Symmetry breaking: by what mechanism is left/right asymmetry initially established in bilateria?

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Abstract

In bilateria development, initial left-right (LR) symmetry breaking event is a big controversial with different models backed up with compelling evidence in different organisms. Planar cell polarity (PCP) has great potential in producing intracellular polarisation thus morphological asymmetry. Recent genetic studies show PCP is essential for posterior positioning of node cilia, leftward nodal flow and in some case LR asymmetry. There is still much to discover.

1 Introduction

Establishment of LR asymmetry is both a medically relevant and conceptually challenging problem. Bilateria appear to be left-right symmetrical in their body lining while their internal visceral organs are both asymmetrically positioned and developed. Though LR asymmetry marker like expression of *Nodal* gene (and its homologs) has been identified to guide the subsequent asymmetrical development, it is not yet clear how early embryos consistently pattern their LR asymmetry with respect to the other two presumably early anteroposterior(AP) and dorsoventral(DV) axes.

One of the promising player in LR asymmetry initiation is PCP, which play conserved roles in Drosophila development and in vertebrate development. The term refers to cell. This intracellular polarisation is then interpreted by various PCP effectors to achieve morphological polarisation like cilia polarisation, convergent extension and directed cell movement.

As reviewed in (Aw & Levin 2009), PCP have great potential in both calculation of LR asymmetry since it fits perfectly Wolpert's definition of 'F-molecule' (Brown & Wolpert

1990) in that the PCP occurs at apical surface (DV axis) and along a global axis (AP axis), potentiating it to derive the LR axis from the two. Originally discovered in Drosophila (Goodrich & Strutt 2011), core PCP proteins are polarised along a specific axis on the apical side of usually epithelial cells due to the antagonistic effect separating 2 groups of protein. After initially set up with a global cue, this separation is further propagated and stabilised with the intercellular interaction between transmembrane *frizzled (fz)* and s*trabismus (stbm,* also known as *Van Gogh)*, which is then interpreted by PCP effectors like *Inturned* and *Fuzzy*.

Indeed, polarisation of PCP proteins (Table 2) have been widely related to LR patterning (Table 1). While ample evidence supports a flow-dependent role in mouse, such evidence for Xenopus and Zebrafish is weaker, and impossible for chick, which does not have an equivalent to the motile cilia on the node. Various evidence support a flow-independent role of PCP including mutants that are defective in both PCP and LR asymmetry but otherwise normal. We here remark on these two possibilities.

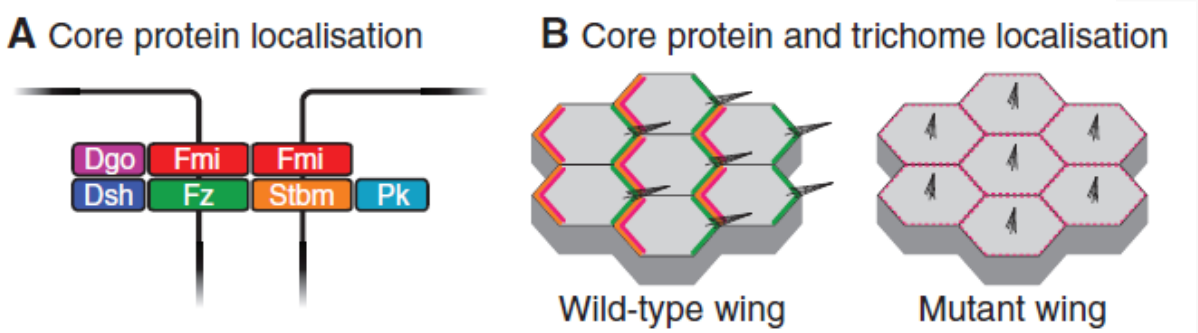

Figure 1 PCP in Drosophila (Adapted from Goodrich, L. V & Strutt, D., 2011. Principles of planar polarity in animal development. Development, *138(10), pp.1877–1892.)*

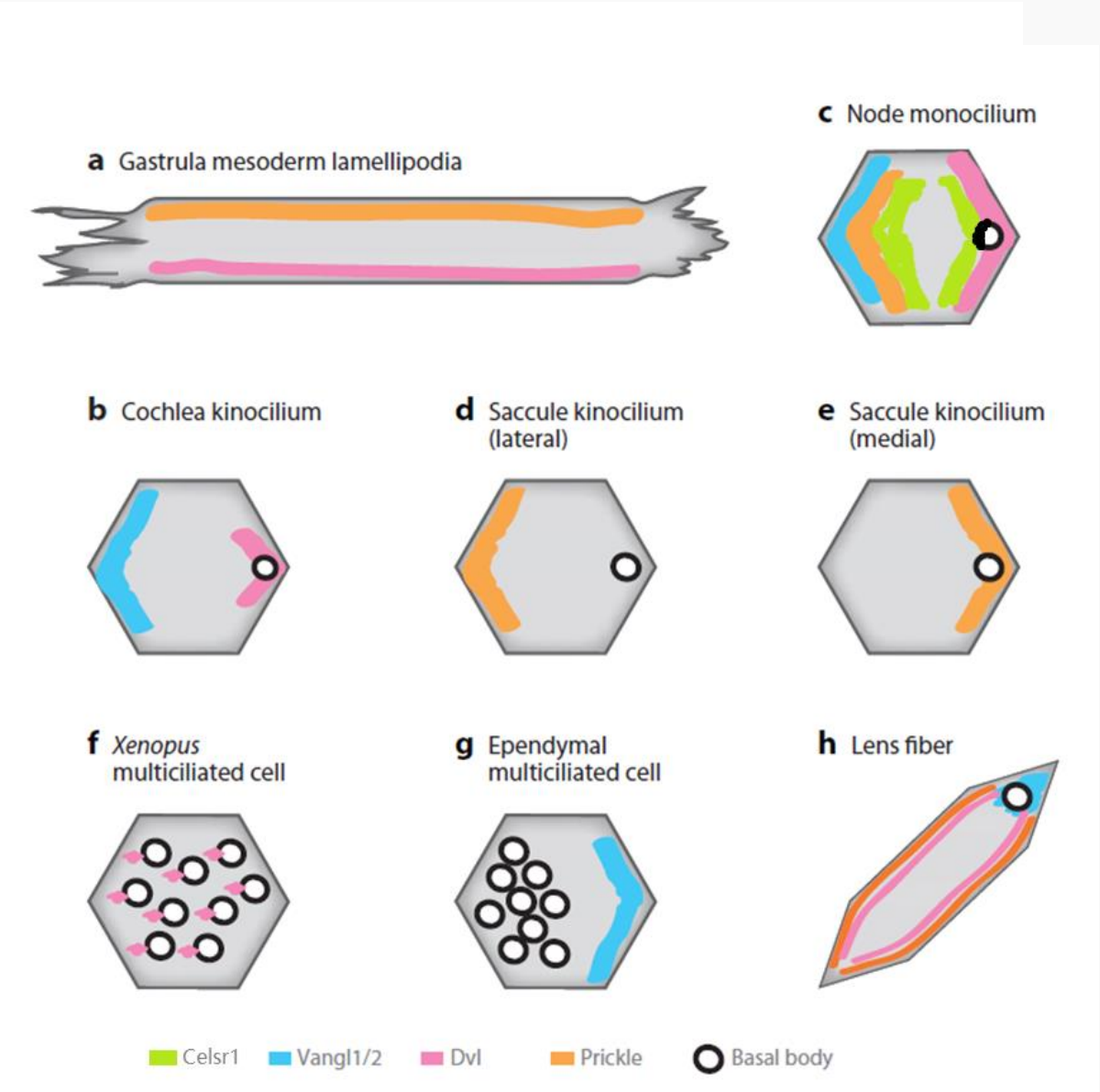

Figure 2 Polarisation of PCP proteins and of basal bodies (at the bottom of cilia) in a variety of vertebrate cells (adapted from Wallingford, J.B., 2012. Planar Cell Polarity and the Developmental Control of Cell Behaviour in Vertebrate Embryos. Annual Review of Cell and Developmental Biology, 28(1), pp.627–653)

Table 1 ***Summary of cilia polarisation defect and LR defects in PCP/Wnt mutants***

Organism	Mutant/treatment	Polarisation defect	LR defect	References
Mouse	Vangl2$^{D/D}$;Vangl1$^{gt/gt}$	Centralised cilia,	Ca2+ signalling: 36% bilateral,36% weak/absent Nodal: bilateral (3/8) or reversed (1/8) Pitx2: bilateral (2/7) Lefty1 and Lefty2: reversed (1/2)	(Song et al. 2010)
	Vangl1$^{gt/gt}$ (PCP defect at 14%)	N/A	Turning failure:(5/5) Pitx2:bilateral(2/5)	
Mouse	Cfl1/Vangl2	Centralised cilia Cytosolic Celsr1	Nodal expression: 4/7 reversed or bilateral	(Mahaffey et al. 2013)

Mouse	Dvl1$^{-/-}$Dvl2$^{+/-}$Dvl3$^{-/-}$	Centralised cilia	Nodal expression: 3/3 wt	(Hashimoto et al. 2010)
Mouse	Dvl1$^{-/-}$Dvl2$^{-/-}$Dvl3$^{+/-}$	Centralised cilia	Nodal expression: 2/2 absent	(Hashimoto et al. 2010)
Mouse	*Ift88$^{-/-}$(Polaris)*	Intact Celsr1 polarisation	Not mentioned	(Mahaffey et al. 2013)
Xenopus	*Vangl2* MO	Centralised cilia	Xnr-1: absent (50% with 15% ctrl) or bilateral (24% with 20% ctrl)	(Antic et al. 2010)
Xenopus	*Vangl2* RNA	Centralised cilia	N/A	
Xenopus	Wnt11b MO	Centralised cilia	Pitx2 57% absent,10% disrupted, 33% wt Coco: 85% reduced over a control at 20% Xnr1:63% absent or reduced, 37% after rescued* with Wnt11b DNA, over a control at 10% absent	(Walentek et al. 2013)
Xenopus	Ectopic Wnt11b DNA	Less posterior cilia	Pitx2 60% bilateral ,12% wt	(Walentek et al. 2013)
Xenopus	Vangl2MO	N/A	Xnr-1: bilateral or reversed (28% over 1%) or absent (10% over 5%)	(Vandenberg & Levin 2012)
Xenopus	DNPar6(disrupt ABP)	N/A	Xnr-1: aberrant (26%, control of 6%)	(Vandenberg & Levin 2012)
Zebrafish	vangl2$^{-/-}$	N/A	Lefty2: absent (20%)	(Borovina et al. 2010
Zebrafish	MZvangl2	60% centralised (10% control) 49% posterior tilted (89% control)	Left2: bilateral(24%),reversed(6%) ctrl<5%	(Borovina et al. 2010
Zebrafish	MZknypek	40% centralised (10% control)	N/A	(Borovina et al. 2010)
Chick	Vangl2 MO	No cilia in wild type	*Shh*: bilateral (25% over 6%) or absent (16% over 6%)	(Zhang & Levin 2009)

* Walentek et al. 2013 is ambiguous about the coinjection of Wnt11b DNA thus inconvincible.

Table 2 ***Vertebrate homologues of planar polarity genes identified in Drosophila (Adapted from Goodrich, L. V. & Strutt, D., 2011. Principles of planar polarity in animal development. Development, 138(10), pp.1877–1892,)***

Drosophila gene	Identified homologues known to function in planar polarity	Asymmetric localisation observed?	Key references
fz	*Fz1*, *Fz2*, *Fz3*, *Fz6* (mouse)	Fz3 (ependymal, inner ear); Fz6 (inner ear, hair follicles)	
stbm/*Vang*	*Vangl1*, *Vangl2* (mouse) *stbm*/*vangl2* (zebrafish, *Xenopus*)	Vangl1, Vangl2 (anterior in mouse node, ear, hair follicles); Vangl2 (ependymal); Vangl2 (proximal to chick node on both sides) Vangl1/Vangl2(functional in Xenopus GRP,not detected yet)	Antic et al., 2010; Song et al., 2010; Borovina et al.,2010
fmi/*stan*	*Celsr1*, *Celsr2*, *Celsr3* (mouse) *fmi*/*celsr* (zebrafish) *C-fmi-1* (chick)	Celsr1 (along AP axis, most likely anterior in mouse node, hair follicles); C-fmi-1 (chick ear)	Mahaffey et al. 2013;Davies et al., 2005; Wada et al., 2006;Devenport and Fuchs, 2008;Carreira-Barbosa et al., 2009
dsh	*Dvl1*, *Dvl2*, *Dvl3* (mouse) *dsh* (zebrafish, *Xenopus*)	Dvl2, Dvl3 (posterior in mouse node)	Hashimoto et al, 2010
pk	*Pk1*, *Pk2* (mouse) *pk1* (zebrafish) *Pk* (*Xenopus*)	Pk1 (absent in mouse node) Pk2 (anterior in mouse node, ear); Pk (mesoderm, neural tube)*	Carreira-Barbosa et al., 2003; Takeuchi et al., 2003; Veeman et al., 2003b; Ciruna et al., 2006; Deans et al., 2007; Antic et al., 2010; Yin et al., 2008
dgo	Inversin (*Invs*), diversin(*Ankrd6*) (mouse)		
ft	*Fat4* (mouse)		
ds	*Dchs1* (mouse)		
fj	*Fjx1* (mouse)		
dachs	None?		
in	*Intu* (mouse) *Int* (*Xenopus*)		
fy	*Fuz* (mouse) *Fy* (*Xenopus*)		
frtz	*Fritz* (*Xenopus*)		

*Note that the asymmetric localisation of Pk observed in zebrafish and *Xenopus* (Ciruna et al., 2006; Yin et al., 2008) was seen using expression of a fusion of *Drosophila* Pk to GFP not using native zebrafish and *Xenopus* proteins.
Celsr, Cadherin, EGF-like, LAG-like and seven-pass receptor; *ds*, *dachsous*; *dgo*, *diego*; *dsh*, *dishevelled*; *Dvl*, dishevelled; *fj*, *four-jointed*; *Fjx1*, four jointed box 1; *fmi*/*stan flamingo*/*starry night*; *frtz*, *fritz*; *ft*, *fat*; *Fuz*, fuzzy homologue; *fy*, *fuzzy*; *fz*, *frizzled*; *in*, *inturned*; *Int*, inturned; *Intu*, inturned; *pk*, *prickle*; *stbm*/*Vang*, *Van Gogh*; *Vangl*, Van
Underlined are where protein localisation at LR organisers and relevant reference

2 *PCP is instructive for a leftward nodal flow*

Among the contentions attempted to describe the earliest symmetry breaking event, an extraembryonic leftward nodal flow generated by the rotational movement of monocilia on the LR organiser has been successful in reconciling experimental

results (reviewed in Hirokawa et al. 2012), especially in mouse where application of artificial flow reverses LR asymmetry (complete *situs inversus)* (Nonaka et al. 2002) and reproducible delicate characterisation of flow allows quantitative predictions(Shinohara et al. 2012; Yoshiba et al. 2012). As to the downstream interpretation of the nodal flow, elegant conditional knockout and restoration of Pkd2 have shown perinodal crown cells to asymmetrically pattern Ca^{2+} signalling, *Cerl2* (an inhibitor of *Nodal*) expression and thus *Nodal* expression.

Importantly, 9+0 monocilia at central node produce this flow not in a forward-back fashion as 9+2 cilia on ependymal cells or airway cells do to generate a flow (Norris 2012). Instead, theoretical modelling and experimental result support that the posterior positioning of cilia and thus the posterior tilting allows cilia to generate an efficient leftward flow. This idea dovetails nicely with the notion of cilia act as a conceptual 'F-molecule' proposed by (Brown & Wolpert 1990) to calculate LR axis by aligning to AP and DV axes, as fulfilled with the posterior positioning of cilia and constriction of monocilia to the apical/ventral surface respectively. It's natural to explain this translational polarisation with PCP provided its extensive involvement in both cilia positioning in both Drosophila and vertebrates (Wallingford 2012). Indeed, this aligning to AP axis has recently been shown defective if PCP is disrupted (Table 1).

Building on the well-established 2-cilia model of nodal flow sensing, asymmetrical cellular polarisation of core PCP proteins have recently been demonstrated in mouse monociliated node cells (see Table 2 **Vertebrate homologues of planar polarity genes identified in Drosophila (Adapted from Goodrich, L. V. & Strutt, D., 2011. Principles of planar polarity in animal development. Development, 138(10), pp.1877–1892,**)Table 2 and Figure 3). Whereas *Dsh2/3* localise to the posterior side of the node cell evidenced by protein localisation in mosaic node,(Hashimoto et al. 2010), *Vangl1, Vangl2* and *Pk2* appear to localise to the anterior side (Song et al. 2010; Antic et al. 2010), though mosaic analysis are required to confirm the localisation of *Vangl1, Vangl2, Pk2* and *Celsr1*, Though intercellular interaction is not yet evident, an antagonistic effect between two groups is emerging.

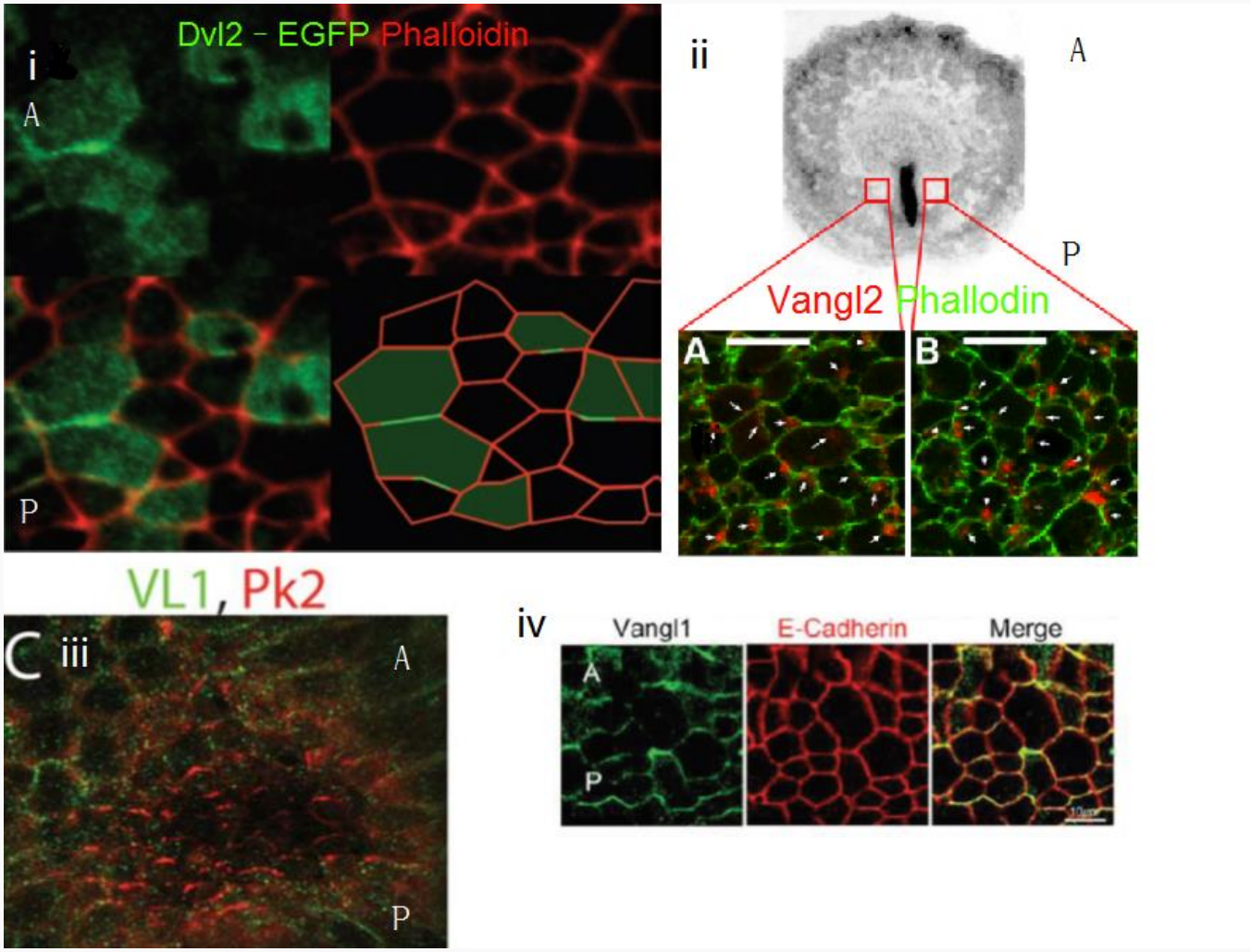

Figure 3 Exemplar localisation of core PCP proteins in LR organiser. (I) Dvl2-EGFP (green) in a chimeric mouse node (ii)Vangl2(red) and around Hensen's node in chick (iii) Vangl1(green) and Prickled2(red) in mouse node (iv) Vangl1(red) in mouse node. A: anterior, P: posterior. Adapted from:
(i) Hashimoto, M. et al., 2010. Planar polarization of node cells determines the rotational axis of node cilia. Nature cell biology, 12(2), pp.170–176.
(ii)Zhang, Y. & Levin, M., 2009. Left-right asymmetry in the chick embryo requires core planar cell polarity protein Vangl2. Genesis, 47(11), pp.719–728.
(iii)Antic, D. et al., 2010. Planar cell polarity enables posterior localization of nodal cilia and left-right axis determination during mouse and Xenopus embryogenesis. PLoS ONE, 5(2).
(iv)Song, H. et al., 2010. Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. Nature, 466(7304), pp.378–382.

PCP is functional in posterior positioning cilia. While nearly all of the mutants display centralised cilia and disrupted nodal flow (Table 1), LR markers are not the same. $Vangl2^{D/D}$&$Vangl1^{gt/gt}$, $Vangl1^{gt/gt}$, and Cfl1/Vangl2 exhibit bilateral Pitx2 expression and bilateral or reversed Nodal expression in LPM (lateral plate mesoderm).In $Dvl1^{-/-}$ $Dvl2^{+/-}Dvl3^{-/-}$, Nodal expression is normal, while in $Dvl1^{-/-}Dvl2^{-/-}Dvl3^{+/-}$, Nodal is absent in LPM, contrast to a common aberrant phenotype. Provided Wnt11bMO has a flow-independent effect on Xnr1/Coco expression in lateral GRP in Xenopus (Walentek et al. 2013), it's curious to examine Dvl for such effect.

During the development of node, the basal bodies dock to the central apical surface of the node cells and subsequently relocate to the posterior end (Hashimoto et al). This phenotype establish an instructive role of posterior polarisation upon permissive role of apical docking of the basal bodies. Indeed, Vangl2 is shown to regulate trafficking apical PCP proteins to the cell cortex (Mahaffey et al. 2013), including Celsr1.The exact function of Dvl remains elusive To examine whether ciliogenesis interfere with PCP polarisation, Mahaffey et al. generated *Ift88(Polaris)*-null embryo and demonstrated an intact polarisation of Vangl2/Celsr1 along AP axis, thus confirm PCP can establish even in absence of a primary cilium.

Building up the model, initial molecular cue setting up PCP has been probed. In mouse node, Mahaffey et al. repress secretion of Wnt ligands pharmacologically and observed intact cellular polarisation but failed node-wise coordination of *Celsr1*. In Xenopus GRP, reducing or increasing Wnt11b activity disturbs polarised positioning of cilia (Walentek et al. 2013).Unfortunately, direct observation of localisation of PCP proteins is either undisclosed or unavailable, possibly due to inability of immunohistochemistry in Xenopus.

3. Support for a flow-independent role of PCP

Despite the discrepancy between flow defect and LR defect, Vangl2 plays a role in chick LR patterning where a ciliary flow is absent in normal development (Zhang & Levin 2009) The proximal-distal polarisation of Vangl2 in chick is indeed the first evidence of intracellular PCP protein's polarisation along the LR axis, allowing perinodal PCP's a role in at least containing LR asymmetry information. Indeed, they propose desynchronized *Shh* expression as observed results from disrupted PCP. No recent study further this observation. It would be interesting to treat half of the embryo with Vangl2-MO.to examine a causative connection

Another compelling evidence comes from MO studies of Xenopus(Vandenberg & Levin 2012) . Embryo-wide MO-mediated disruption of ABP and PCP both led to heterotaxia (23% and 26% respectively). Specifically, when MO is targeted to progenitor of non-GRP cells, heterotaxia is still induced (30% and 15% respectively) whereas cilia positioning is unaltered. Moreover, they showed intact PCP/ABP is required for an ectopic organiser in a conjoined twin to correctly pattern LR asymmetry.

4. Conclusion and future

Taken together, polarised PCP localisation is evidenced in the node and abrogating core PCP proteins disrupts the posterior positioning of cilia without affecting

ciliogenesis, which in turn abolishes the leftward flow, evidencing an instructive role of PCP. However, defective leftward flow does not always lead to LR defect and sometimes no LR defect. While robustness LR patterning could accommodate this discrepancy (Shinohara et al. 2012), a flow-independent role of PCP is also possible (Walentek et al. 2013) since PCP polarisation is at cellular level and not specific to cilia. Indeed, flow-independent role is emerging (Zhang & Levin 2009; Vandenberg & Levin 2010), though it is challenging to link such data to existing knowledge of PCP (Blum et al. 2014; Wallingford 2012) and little continuation studies was done.

Provided disconcordance between flow defect and LR defect, it would be worthwhile to examine PCP mutants previously reported for LR normal for cilia polarisation(Hashimoto & Hamada 2010) both to see whether it could bridge the gap and to refine the molecular details of this PCP system. Hensen's node in chick would remain to be explored for novel PCP function(Zhang & Levin 2009). New technique is available for zebrafish (Borovina et al. 2010) and MO-treatment in Xenopus enables statistical analysis. There is yet much to explore.

5. References

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