15254551]
32. Goodrich LV, Strutt D. Principles of planar polarity in animal development. *Development*. 2011; 138:1877–1892. [PubMed: 21521735]
33. Juriloff DM, Harris MJ. A consideration of the evidence that genetic defects in planar cell polarity contribute to the etiology of human neural tube defects. *Birth defects research Part A, Clinical and molecular teratology*. 2012; 94:824–840.
34. Wu J, Mlodzik M. A quest for the mechanism regulating global planar cell polarity of tissues. *Trends in cell biology*. 2009; 19:295–305. [PubMed: 19560358]
35. Gao B. Wnt Regulation of Planar Cell Polarity (PCP). *Current topics in developmental biology*. 2012; 101:263–295. [PubMed: 23140633]
36. Heisenberg CP, Tada M, Rauch GJ, Saude L, Concha ML, Geisler R, Stemple DL, Smith JC, Wilson SW. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature*. 2000; 405:76–81. [PubMed: 10811221]
37. Jessen JR, Topczewski J, Bingham S, Sepich DS, Marlow F, Chandrasekhar A, Solnica-Krezel L. Zebrafish trilobite identifies new roles for Strabismus in gastrulation and neuronal movements. *Nature cell biology*. 2002; 4:610–615.
- **38. Gao B, Song H, Bishop K, Elliot G, Garrett L, English MA, Andre P, Robinson J, Sood R, Minami Y, et al. Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. *Developmental cell*. 2011; 20:163–176. First molecular evidence showing that chondrocytes are polarized and Wnt5a is required for PCP establishment; Demonstrates how signal is translated from Wnt ligand to PCP core protein. [PubMed: 21316585]
39. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Developmental cell*. 2009; 17:9–26. [PubMed: 19619488]
40. Veeman MT, Axelrod JD, Moon RT. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Developmental cell*. 2003; 5:367–377. [PubMed: 12967557]
41. van Amerongen R, Mikels A, Nusse R. Alternative wnt signaling is initiated by distinct receptors. *Science signaling*. 2008; 1:re9. [PubMed: 18765832]
42. Wang Y, Guo N, Nathans J. The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006; 26:2147–2156. [PubMed: 16495441]
43. Yu H, Smallwood PM, Wang Y, Vidaltamayo R, Reed R, Nathans J. Frizzled 1 and frizzled 2 genes function in palate, ventricular septum and neural tube closure: general implications for tissue fusion processes. *Development*. 2010; 137:3707–3717. [PubMed: 20940229]
44. Yu H, Ye X, Guo N, Nathans J. Frizzled 2 and frizzled 7 function redundantly in convergent extension and closure of the ventricular septum and palate: evidence for a network of interacting genes. *Development*. 2012; 139:4383–4394. [PubMed: 23095888]
- *45. Li Y, Dudley AT. Noncanonical frizzled signaling regulates cell polarity of growth plate chondrocytes. *Development*. 2009; 136:1083–1092. Demonstrates PCP may regulate oriented division of chondrocytes in growth plate. [PubMed: 19224985]

46. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development*. 1999; 126:1211–1223. [PubMed: 10021340]
47. DeChiara TM, Kimble RB, Poueymirou WT, Rojas J, Masiakowski P, Valenzuela DM, Yancopoulos GD. Ror2, encoding a receptor-like tyrosine kinase, is required for cartilage and growth plate development. *Nature genetics*. 2000; 24:271–274. [PubMed: 10700181]
48. Takeuchi S, Takeda K, Oishi I, Nomi M, Ikeya M, Itoh K, Tamura S, Ueda T, Hatta T, Otani H, et al. Mouse Ror2 receptor tyrosine kinase is required for the heart development and limb formation. *Genes to cells : devoted to molecular & cellular mechanisms*. 2000; 5:71–78. [PubMed: 10651906]
- *49. Wang B, Sinha T, Jiao K, Serra R, Wang J. Disruption of PCP signaling causes limb morphogenesis and skeletal defects and may underlie Robinow syndrome and brachydactyly type B. *Human molecular genetics*. 2011; 20:271–285. Shows genetic interaction between Wnt5a and Vangl2 in limb morphogenesis. [PubMed: 20962035]
50. Afzal AR, Rajab A, Fenske CD, Oldridge M, Elanko N, Ternes-Pereira E, Tuysuz B, Murday VA, Patton MA, Wilkie AO, et al. Recessive Robinow syndrome, allelic to dominant brachydactyly type B, is caused by mutation of ROR2. *Nature genetics*. 2000; 25:419–422. [PubMed: 10932186]
51. van Bokhoven H, Celli J, Kayserili H, van Beusekom E, Balci S, Brussel W, Skovby F, Kerr B, Percin EF, Akarsu N, et al. Mutation of the gene encoding the ROR2 tyrosine kinase causes autosomal recessive Robinow syndrome. *Nature genetics*. 2000; 25:423–426. [PubMed: 10932187]
52. Person AD, Beiraghi S, Sieben CM, Hermanson S, Neumann AN, Robu ME, Schleiffarth JR, Billington CJ Jr, van Bokhoven H, Hoogeboom JM, et al. WNT5A mutations in patients with autosomal dominant Robinow syndrome. *Developmental dynamics : an official publication of the American Association of Anatomists*. 2010; 239:327–337. [PubMed: 19918918]
53. Song H, Hu J, Chen W, Elliott G, Andre P, Gao B, Yang Y. Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. *Nature*. 2010; 466:378–382. [PubMed: 20562861]
54. Olsen BR, Reginato AM, Wang W. Bone development. *Annual review of cell and developmental biology*. 2000; 16:191–220.
55. Randall RM, Shao YY, Wang L, Ballock RT. Activation of Wnt Planar Cell Polarity (PCP) signaling promotes growth plate column formation in vitro. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*. 2012; 30:1906–1914. [PubMed: 22674351]
56. Parr BA, Shea MJ, Vassileva G, McMahon AP. Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. *Development*. 1993; 119:247–261. [PubMed: 8275860]

Figure 1.

Schematic presentation of the generation of planar polarity from a group of homogeneous cells under the influence of a global cue. As the result of PCP, the core PCP proteins become asymmetrically localized to proximal (P) and distal (D) cell membranes.

Figure 2. Vangl2 proteins are asymmetrically localized to the proximal side of chondrocytes in E12.5 mouse distal limb (green, white arrows). Chondrocytes are marked by Sox9 protein expression (red). DAPI (blue) stains the nucleus. D, distal; P, proximal.

Figure 3.

Diagram of limb morphogenesis showing the roles of Wnt5a/PCP at different development stages. Wnt5a is required for polarized cell behaviors in the early limb bud and PCP establishment around E12.5. It is unknown whether core PCP itself has an effect on cell migration or division in the early limb bud, and its direct role on columnar chondrocytes organization needs further genetic investigation.