Developmental Biology 377 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review

The mechanisms of planar cell polarity, growth and the Hippo pathway: Some known unknowns

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ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form
26 January 2013
Accepted 28 January 2013

Keywords:
Planar cell polarity
Growth
Gradients
Morphogens
Hippo
Fat
Dachsous
Wnt
Frizzled
Van Gogh
Starry night

ABSTRACT

Planar cell polarity (PCP) is a small but important area of research. In this review we discuss a limited number of topics within the PCP field, chosen because they are difficult, unsolved, controversial or just because we find them interesting. Because *Drosophila* is the best studied and technically most amenable system we have concentrated on it, but also consider some examples from work on vertebrates. Topics discussed include the number of genetic pathways involved in PCP, as well as the causal relationship between embryonic axes, gradients of morphogens and PCP itself. We consider the vexed question of the roles of the Wnt genes in PCP in both vertebrates and *Drosophila*. We discuss whether the proteins involved in PCP need to be localised asymmetrically in cells in order to function. We criticise the way the Hippo pathway is described in the literature and ask what its wildtype function is. We explore afresh how the Hippo pathway might be linked both to growth and to PCP through the gigantic cadherin molecule Fat. We offer some new ways of making sense of published results, particularly those relating to the Frizzled/Starry night and Dachsous/Fat systems of PCP.

Contents

Introduction . . . 1
Operational approach to the mechanisms of PCP . . . 2
How many genetic pathways in PCP? . . . 2
PCP, axes, compartments, morphogens and gradients . . . 3
Using genetic mosaics to map polarity gradients . . . 3
Polarised denticles in the larval abdomen . . . 5
What are the polarising signals in *Drosophila* and vertebrates? . . . 5
The asymmetric localisation of PCP proteins: Is this a cause or an effect of PCP? . . . 5
What is the wildtype function of the Hippo pathway? . . . 6
Acknowledgements . . . 7
References . . . 7

> "There are known knowns; there are things we know we know. There are known unknowns; we know there are some things we do not know. There are also unknown unknowns; we don't know we don't know."
>
> Donald Rumsfeld (United States Secretary of Defense), February 12th 2002.

Introduction

Planar cell polarity (PCP) refers to the polarity of a cell *within* the plane of an epithelium (Nübler-Jung et al., 1987); it is different from apico-basal polarity both conceptually and mechanistically. PCP is an over-reviewed subject; the many reviews mostly rehash the same experimental findings, testing the patience of the reader (for a comprehensive list of recent reviews see Yang, 2012). Our aim is to test the patience of the reader in an alternative way; in reviewing PCP we emphasise uncertainties which have been forgotten or ignored. We also

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0012-1606/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2013.01.030

discuss the relationship between PCP and growth, a topic that resembles a minefield.

Over the last 100 years or more, embryologists have concentrated on how cells know their place in the embryo, on how such positional information (Wolpert, 1996) is conveyed and interpreted to determine a cell's identity as well as the fates of its daughter cells. Positional information is usually encoded in a pervasive gradient, the concentration of a morphogen at each locale giving scalar information to the cells (Lawrence, 2001a). But identified cells in embryos also need to move in one particular direction or send an axon in one direction or divide and migrate to grow preferentially in one direction. Thus, to build an animal properly, embryonic cells must have access also to vectorial information. This vectorial information can be directly and simply expressed in the orientation of subcellular and/or multicellular structures such as stereocilia in the inner ear, bristles on a fly or mammalian hairs (Goodrich and Strutt, 2011). But orienting a cell is not simple and depends on diverse inputs and processes — a hidden complexity that has lead to confusion and disagreement amongst experts.

During the history of embryology few scientists have studied PCP, and this is largely because of the dictates of fashion, but also because research into PCP has proved difficult. Why? One reason is that PCP is a contextual phenomenon — what matters is the alignment of a cell with respect to the axis of an appendage (distal or proximal?) or of an embryo (anterior or posterior, dorsal or ventral?). Thus PCP needs to be studied in context, *in situ* and *in vivo* and these can be demanding requirements. Also there is another hindrance, studies of PCP have been limited because, although some cells make conspicuous and oriented outgrowths, the polarity of most cells is concealed. This difficulty can sometimes be overcome: noone had seen PCP in the *Drosophila* blastoderm and yet, if one protein, Slam, is artificially over-expressed at that early embryonic stage, these apparently unpolarised cells place Slam along the antero-posterior axis of the cell (Lecuit et al., 2002; Zallen and Wieschaus, 2004) suggesting that components of a PCP machinery are present and active. Nevertheless, PCP has been mostly investigated in systems in which the polarity of each cell (or group of cells) is signalled by oriented structures. This restriction of itself is benign, but it can foster the dubious assumption that plain epithelial cells, those that have no outgrowths, are unpolarised. The number of developmental phenomena recognised as depending on PCP has increased massively in recent years. The phenomena include cell migration, as in convergent extension and in neurulation, neurogenesis, axonal guidance, dendritic branching, kidney morphogenesis and vasculogenesis (Wang and Nathans, 2007; Gao, 2012).

It is not yet clear whether the basic mechanisms of PCP are universal, although this is argued by the conservation of the main genes from flies to mammals. But, in any case, it always makes sense to focus research on the most convenient system. For PCP there is no doubt this system is *Drosophila* and the reasons are mainly technical. *Drosophila* of course has plenty of genetics but also has tissues consisting of simple monolayers of cells, with each cell displaying its polarity in cuticular structures. Also, no system has better methods of marking genetic mosaics, cell by cell. For these reasons we will concentrate here on flies, with short excursions to mammals.

Operational approach to the mechanisms of PCP

Cell interaction is at the heart of PCP. Cells are polarised in response to information coming from other cells: this can be of two kinds. There can be long range information defining an embryonic axis that derives from a morphogen gradient. There can be short range information that coordinates the polarity of neighbouring cells. We need to understand the nature of these types of polarising information and ask how they are sent and received. One approach is to try to identify the genes needed in sending cells and discriminate them from those needed in the receiving cells. To do this genetic mosaics have proved essential, both in *Drosophila* (see for example Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987; Taylor et al., 1998; Wolff and Rubin, 1998; Chae et al., 1999; Usui et al., 1999; Casal et al., 2002; Strutt and Strutt, 2002; Yang et al., 2002) and in vertebrates (see for example Jessen et al., 2002; Wada et al., 2005, 2006; Devenport and Fuchs, 2008).

How many genetic pathways in PCP?

In *Drosophila*, spontaneous mutations that cause bristle disorientation such as *frizzled* (*fz*) (Gubb and Garcia-Bellido, 1982; Adler et al., 1987; Vinson and Adler, 1987), *dachsous* (*ds*) (Adler et al., 1998) and *fat* (*ft*) (Casal et al., 2002; Strutt and Strutt, 2002; Yang et al., 2002) were later augmented by genes discovered through dedicated screens, such as *starry night* — *stan*, also known as *flamingo* — (Chae et al., 1999; Usui et al., 1999) and *Van Gogh* — *Vang,* also known as *strabismus* — (Taylor et al., 1998; Wolff and Rubin, 1998). Studies on these genes have established that there are (at least) two sets of genes that drive PCP:

1. the Ds/Ft system which incorporates at least two other key proteins, Dachs and Four-jointed (for a review see Thomas and Strutt, 2012).
2. the Fz/Stan system that incorporates at least one other key protein, Vang (for a review see Adler, 2012).

In many recent papers the number of independent PCP systems (one or two?), a central issue, is usually described simply as controversial and left unresolved. In our opinion the one-pathway hypothesis, that the proteins of the Ds/Ft system act upstream to drive the Fz/Stan system, is justified more by tradition than by logic. The arguments for this hypothesis are weak and the experimental evidence flawed — discussed in Lawrence et al. (2007). Against this hypothesis there is one piece of evidence that trumps all the other less persuasive arguments that can be marshalled on both sides: this is the demonstration that, in the absence of a functioning Fz/Stan system, cells containing different amounts of Ds or Ft can polarise responding cells effectively and *in vivo* (Casal et al., 2006). Thus the Ds/Ft system can act very well without the Fz/Stan system. However others do not agree with this interpretation and have argued that the Stan mutant genotype we used to inactivate the Fz/Stan system might not do so sufficiently (see Axelrod, 2009; Peng and Axelrod, 2012). We find that argument feeble, for two reasons: (1) the Ds/Ft signal can still repolarise cells of this Stan mutant genotype even when, in addition, Fz is completely removed from the fly and (2) the same Stan mutant genotype we used completely blocks the ability of fz^- cells or cells that over-express *fz* to polarise the responding cells *in vivo* (Casal et al., 2006). And there is more evidence in favour of the independence of the two systems that comes from the adult abdomen. Although in the *A* compartment the orientations of the Ds/Ft and Fz/Stan systems are concordant (as they should be if they were part of one pathway), they oppose each other in the *P* compartment (see below).

Others maintain that, since our two-pathway conclusion depends on results in the abdomen, it might not apply to other organs such as eye and wing. This opinion could be correct, but it makes little sense to us as fundamental mechanisms are normally conserved from organ to organ and usually from species to species. Indeed, there is some evidence for two pathways acting