Bad hair days for mouse PCP mutants

Jeffrey D. Axelrod

Mammalian hairs have characteristic patterns of orientation, with a predominantly rostral to caudal direction, occasional swirls and a high level of local correlation between hairs. A detailed new study demonstrates that the polarity of hairs derives from an underlying planar polarity of the basal epidermal cells from which hair follicles arise.

Mammalian body hairs begin as placodes in the basal epidermis. The placodes, separated from one another by basal epidermal cells, invaginate into the dermis to form hair germs and ultimately hair follicles from which the hairs emerge. Early in their development, the hair germs tilt, causing the hairs to grow at an angle. Projection of the hairs onto the plane of the epithelium defines vectors representing their polarities, and they are far from random. On a given region of skin, these vectors all point in the same direction, producing an ordered, polarized array. On page 1257 of this issue, Devenport and Fuchs report progress in understanding how this coordinated polarization arises[1].

Many epithelial cells are individually polarized orthogonal to their apical–basal axes, a phenomenon called planar cell polarity (PCP). The most detailed studies of PCP have looked at the epidermis of *Drosophila melanogaster*, where individual cells produce trichomes, which emerge from the distal side of the cell and point distally (Fig. 1a)[2]. PCP in *Drosophila* is characterized by the asymmetric accumulation of specific PCP proteins on proximal or distal sides of each cell. Among these, the serpentine receptor Frizzled (Fz)[3], accumulates on the distal side, the four-pass transmembrane protein Van Gogh (Vang or Strabismus/Stbm)[4], accumulates on the proximal side, whereas the seven-transmembrane atypical cadherin Flamingo (Fmi or Starry night/Stan)[5], accumulates on both sides. The axis of these asymmetric subcellular distributions predicts the trichome polarity pattern[2]. These proteins are thought to communicate across intercellular boundaries, recruiting one group to the distal side of cells, and the other to the proximal side, through the function of a poorly understood feedback mechanism[6]. The results are the tendency of adjacent cells to polarize in the same direction, like dominoes, and the local propagation of polarity from cell to cell. The same proteins adopt asymmetric subcellular distributions in specific cells of the ommatidia of the *Drosophila* eye, where they polarize an intercellular signal that determines differential cell fates (Fig. 1b). Although the specifics are controversial, a global directional cue must guide the directionality of the self-organizing system mediated by Fz/Vang/Fmi[7,8].

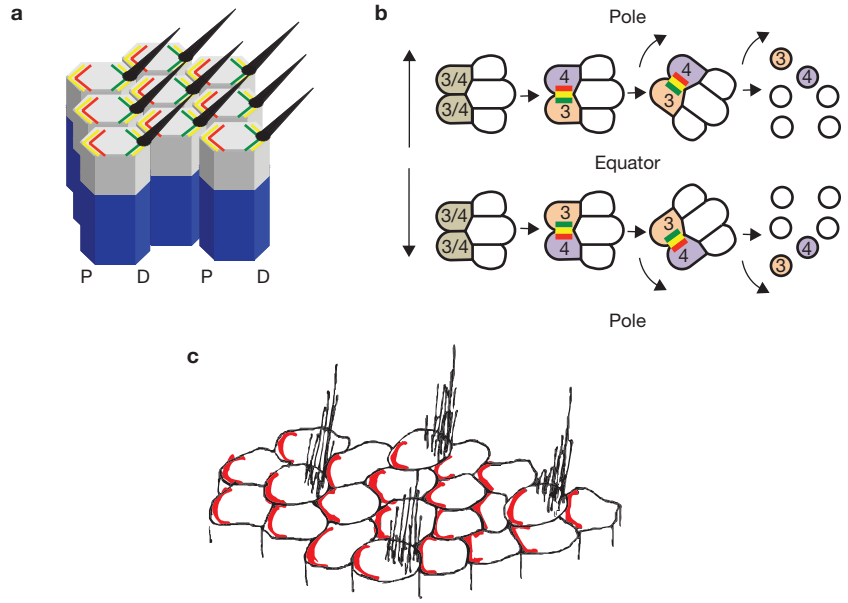

Figure 1 PCP in the *Drosophila* wing and eye, and the mammalian vestibular epithelium. (**a**) *Drosophila* wing epithelial cells elaborate a trichome from their distal vertex. The PCP proteins Fmi (yellow), Fz (green) and Vang (red) accumulate at proximal (P) and distal (D) cell boundaries and are thought to participate in distinct proximal (Fmi and Vang) and distal (Fmi and Fz) complexes that communicate with and stabilize each other across the boundary. (**b**) In the *Drosophila* eye, the prospective R3/R4 photoreceptors are recruited into developing ommatidia. The same PCP protein complex establishes an asymmetric complex between the prospective R3 and R4 cells, biasing a Notch signal that directs adoption of the R3 fate in the equatorial cell and the R4 fate in the polar cell. Subsequent differentiation and rotation of the ommatidium depends on the relative positions of R3 and R4. (**c**) In the mammalian vestibular epithelium, sensory hair cells differentiate within a supporting epithelium. The sensory hair cells elaborate asymmetrically placed ciliary bundles whose orientation is determined by the PCP mechanism. The entire epithelium shows polarized distributions of PCP proteins before sensory hair cell differentiation.

In vertebrates, PCP is readily apparent in the sensory epithelium of the inner ear, where the sensory hair cells show a striking planar polarity evident in their asymmetric placement of ciliary bundles on their apical surfaces. Asymmetric localization of Fz, Vangl2, Celsr1/Fmi and other PCP proteins seems to recapitulate that seen in *Drosophila*, and disruption of genes encoding these proteins produces abnormal PCP of the sensory hair cells (Fig. 1c), suggesting a well conserved mechanism[9]. Genetic analyses indicate that PCP is involved in a growing list of developmental events, and its disruption is associated with open neural tube defects, polycystic kidney disease, deafness syndromes, ciliary dyskinesia, situs inversus and conotruncal heart defects. In addition, PCP is involved in normal vasculogenesis and wound healing, and is implicated in the invasive and metastatic behaviour of malignant neoplasms[10].

An earlier study had shown that mouse Fz6 is required for normal body hair polarity, as mutants show abnormal swirling hair patterns[11].

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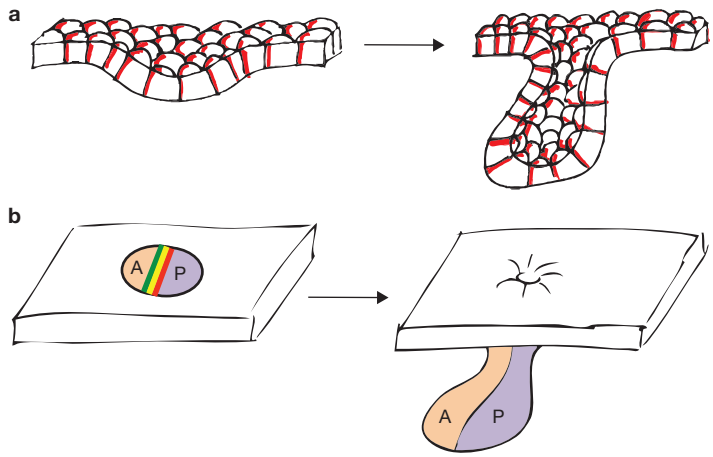

Figure 2 PCP in mammalian hair germs. (**a**) The hair placode begins as an invagination of the basal epidermal layer. Asymmetric distribution of the PCP proteins Vang (red), Fz and Celsr1/Fmi are evident at this stage. The placode invaginates to form a hair germ. (**b**) Model for early polarization of the hair germ: two germ founder cells may acquire PCP in the context of the polarized basal epidermal cell layer. The founder cells may then communicate with each other using a polarized signal whose direction is biased by the PCP mechanism, to determine an anterior (A) and posterior (P) cell. The A and P cells would then give rise to anterior and posterior compartments with different developmental properties that cause the hair germ to tilt.

This observation suggested that the same PCP mechanism may polarize hair growth, though a role for β-catenin-dependent Wnt signalling was not ruled out, and the mechanism was not characterized. Devenport and Fuchs have now shown that a conserved PCP pathway polarizes the basal epidermal cells from which hair germs develop, and this polarity somehow affects hair germ gene expression and morphogenesis to produce coordinated tilting and thus hair polarity[1].

Devenport and Fuchs first characterized changes in the hair germ that correspond to polarization. Early in hair germ development, anterior–posterior differences in cellular morphology and corresponding changes in actin and keratin organization are observed. Remarkably, they also found that patterns of gene expression are restricted to anterior or posterior regions of the hair germ. Thus, anterior and posterior cells somehow acquire information that specifies their location in this otherwise symmetric structure, and it seems that differential cytoskeletal reorganization in the germ cells controls tilting.

They then asked what role the PCP mechanism might have in this process. Examining the localization of Vangl2, Celsr1 and Fz6, they found that each of these proteins adopts an anterior–posterior asymmetric subcellular localization both in the developing hair germ and in the surrounding basal epidermal layer (Fig. 2a). This polarity is evident when the hair placode begins its invagination, before tilting, but does not depend on these events. Furthermore, skin of Vangl2 or Celsr1 mutant embryos shows abnormalities in polarity. Whereas wild-type hair germs show an early posterior tilt, the mutant hair germs grow straight down, tilting much later and not in the correct direction. Correspondingly, gene expression and cytoskeletal organization become symmetric and the germs adopt symmetric morphology. Thus, body hairs develop in an epithelium that already shows molecular evidence of PCP, and disrupting PCP perturbs hair germ tilting.

To further test the idea that a conserved PCP mechanism somehow polarizes hair germ tilting, the authors examined the interdependence of Vangl2, Fz6 and Celsr1 subcellular localization *in vivo*, and found an interdependence very similar to that observed in flies. They also found that, in transfected keratinocytes, Celsr1 is strongly recruited to points of cell contact between neighbouring cells only when both cells express it, suggesting homodimerization by this atypical cadherin as previously shown in flies[5]. For unknown reasons, the C-terminal tail is required for homodimerization, as is at least one of the cadherin repeats, where Crsh, the mutation causing neural tube, inner ear and body hair defects, maps. Consistent with these proteins forming a complex that bridges neighbouring cells, *Drosophila* Fmi and Fz have been shown to physically interact[12]. The authors demonstrate a similar interaction between co-transfected Celsr1 and Vangl2. The Lp mutation in Vangl2 blocks this interaction, suggesting its importance *in vivo*.

Taken together, these results show that a PCP mechanism strongly resembling that identified in *Drosophila* operates in the mouse basal epidermis. When hair germs develop in this epidermis, they rely on PCP to differentiate their anterior and posterior sides to produce the correct tilt.

A key feature of hair polarization is its coordination across the tissue, despite the fact that hair germs are isolated from one another by intervening basal epidermal cells. This situation resembles the vestibular epithelium, in which sensory hair cells show coordinated polarization despite intervening supporting cells. It seems that the coordination problem is solved in both tissues by polarizing the entire epithelium in a concerted fashion, and then using the polarity information to drive morphological polarization in the relevant cells.

The skin faces an additional problem. In the vestibular epithelium, individual sensory hair cells polarize, whereas in the skin, large multicellular structures become polarized and different cells in the structure show unique behaviours. Although it remains to be determined how this occurs, this situation is analogous to polarization of ommatidia in the *Drosophila* eye, and may share mechanistic features. In the eye, two neighbouring cells, the R3 and R4 precursors, adopt different fates depending on a directional Notch signal between them. Once differentiated, these two cells trigger the subsequent development of asymmetry within the ommatidium. The PCP proteins Fz, Vang and Fmi set up an asymmetric boundary between the precursors and bias the direction of Notch signalling, thereby determining the polarity of the R3/R4 fate decision[13,14]. This suggests an appealing, though speculative, model for hair germ polarization, in which two of the earliest cells of the hair placode signal between each other to determine anterior and posterior fates (Fig. 2b). The direction of this signal may be regulated by PCP. Early specification of anterior and posterior fates could then be transmitted by lineage, and this would be consistent with the apparent compartmentalization the authors observed by lineage tracing.

Some intriguing results in the study by Devenport and Fuchs[1] raise additional questions. As seen in *Drosophila*, non-autonomous effects of Vangl2^{Lp} mutant tissue were observed:

in chimaeric skin, a wild-type follicle, surrounded by Vangl2^{Lp} mutant cells, failed to polarize. In a related experiment, a patch of embryonic skin, explanted after PCP was first evident, developed normal polarity in culture. However, if the graft was rotated and re-implantated into adults, the polarity realigned to match the host. Whether this realignment results from non-autonomy through the Fz/Vang system, or whether the graft re-acquires its global directional cue from the neighbouring tissue is not known.

Much like Vangl2^{Lp}, Fz6 mutant hair germs initially grow straight down, and then tilt randomly. Fz6 hairs then undergo a substantial realignment to produce a coordinated though abnormally swirling pattern[15]. A similar phenotype is observed for trichomes on the *Drosophila* epidermis. The mechanism by which this late coordination occurs is unknown.

With the work reported by Devenport and Fuchs as a foundation, and the many experimental tools available in this system, these and other questions may soon be answered.

1. Devenport, D. & Fuchs, E. *Nature Cell Biol.* **10,** 1257–1268 (2008).
2. Zallen, J. A. *Cell* **129,** 1051–1063 (2007).
3. Vinson, C. R., Conover, S. & Adler, P. N. *Nature* **338,** 263–264 (1989).
4. Taylor, J., Abramova, N., Charlton, J. & Adler, P. N. *Genetics* **150,** 199–210 (1998).
5. Usui, T. *et al. Cell* **98,** 585–595 (1999).
6. Amonlirdviman, K. *et al. Science* **307,** 423–426 (2005).
7. Ma, D., Yang, C. H., McNeill, H., Simon, M. A. & Axelrod, J. D. *Nature* **421,** 543–547 (2003).
8. Casal, J., Lawrence, P. A. & Struhl, G. *Development* **133,** 4561–4572 (2006).
9. Wang, Y. & Nathans, J. *Development* **134,** 647–658 (2007).
10. Simons, M. & Mlodzik, M. *Annu. Rev. Genet.* (2008).
11. Guo, N., Hawkins, C. & Nathans, J. *Proc. Natl Acad. Sci. USA* **101,** 9277–9281 (2004).
12. Chen, W. S. *et al. Cell* **133,** 1093–1105 (2008).
13. Cooper, M. T. & Bray, S. J. *Nature* **397,** 526–530 (1999).
14. Fanto, M. & Mlodzik, M. *Nature* **397,** 523–526 (1999).
15. Wang, Y., Badea, T. & Nathans, J. *Proc. Natl Acad. Sci. USA* **103,** 19800–19805 (2006).

A new dawn for Aurora?

Andrea H. Brand

The balance between proliferation and differentiation is essential not only for the generation and maintenance of tissues, but also to prevent uncontrolled cell division and tumorigenesis. The mitotic kinase Aurora A coordinates cell-cycle events and asymmetric division by regulating localization of the cell fate determinant Numb through remodelling of the conserved PAR polarity complex.

Stem cells of the *Drosophila melanogaster* central nervous system, called neuroblasts, divide asymmetrically to produce a self-renewing cell and a daughter cell that divides only once to give two post-mitotic neurons. At each division, cell fate determinants are segregated to the daughter cell, where they inhibit self-renewal and promote differentiation. Interest in the molecular mechanism of asymmetric division has peaked recently with the discovery that defects in asymmetric cell division can lead to tumours[1] (the 'cancer stem-cell hypothesis'). Loss of any one of three asymmetrically segregated cell fate determinants, Numb, Prospero or Brat, enables the daughter cells to self-renew, resulting in tumours in embryos[2], larvae and adults[3–5]. In addition, in the *Drosophila* peripheral nervous system, sensory organ precursors (SOPs) undergo a limited number of cell divisions and do not self-renew, but determinants are asymmetrically segregated at each division to produce the four cell types that form the sensory organ (neuron, sheath, hair and socket cells). As such, they do not conform to the present definition of stem cells. Nonetheless, many of the molecules that direct asymmetric cell division are conserved between SOPs and neuroblasts[6]. Understanding the mechanism by which fate determinants are accurately partitioned between cells is an important step towards understanding how self-renewal and differentiation are correctly balanced and, perhaps, how tumorigenesis may be prevented or reversed.

The Par complex, which consists of Bazooka (Baz, the *Drosophila* homologue of Par-3), Par-6 and atypical protein kinase C (aPKC), is known to regulate polarity within neuroblasts in the central nervous system and in SOPs in the peripheral nervous system. The Par complex localizes to the apical cortex of neuroblasts and to the posterior cortex of SOPs from where it controls asymmetric segregation of cell fate determinants through regulation of the tumour suppressor Lethal (2) giant larvae (Lgl).

In a recent paper, Witrz-Peitz *et al.*[7] investigated the molecular interactions that enable the Par complex to inactivate Lgl and how this, in turn, leads to asymmetry in Numb localization in neuroblasts and in SOPs. They showed that the mitotic kinase Aurora-A (AurA) initiates a phosphorylation cascade that ultimately leads to phosphorylation of Numb, its release from the posterior cortex and accumulation at the anterior cortex, thereby promoting its segregation to the differentiating daughter where it prevents proliferation.

Recently, two mitotic kinases, AurA and Polo, have been shown to link asymmetric division to cell-cycle progression[8–11]. In *aurA* mutant SOPs, Numb is found around the entire cell cortex, rather than solely at the anterior cortex, and is partitioned to both daughter cells at mitosis, resulting in cell fate transformations in the SOP lineage[8]. In the larval brain, AurA acts as a tumour suppressor[9,10] and in *aurA* mutants, Numb and aPKC are mislocalized to the entire neuroblast cortex, mitotic spindles are misoriented and ectopic neuroblasts are generated at the expense of differentiated neurons, a phenotype observed also in *numb* mutants. Thus, in wild-type, AurA acts through aPKC and Numb to inhibit neuroblast self-renewal and to regulate asymmetric segregation of Numb[9,10]. Supporting this conclusion, the *aurA* mutant phenotype can be suppressed by ectopic expression of Numb.

Polo also acts as a tumour suppressor in the larval brain[11], and *polo* mutants have very similar phenotypes to *aurA*: uncontrolled proliferation of neuroblasts at the expense of neurons, mislocalization of aPKC, Numb and its binding partner Pon (partner of Numb). Polo

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