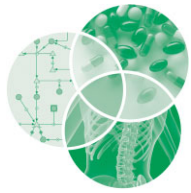
Advanced Review



Modeling the control of planar cell polarity

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A growing list of medically important developmental defects and disease mechanisms can be traced to disruption of the planar cell polarity (PCP) pathway. The PCP system polarizes cells in epithelial sheets along an axis orthogonal to their apical–basal axis. Studies in the fruitfly, *Drosophila*, have suggested that components of the PCP signaling system function in distinct modules, and that these modules and the effector systems with which they interact function together to produce emergent patterns. Experimental methods allow the manipulation of individual PCP signaling molecules in specified groups of cells; these interventions not only perturb the polarization of the targeted cells at a subcellular level, but also perturb patterns of polarity at the multicellular level, often affecting nearby cells in characteristic ways. These kinds of experiments should, in principle, allow one to infer the architecture of the PCP signaling system, but the relationships between molecular interactions and tissue-level pattern are sufficiently complex that they defy intuitive understanding. Mathematical modeling has been an important tool to address these problems. This article explores the emergence of a local signaling hypothesis, and describes how a local intercellular signal, coupled with a directional cue, can give rise to global pattern. We will discuss the critical role mathematical modeling has played in guiding and interpreting experimental results, and speculate about future roles for mathematical modeling of PCP. Mathematical models at varying levels of inhibition have and are expected to continue contributing in distinct ways to understanding the regulation of PCP signaling. *WIREs Syst Biol Med* 2011 DOI: 10.1002/wsbm.138

INTRODUCTION

The orientation of epithelial cells along an axis orthogonal to the apical–basal axis is referred to as planar cell polarity (PCP). PCP signaling controls the polarity of numerous epithelial cell types in both *Drosophila* and vertebrates (Figure 1). In *Drosophila*, the most intensively studied planar polarized tissue is the fly wing, in which each cell produces a trichome ('hair'), that in wild type, emerges from the distal side of the cell and points distally.[1] PCP mutants cause disruption of this pattern. PCP also orients asymmetric cell divisions such that fate determinants are properly inherited by a single daughter (i.e., the *Drosophila* pI sensory organ precursor cells[2–4]), and determines rotation and chirality of cell clusters in the *Drosophila* eye.[5–7] In vertebrates, the PCP signaling modules identified in flies are conserved, and interact with diverse systems to produce planar polarized structures.[8–12] Defects in PCP result in a range of developmental anomalies and diseases including open neural tube defects,[13] polycystic kidneys,[8,11,13,14] conotruncal heart defects,[15–18] and disruption of sensory hair cell polarity causing deafness.[19–29] PCP is also believed to underlie the directed migration of malignant cells during invasion and metastasis.[30–34] A growing body of evidence suggests that the molecular mechanisms controlling PCP in flies are well conserved in vertebrates, albeit with some additional layers of regulation (reviewed in Refs 8, 12). Unfortunately, the PCP signaling modules and their interactions are as yet poorly understood, thereby limiting the potentially substantial opportunities for therapeutic interventions.

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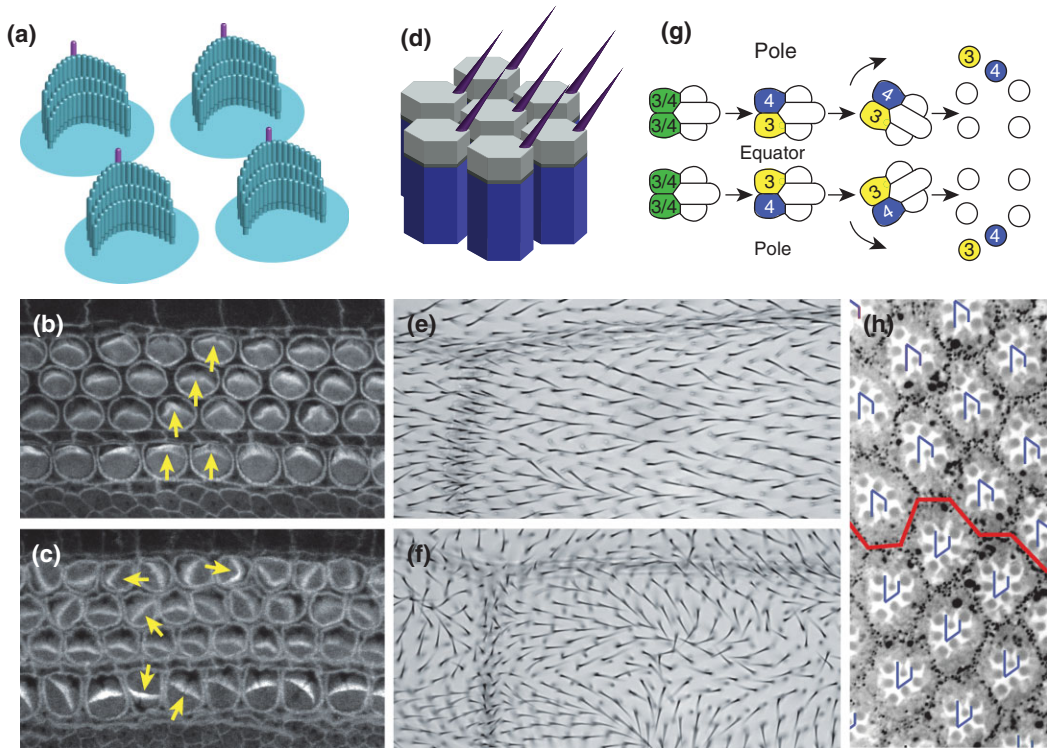

FIGURE 1 | Planar cell polarity. (a) Cartoon of cochlear hair cells showing asymmetrically localized and coordinately oriented kinoclila (purple) and stereocilia (blue). (b) Wild-type and (c) *Lp/Lp* mutant cochlear hair cells stained with phalloidin to show actin. Orientation of some cells is denoted with arrows. (d) Cartoon of Drosophila pupal wing cells with nascent hairs emerging from the distal vertex and pointing distally. (e) Wild-type and (f) dishevelled-1 wings showing hair polarity (distal is right). (g) Chiral and oriented ommatidia in the fly eye. Equipotent R3/R4 cells (green) differentiate to R3 (equatorial; yellow) and R4 (polar; blue) according to their relative position along the equatorial-polar axis. Ommatidia then rotate in opposite directions in the dorsal (top) and ventral (bottom) eye fields. (h) Wild-type eye showing ommatidia in the dorsal (top) and ventral (bottom) eye fields. Orientation is noted with blue arrows. Images in (b) and (c) were kindly provided by M. Montcouquiol and M. Kelley.

The planar polarity of cells in an epithelium constitutes a large scale pattern. The typical wild-type pattern is a simple parallel array, but mutations of PCP signaling components may produce more complex patterns. Clonal disruption of the PCP signaling mechanism not only disrupts polarity within the clone, but in some cases causes characteristic changes in the polarity of neighboring, nonmutant cells. These mutant patterns, described below, hold important clues to understanding the signaling mechanisms governing PCP.

The goal of this article is to describe how various forms of mathematical modeling have contributed in different ways to understanding the relationship between the molecular signaling events underlying PCP and the large scale patterning that is observed in wild-type and mutant animals. Mathematical modeling has allowed investigators to test predictions concerning perturbation of the molecular machinery, and therefore better understand those molecular mechanisms. In addition, it has provided a window into understanding control features that allow the system to function robustly. We will highlight the role mathematical modeling has played in this process, and suggest that mathematical modeling will become an increasingly important tool in deciphering the relationship between molecular mechanisms and the resultant developmental patterns they control. We will not attempt to provide an exhaustive discussion of what is known and not known about the molecular details of PCP signaling, either in vertebrates or in *Drosophila*, nor to describe the variations observed in different planar polarized tissues, though these are important topics. Numerous reviews discuss these topics.[6,9,35–37]

PLANAR CELL POLARITY

Drosophila as a Model System for PCP

The greatest strides in understanding PCP have been made using *Drosophila* as a model system. A powerful set of genetic, molecular and cell biological tools, together with easily observable phenotypes, make *Drosophila* an extremely attractive system for investigating the controls governing PCP.

The Genetic Basis for PCP

PCP in *Drosophila* is seen in most cells secreting the adult cuticle.[38] Cells on most of the body surface produce a single trichome, or hair, and the hairs are polarized with respect to the axis of the body or appendage (Figure 1). Hair polarity has been most intensively studied in the wing and to a lesser extent in the abdomen. Studies of the hair producing cells of the wing have been the most informative in revealing the connections between molecular signaling and large scale patterning, and most mathematical modeling of PCP has been based on hair polarity, so these will be the focus of our discussion, with some reference also to work in the eye and abdomen.

Genetic and molecular analyses in *Drosophila* suggest that the PCP signaling components may be divided into three interacting groups that we refer to as modules: a global directional module, a core module, and a suite of tissue specific effector modules that respond to the upstream modules to produce morphological asymmetry in individual tissues. The 'core' PCP module coordinates polarity between neighboring cells in all tissues, and includes Frizzled (Fz[39]), Flamingo (Fmi; aka Starry night[40,41]), Van gogh (Vang[42,43]), Prickle-spiny-legs (Pk[44]), Dishevelled (Dsh[45,46]), and Diego (Dgo[47,48]). Early genetic analyses of the core PCP genes led to the view that the core components signal in a linear fashion from the receptor, Fz,[49] through a downstream, cytoplasmic transducing protein, Dsh,[50] providing output to the various effector modules (reviewed in Refs 50, 51), although it now appears to be nonlinear in nature. Most effector components show some tissue specificity, and while less is known about them as compared to the upstream components, a reasonable view is that they couple signaling from the core proteins to the cell-type specific responses required to generate PCP in the individual tissues (reviewed in Refs 7, 50, 52). Like the core module, the global module also influences PCP in all tissues, and comprises the golgi protein Four-jointed (Fj[53]), and the atypical cadherins Dachsous (Ds[54]), and Fat (Ft[55–59]). These proteins were proposed to function upstream of the core components, transducing global directional information to the core PCP proteins.[56,60–62] While this view has been challenged, with some arguing that the global and core modules function in parallel,[63,64] we believe the arguments for the parallel model are not compelling (reviewed in Ref 37).

Cellular Phenotypes Correlate with Activity of the Core PCP Module

The cellular phenotypes of wild-type and mutant hair cells in the wing, first characterized in detail by Wong and Adler,[1] are diagnostic for the three modules of the mechanism, and as described below, correspond to molecular asymmetry and orientation (or lack thereof) within the cell. The location for prehair initiation is the critical event in establishing polarity: for hairs emerging away from the center of the cell, the hair is always observed to point outward from the center.[1] In wild type, the uniform placement of the nascent prehairs at the distal edge of the cell reflects the ability of the cell to distinguish that edge on a molecular level. Mutation of the core PCP genes causes the prehairs to initiate and grow at (*dsh*) or at a variable distance from (*fz*, *dsh*, *Vang*, *pk*, *fmi*, and *dgo*) the center of the cell, reflecting a complete or partial failure to achieve molecular asymmetry[1,37,65] (Figure 2). Mutants affecting the downstream effector module in wing cells (*inturned*, *fuzzy*, *multiple wing hairs*, the *Drok* pathway) typically produce multiple prehairs that often initiate at aberrant locations around the cell periphery[1,66] (Figure 2), reflecting uncoupling of the effector systems from the molecular asymmetry produced by the core proteins. Mutants in the global components *fj*, *ds*, and *fat* do not abolish cellular asymmetry, invariably producing prehairs at the periphery as in wild-type cells.[61,67] However, the prehairs often fail to correctly choose the distal edge. Furthermore, the prehair locations chosen by neighboring cells are highly correlated in swirling patterns, reflecting a loss of overall directionality, yet keeping intact the ability of the core module to produce subcellular asymmetry and to coordinate local alignment.

Considerable attention has been paid to the fact that in mutants of the core PCP genes, the prehairs do

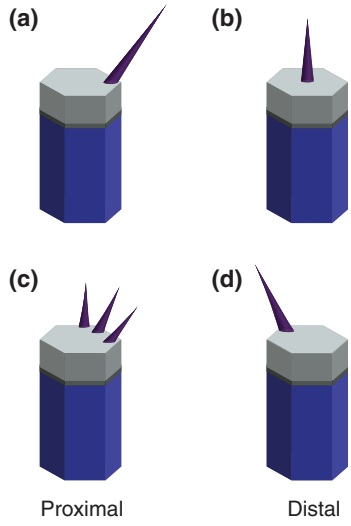

FIGURE 2 | Cellular phenotypes of wing hair cells. (a) Wild-type, (b) core gene mutants, (c) downstream effector mutants, and (d) global directional mutants.

not adopt a random polarity, instead forming stereotypical patterns in which hairs on a given region of the wing tend to point in a given direction. These mutants produce little or no molecular asymmetry of PCP proteins, and prehairs tend to grow at or near the center of the cell; thus they are distinct from those observed in the global mutants. Furthermore, these patterns are largely the same in double mutants of the core and global modules.[56] Therefore, a reasonable conclusion is that the stereotypical patterns of hair orientation seen in core mutants arise through an unknown mechanism not related to known components of the PCP signaling pathway (see for example Ref 68).

Clonal Phenotypes: Autonomy and Nonautonomy

In wild-type fly wing and abdomen, the observed polarity pattern is a simple parallel array of polarized cells, each producing its hair on the same side of the cell. However, the patterns become more interesting when clones of cells lose or overexpress one or more components of the system, as described below (Figure 3). The resulting patterns indicate transmission of polarity information between cells, and have served as a rich source of clues to the mechanism that underlies polarization.

Clones mutant for the core PCP genes *fz* and *Vang* produce misorientation of neighboring, nonmutant tissue, a phenomenon referred to as domineering nonautonomy.[42,69,70] Remarkably, *fz* mutants induce polarity reversals on the distal side of clones, while *Vang* induces polarity reversal on the proximal side (Figure 3). While most *fz* clones display domineering nonautonomy, some alleles produce nearly autonomous polarity defects,[70,71] as do the remaining core PCP genes.[40,45,46,69,72] Overexpression clones of the core components each produce a characteristic nonautonomous effect as well (summarized in Ref 65). Clones of the upstream components *ds* and *fat* often, but do not always produce PCP defects.[56,61,73] However, those that do produce a variable domineering nonautonomy, with much less directional bias than is seen around *fz* and *Vang* clones.[56,61] Results are largely similar for the abdomen.[62,74,75]

Domineering Nonautonomy and the Diffusible Factor ('Factor X') Models

The observation of domineering nonautonomy prompted researchers to propose models in which cells respond to an initial cue by making and secreting a diffusible second factor, resulting in a distribution gradient that determines polarity[38,74,76–87] (Figure 4).

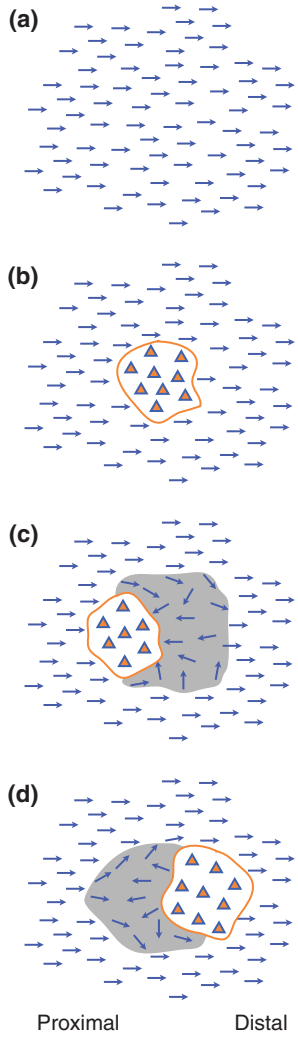

FIGURE 3 | Cartoons of clonal phenotypes. (a) Wild-type polarity with all hairs pointing distally. (b) A cell autonomous mutant clone (orange outline) showing loss of polarity within the clone (triangles) and normal polarity surrounding the clone. (c) Mutant clone with distal domineering nonautonomy (gray shaded area) characteristic of *fz* mutant clones. (d) Mutant clone showing proximal domineering nonautonomy characteristic of *Vang* mutant clones. See Figure 7 for phalloidin stained pupal wing clones showing these phenotypes.

These models, referred to as the 'factor X' models for the unidentified diffusible factor, were first formalized with reference to phenotypes in the eye.[77] In the eye, Wingless (Wg; a Wnt protein) signals in a gradient that is highest at the poles and lowest at the equator. Wehrli et al. proposed that Wg signaling induces the dose dependent secretion of a diffusible factor X, which in turn regulates Fz signaling via the gradient of factor X distribution. Clonal disruption of the response to Wg would perturb the factor X gradient both in the mutant and the neighboring wild-type tissue, inducing a gradient reversal that would cause the

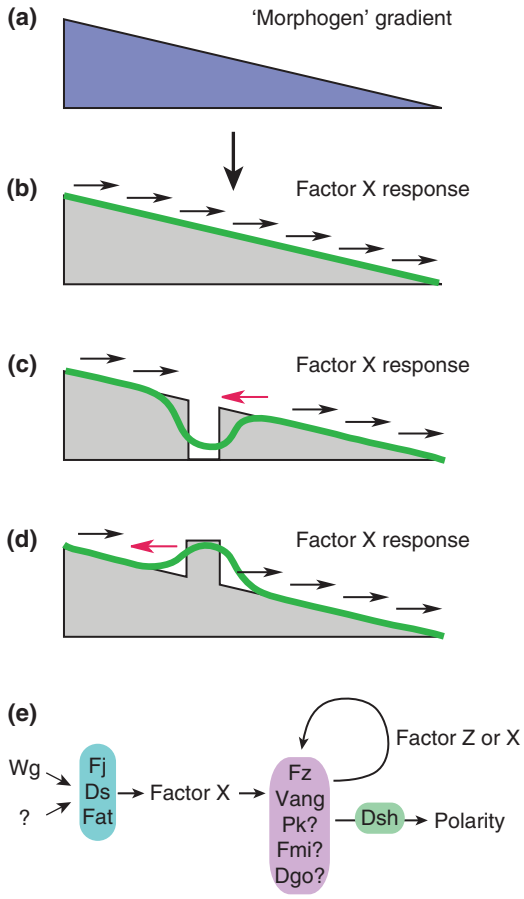

FIGURE 4 | Diffusible factor model. An upstream signal (a; blue), such as Wg in the eye, induces a proportional expression of a diffusible factor X (gray). (b) In wild type, factor X distributes in a gradient (green) whose slope determines polarity (arrows). (c) In clones that do not respond to the upstream signal, the factor X expression profile results in a distribution profile (green) with slope reversal, causing polarity reversal distal to the clone (red arrow). (d) Clones with augmented response to the upstream factor result in a distribution profile showing polarity reversal on the proximal side of the clone. (e) Factor X and factor Z signaling scheme.

observed nonautonomy. A variation of this model was subsequently offered to explain the *fz* and *Vang* clonal phenotypes in the wing. In response to an unknown, graded, upstream signal, Fz and Vang were proposed to regulate production of a diffusible 'factor Z' whose gradient is read out to produce polarity.[38,79] The factor X and factor Z models may be unified: since factor Z might feed back through Fz, factors X and Z could be the same molecule.

These models have also been asked to account for the observation that clones of cells mutant for most *fz* alleles, including null alleles, produce domineering nonautonomy, but clones of cells mutant for certain *fz* alleles produce a nearly cell autonomous phenotype.[69–71] In addition, *dsh* mutant cell clones produce nearly cell autonomous polarity disruptions.[45,46,65] The diffusible factor models explain these observations by positing that Fz mediates two separably mutable signaling functions: a cell autonomous, Dsh dependent, signaling function and a Dsh independent nonautonomous signal.[41,42,73,88] The nonautonomous signal might be mediated by factor Z, that is produced in a dose dependent response to Fz signaling, while the autonomous Dsh dependent signal would initiate cell polarization.[38,79]

Despite its attractiveness, challenges exist for the diffusible factor model. Although the model proposes an autonomous Dsh dependent Fz signaling pathway and a separably mutable nonautonomous Fz signal, *dsh* clones show a small though significant amount of nonautonomy[65,89] (P. Adler, personal communication) that is difficult to reconcile with this distinction. Furthermore, while the molecular mechanism of Fz signal transduction is not well understood, there is no molecular evidence for separable signals upstream of Dsh. The controversial proposal that Fz signals through a heterotrimeric G protein suggests that the G protein would function in Dsh dependent pathways[90] (see Ref 91 for review of evidence from higher organisms). Finally, despite tremendous efforts, diffusible factors X or Z have not been identified.[74] A Wnt protein is a logical candidate for factor X or Z, but no Wnt has been identified to play this role in the fly.[74,92] In contrast, Wnt proteins have been shown to function in vertebrate PCP pathways (see Refs 93–95 for examples). The evidence, while somewhat difficult to interpret, is most consistent with Wnts being permissive rather than having an instructive role as predicted by factor X models.[93,95–97]

The Molecular Basis of Subcellular Asymmetry

The finding that core PCP proteins localize asymmetrically within the cell paved the way for a more mechanistic understanding of subcellular asymmetry. Uemura et al. found that *fmi* mutants display PCP phenotypes, and that Fmi protein appears to localize to both the proximal and distal sides of wing cells prior to prehair formation, where it most likely forms a homodimer spanning the intercellular boundary.[40] This was the first molecular indication that this boundary becomes specialized during PCP signaling, although its bipolar localization indicated that Fmi could not be the component that distinguishes proximal from distal within the cell. Two subsequent reports identified unipolar protein localization and therefore a molecular basis for distinguishing one side of the cell from the other. Fz and Dsh were both

found to localize to the distal side of the cell before prehair formation.[98,99] Subsequently, Pk and Vang were found to localize proximally, and Dgo distally with Fz and Dsh.[47,100,101] These findings suggested that localization of core proteins might provide cues for morphologic cellular asymmetry.

A Local Signaling Model for Fz Dependent PCP Signaling

The possibility that distally localized Fz and Dsh designate the distal side of cells for prehair initiation raised the critical question of how these proteins achieve their asymmetric localization. Based on genetic mosaics, subcellular localization and biochemical assays, Tree et al.[72] proposed a feedback loop mechanism that mediates a competition between proximal and distal proteins displayed on adjacent surfaces of neighboring cells (Figure 5). This model had features similar to insightful models proposed earlier by Adler et al. that were proposed without the benefit of subcellular localization data.[42,102] Largely on the basis of mosaic analyses of clones lacking or overexpressing individual components, it was deduced that these proteins communicate at cell boundaries, with the distal proteins (Fmi, Fz, Dsh, Dgo) recruiting the proximal proteins (Fmi, Vang, Pk) to adjacent cell boundaries, and vice versa. Through the function of a feedback mechanism, these proteins become strongly polarized.[65,100] Mutual exclusion of proximal and distal complexes appears to promote an all-or-none accumulation in one or the other orientation. This module therefore behaves as a bistable switch, producing a strongly biased accumulation of complexes oriented in one direction, and producing local alignment between neighboring cells.

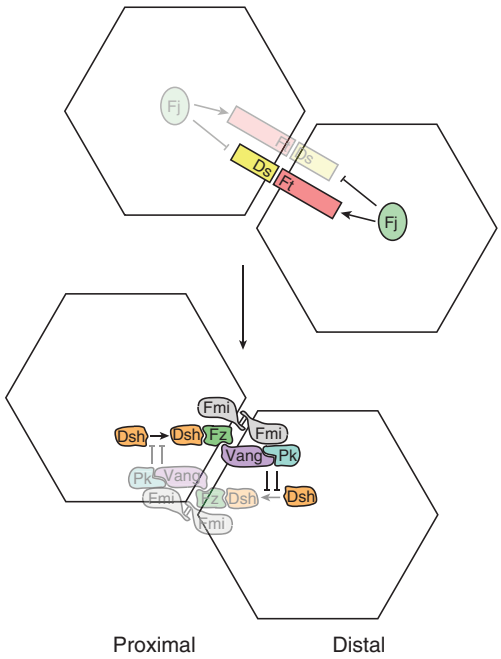

FIGURE 5 | Cartoon of the Fz feedback loop proposed by Tree et al. (without Fmi and Vang) as subsequently modified by Amonlirdviman et al.[65,100](with Fmi and Vang; bottom). A cartoon of a possible mechanism for Fj, Ds, and Ft function that biases Fz feedback loop function is also shown (top). Pale symbols represent species with reduced concentration.

Specific roles for the core proteins in this process are not well understood. Fmi, Fz, and Vang are thought to communicate information between cells, while the remaining proteins appear to participate in amplifying asymmetry.[40,75,92,103] Functionally asymmetric Fmi homodimers have been proposed to communicate information between adjacent cells,[92] though others have suggested that Fz and Vang communicate directly.[104] Dgo competes with Pk for binding to Dsh,[48,105] suggesting that Dgo may reinforce the difference between the proximal and distal complexes. Aside from the demonstration of some binding interactions *ex vivo,* the feedback competition mechanism is very poorly understood at a biochemical level.

More recently, directed vesicular transport and recycling of PCP components have been implicated as intrinsic parts of this mechanism.[37,106–109] While it is likely that these are important elements of the mechanism, and will undoubtedly attract considerable attention in the future, they are just now being incorporated into mathematical modeling efforts, so will not be discussed at length here.

Though not as extensive, studies of the fly eye are consistent with a similar mechanism.[48,80,110,111] Subcellular localization has not yet been examined in the abdomen, though it seems likely that findings will be similar. It is important to point out that most of the observations that contributed to the local feedback model could also be consistent with a factor X model. Furthermore, it is not intuitively obvious to all observers[38,73] that the local feedback loop model can explain the range of autonomous and nonautonomous clonal phenotypes that have been observed.

The Global Directional Cue

In addition to a local alignment mechanism, the PCP process requires a global directional cue to orient cell polarity with respect to the tissue axes. Based on data from both eye and wing, a model involving Fat, Ds, and Fj was proposed as an alternative to hypothetical diffusible factors in providing a global directional signal.[60,61] This global module is proposed to function by converting expression gradients of Fj and Ds into

asymmetric subcellular arrays of Fat-Ds heterodimers. Fat and Ds colocalize in uniformly distributed puncta in the marginal zone, forming heterodimers that are proposed to occur in a biased orientation along the proximal-distal axis.[61] Golgi localized Fj acts on both Fat and Ds, perhaps via an ectokinase activity to make Fat a stronger ligand, and Ds a weaker ligand, for the other.[112–114] The graded expression of Fj and Ds[58,60–62,75] therefore produce an excess of Fat-Ds heterodimers in one orientation relative to the other at proximal-distal boundaries, according to the Fj and Ds gradients[61] (Figure 5). Directional information present in the transcriptional gradients of Fj and Ds is thereby converted into subcellular gradients of Ds and Fat, which then signal to downstream components by an unknown mechanism to regulate orientation of polarization. An attractive model is that these components orient or polarize the apical microtubule web along which PCP components traffic.[37,108,115,116] The protein phosphatase subunit Wdb, the transcription factor Atrophin, and the Par-1 kinase may have roles in transducing the Fat signal.[81,109,116]

Gradients of Fj and Ds are present in eye, wing and abdomen, and are therefore good candidates for providing a global directional signal.[58,60–62] Interestingly, these gradients may also control tissue size through a mechanism that senses their steepness.[117,118] This model leaves several additional questions unanswered. If directional information is encoded in the gradients of Fj and Ds, then flattening these gradients should disrupt polarity, and this is seen in the eye.[119] However, the same intervention in the wing produces only a mild polarity phenotype, indicating that in this tissue, another source of directional information is likely to exist.[119,120] Furthermore, in the abdomen, the gradients of Fj and Ds are reversed in anterior and posterior compartments, yet the hairs point posteriorly in both. Similarly, these gradients are oriented oppositely with respect to the direction of Fz accumulation in the eye and wing. Therefore, if these gradients signal direction, the signal must be interepreted differently in different compartments.

Disruption of the global module highlights an important feature of the core module. Activity of the core module ensures that within small *fat* clones, cells almost always have normal polarity, and even within large clones, cells have a strong tendency to local alignment with their neighbors.[61,73] This tendency toward local alignment is also evident in the domineering nonautonomy seen in wild-type tissue near some mutant clones. Mutant clones that reverse the direction of core protein recruitment in wild-type cells at a clone boundary will propagate the reversed polarity for some distance through wild-type tissue.[61]

As noted above, some have argued that the Fat/Ds/Fj global module signals directly to effector modules, and does not provide directional input to the core module.[63,64] While we find these arguments uncompelling (reviewed in Ref 37), for the purposes of the following discussion of mathematical modeling, one can consider the modeled directional signals to be generic in nature, as no explicit assumptions were made as to whether they represent the Fat/Ds/Fj module or some other uncharacterized directional signal.

MATHEMATICAL MODELING OF PCP

A variety of modeling strategies to understand PCP have been adopted, with a range of strengths and weaknesses. At one extreme, models have been developed to relate the activities or localization of specific PCP proteins to pattern formation. Among the limitations of these is that they require computationally intensive numerical methods to analyze the model and identify parameters. Investigators interested in exploring higher-level properties of the PCP signaling system have developed a number of more abstracted models that sacrifice capturing (and therefore predicting) molecular detail, but allow analytical exploration of parameters and larger scale model behaviors.

Testing Molecular Models of PCP with Mathematical Modeling

A persistent challenge in understanding PCP has been to relate molecular interventions and observations to the emergent polarity patterns evident in and around mutant clones, and therefore to draw inferences about molecular relationships from the clonal phenotypes. Several distinct 'bottom-up'[121] mathematical models based on proposed functions of the core PCP protein have been used in efforts to understand the relationship between molecular mechanisms and patterning.

Lawrence et al.[75] created a simple algebraic model to test their proposal that Fz activity can be represented by a scalar value in each cell, and that a comparison of Fz activation levels between cells determines the direction of polarization (Figure 6). This model is intuitively appealing, but notably, it discards the notion that asymmetrically localized proteins play a role in polarization, and instead suggests that these localizations are a mechanistically unimportant consequence of polarization. Forced expression gradients of Fz are known to direct polarity from high toward low Fz levels.[84] Drawing on this observation, as well as a variety of other results, Lawrence et al. proposed that factor X, in an unspecified way involving, and most likely resulting

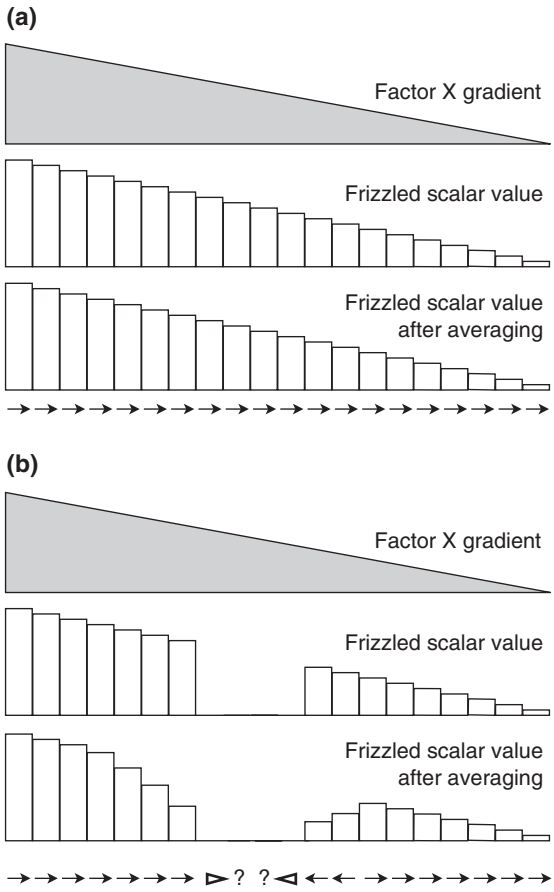

FIGURE 6 | Schematics of the Lawrence et al. algebraic model. (a) In wild type, the factor X gradient (gray) initiates Fz activation at scalar levels in each cell. After averaging, the resulting Fz levels are compared with neighbors to produce a vector determining polarity (arrows). (b) A wing with a *fz* clone cannot produce Fz within the clone, and after averaging, the scalar levels of Fz in neighboring cells are affected, altering the resulting polarity. Cells at the edges of the clone may be repolarized, depending on the mechanism of comparison and vector determination (arrowheads).

from Fj, Fat, and Ds function, induces a scalar level of Fz activation in individual cells, depending on their relative position with respect to the factor X gradient. Subsequently, each cell compares its level of Fz activation with its neighbors, using a mechanism requiring Fmi in each cell and Vang to sense the Fz activity level in its neighbor. Each cell is then proposed to adjust its Fz activity level from that determined by the initial scalar level toward an average of that in each of its neighbors. In a rough gradient, this would have the effect of smoothing the gradient. However, in and around clones that either reduce or abolish Fz activity (*fz* clones), or conversely elevate Fz activity (*Vang* clones), the gradient would be modified such that it would reverse its slope on either the distal or proximal sides, respectively. Finally, at the conclusion of the averaging process, the comparison process would determine a vector that describes the cells' polarity.

One-dimensional lines of cells were numerically simulated to demonstrate that this mathematical model recapitulates the distal domineering nonautonomy near a *fz* clone (Figure 6). By proposing that Vang antagonizes Fz activity cell autonomously, it also recapitulates the proximal domineering nonautonomy near a *Vang* clone. Furthermore, the distance to which domineering nonautonomy reverses polarity is controlled by a variable that represents the relative amount by which the averaging between neighbors and the reading of the original factor X gradient influence the adjusted scalar Fz value at each step. The model is attractive in that it provides an intuitive connection between the biological model and the resulting polarity, and it provides a demonstration that a model including factor X plus scalar Fz activation might explain at least some of the observed PCP phenotypes. Note that, depending on the mechanism by which the comparison and vector determination is accomplished (not specified), this model might explain the polarization of one row of *fz* or *Vang* mutant cells inside clones. It is not based on specific molecular interactions between signaling components, so it cannot be used to gain a mechanistic understanding of these interactions. Furthermore, there is no experimental evidence for a tissue wide Fz expression gradient, nor is there an assay for the hypothesized Fz activation that could be used to gain evidence supporting the possibility of a Fz activity gradient during planar polarization.[98,102]

Amonlirdviman et al.[65] have described a mathematical model designed to test the feasibility of the asymmetric protein/Fz feedback loop model proposed by Tree et al.[72] This model proposed that the autonomy or domineering nonautonomy of *fz*, *Vang*, *dsh*, and *pk* clones, and a variety of other phenotypes results simply from the dynamics of the proposed interactions (Figure 7). In this model, the global directional cue is provided as an asymmetric input to each cell. Though not explicitly modeled, this signal could result from the graded expression of Fj, Ds and, at least in the wing, other unknown signals, or from some other unidentified source.

The Amonlirdviman model represents the proposed Fz feedback loop solely as reactions between Dsh, Fz, Vang, and Pk to form protein complexes, with a feedback inhibition term and a global asymmetric input. These interactions are represented in a series of reaction–diffusion partial[65] and ordinary[122,123] differential equations that define the concentrations of proteins or protein complexes, and their evolution over time, at each location within each cell of

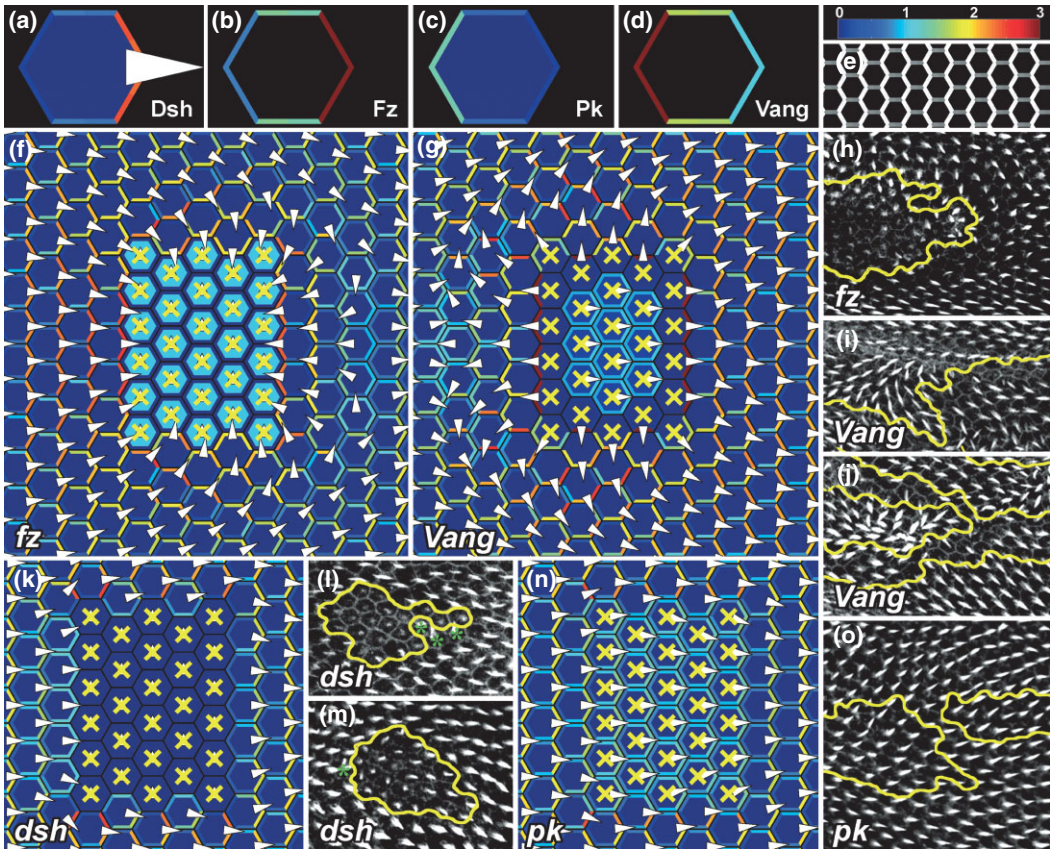

FIGURE 7 | Simulation results from the Amonlirdviman et al. model. Wild-type results showing the final distributions of Dsh (a), Fz (b), Pk (c), and Vang (d). The same color scale is used in all figures, where 1 is scaled to the initial uniform concentration of Dsh and the scale is truncated so that concentrations greater than 3 are shown in red. (e) Simulated distribution of Dsh displayed as an intensity representing total Dsh concentrations, corresponding to the appearance of Dsh::GFP in wild-type experiments. Simulation results of several PCP phenotypes showing the final distribution of Dsh with predicted hair growth directions derived from the vector sum of Dsh (f, g, k, and n) and corresponding pupal wings (h–j, l, m, and o). Greater Dsh asymmetry is represented by hair placement at increasing distances from the cell center. When Dsh asymmetry does not exceed the threshold value, the hair is depicted at the center of the cell. Mutant cells are designated with yellow. (h) fz^{R52} clones. (i and j) $Vang^{A3}$ clones. (l and m) dsh^{V26} mutant clones. (o) *pk-sple*^{13} clones. Asterisks mark nonautonomous effects near *dsh* clones. (Reprinted with permission from Ref 65. Copyright 2005 American Association for the Advancement of Science.)

a simulated two-dimensional cell grid. Given these equations, and a set of starting protein concentrations, one can then simulate the evolution of protein localization over time. Fmi and Dgo were not explicitly modeled, yet the logic of the model could easily be retained if they were included. The final polarity of a cell is taken as the vector sum of Dsh within that cell. Since the values for the parameters needed to instantiate the model are not known, they were identified through an optimization procedure in which the model was asked to simulate a representative set of PCP phenotypes. The first experiment was, therefore, to ask if a set of parameters could be identified that would produce a large set of characteristic PCP phenotypes. The results showed that the proposed feedback loop mechanism can recapitulate all PCP phenotypes that were examined, demonstrating that the Fz competition mechanism, combined with a global directional cue, is sufficient to explain domineering nonautonomy and other phenotypes, despite features of the model that may be counterintuitive[38,73] (Figure 7). The model is robust to parameter perturbation.

The Amonlirdviman model also led to a new insight important for understanding autonomous versus nonautonomous *fz* alleles. The results demonstrated that modeling a Dsh mutant protein rendered nearly incapable of being recruited to the plasma membrane in the presence of Fz (Dsh^1) produced a nearly (though not completely) cell autonomous clonal phenotype, as was known to be the case. It therefore followed that autonomous *fz* alleles that recruit Dsh poorly, should retain the ability to recruit Vang in the neighboring cell. In contrast, nonautonomous *fz* alleles (such as nulls) should exhibit impaired interactions with both Dsh and Vang. Simulations showed this to be the case. On the basis of these predictions, the

cell autonomous *fz* alleles were then experimentally demonstrated to retain their ability to recruit Vang to the boundary of neighboring cells while nonautonomous alleles were shown to lose this ability. The model therefore yielded a prediction that was experimentally confirmed, lending insight into the biological process.

The mode by which global directional information might impinge on the core module is not known. The Amonlirdviman model, and a subsequently modified version[124] allowed the authors to test the feasibility of global inputs in several different forms, including an identical gradient across each cell (as suggested by Ma et al.[61]), a scalar gradient across the tissue (similar to that suggested by Lawrence et al.[75]), and a monotonic gradient across the tissue (Figure 8). The results demonstrated that any of these forms of global directional input can produce correct results in conjunction with the feedback loop model. Therefore, the conclusions drawn from this work do not depend on the specific directional mechanism that is implemented. Additional experimental results will be required to determine how directionality is imposed on the core feedback loop mechanism.

The Amonlirdviman model demonstrates the feasibility of the proposed biological model to explain characteristic phenotypes without invoking diffusible factors, and provides insight into the molecular basis of autonomous and nonautonomous *fz* alleles. The underlying molecular interactions are explicitly defined, though they certainly reflect a simplification of the true molecular mechanism. The cost of increased detail is a potential loss of intuition: with ten differential equations and about 30 parameters, the key shortcoming of the Amonlirdviman model is in its complexity. In addition, it fails to provide testable predictions that might distinguish between models for the mechanism by which a directional signal influences the Fz feedback loop.

A third molecular-based mathematical model, presented by Le Garrec et al., is a reaction–diffusion model using stochastic difference equations.[125] The authors model a system based on a graded extracellular Fz ligand and asymmetric protein localization dependent on intracellular feedback loops (Figure 9). The model aims to test the requirements for such an extracellular ligand, and to ask whether introduction of intercellular interactions might be mediated by Fmi dimerization. The underlying biological model, while similar to that modeled by Amonlirdviman et al., differs in several essential ways. The authors used trial and error to explore how the model would react to a variety of initial ligand conditions, and found that to achieve the correct results for wild-type and mutant clones, the ligand should be assumed to be expressed at a limiting level in a gradient from proximal (high) to

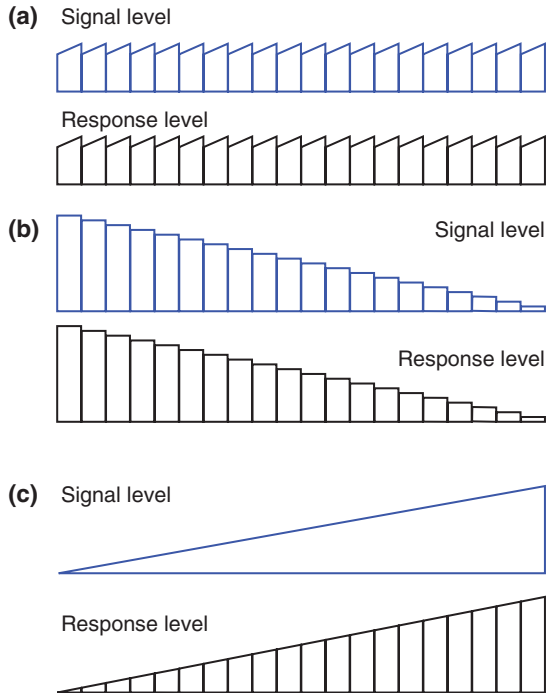

FIGURE 8 | Global signaling paradigms tested by the Amonlirdviman et al. model.[65] (a) Nonglobally varying, constant slope within each cell, as suggested by the global signaling model depicted in Figure 5. (b) Globally varying, uniform within each cell, similar to that proposed by Lawrence et al.[75] (c) Globally varying, constant slope. This signal can be viewed as a combination of the forms shown in (a) and (b). Depending on whether the intercellular or intracellular components predominate, the slope can be in either direction. As shown, the subcellular asymmetry dominates, as in (a).

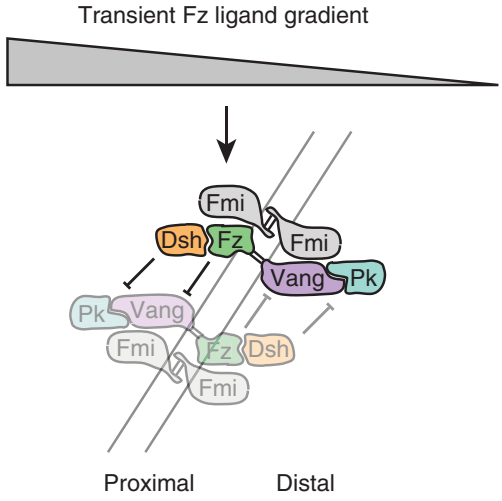

FIGURE 9 | Signaling mechanism modeled by Le Garrec et al.[125] using reaction–diffusion stochastic difference equations. Note that the feedback loops differ from those in Figure 5.

distal (low). Ligand-receptor (Fz) binding is rapid and irreversible, and the free ligand gradient must disappear early in the signaling process. The feedback loops adopt two hypothetical feedback mechanisms, different from those of Tree et al.,[72] as shown in Figure 9. Fmi is modeled as an agent mediating the interaction between Fz and Vang, and has higher affinity for heterotypic FzFmi–FmiVang complexes than for either homotypic complex including only Fz or only Vang. Furthermore, complex assembly is restricted to an ordered set of additions, eliminating some possible species, and finally an activated form of Fz (ligand bound, denoted Fz*) and Dsh (Fz bound, denoted Dsh*) are assumed. Variables were instantiated by manual adjustment to achieve desired wild-type and mutant clonal phenotypes, and to be within reasonable physiological limits.

The model succeeds in capturing many characteristic PCP phenotypes, demonstrating the feasibility of the model assumptions. Importantly, it also demonstrates that Fmi mediated intercellular interactions can produce correct results, as had been suggested though not tested by Amonlirdviman et al.,[65] and which is consistent with numerous experimental results.[40,75,92,98,101,103] The model encodes intrinsic stochastic variation, and the results demonstrate that the model structure is robust. This model has more recently been extended to the fly eye, describing the relationship between PCP and Notch signaling.[126]

The Le Garrec model predicts a number of conditions that have not been experimentally validated. First, the model makes a strong prediction that the initial extracellular gradient of ligand must decrease from proximal to distal, and must be shallow and short lived. This may be at odds with a number of findings. It is not clear that a ligand for Fz exists in PCP signaling in the fly,[74,92] and the irreversibility of ligand binding would suggest an affinity orders of magnitude higher than that measured for Fz and Wnts.[127] Irreversible activition of Fz might occur by a different mechanism that is activated in a transient and graded fashion (see for example Ref 128). A more vexing problem is that the transient nature of the activation is difficult to reconcile with the experimental observation that active Fz can be present in only the latter part (notably, even with forced expression) of the 30 hour polarization period and still achieve reasonably good polarity.[73,129] A second unvalidated condition is the hypothesized intracellular feedback mechanisms that are not based on experimental evidence. It is difficult to know what impact this might have on the simulation results, though it is curious that a feedback mechanism similar to that proposed earlier[65,72] apparently did not produce the correct results in this context.

Understanding Swirling PCP Patterns with Mathematical Modeling

A modified version of the Amonlirdviman model was used, in conjunction with biological experiments, to ask whether the variable swirling patterns seen within *fat* clones could be attributed to irregularities in cell packing.[122] Biological experiments were consistent with a causative effect of irregular cell packing on formation of swirls, and modeling was invoked to ask whether cell packing is sufficient to explain the swirling patterns. The mathematical model was modified to simulate PCP on a cell grid captured from images of wings, and was shown to be capable of predicting the presence of a swirl from the cell geometry, allowing the authors to conclude that one need not postulate anything other than the activity of the core module operating in the context of a given cell packing to account for the swirls. Core module function is therefore sensitive to cell packing, but combination with a distributed global directional cue robustly overcomes the noise produced by irregular packing.

New fast optimization schemes applied to the Amonlirdviman model in Raffard et al.[123] helped to suggest how a global directional signal might affect the core module, yet the detail of the molecular-based modeling approach[65,122,123] can hamper intuitive understanding of the relationship between these two modules. Indeed, we believe that it is the intuition provided by simple models of module interaction, combined with the detailed models of molecular interactions, that will lead to a complete understanding of PCP.

A simple model of macroscopic polarity behavior was reported by Wang et al.[130] The model represents hairs as vectors at regular intervals on a two-dimensional Cartesian grid, and includes an update rule which iteratively re-orients each vector according to a scaled vector sum of its neighboring hairs' orientations. The update rule is designed to mimic PCP, in that the neighboring information is taken only from two concentric circles of locally surrounding vectors, and has stable equilibria corresponding to observed PCP patterns: all vectors are parallel, and vectors have perfect radial or circumferential symmetry. Using an initial orientation of vectors to mimic the action of a global directional cue, various interesting PCP-like behaviors were observed in the model. In the absence of a global directional signal, many small swirls progressively merged to fewer, larger swirls, producing patterns similar to those seen in *fat* or *ds* mutants. An important result was that the global directional cue need only produce a rough alignment. The local consensus rule then brings the pattern to equilibrium.

Similar in spirit is the model developed by Zhu[131] in which cells are described by equations used to represent polarizing dielectric molecules. Again, PCP-like behavior is demonstrated. Simulations showed that anisotropic propagation of molecular orientation produces swirling patterns, but that when present, global cues predominate in determining polarity. The model, designed to test the contribution of cell geometry to producing swirling patterns, showed that within clones of cells, weakened or absent global cues greatly increased the sensitivity of swirling patterns to cell packing, a result similar to that of Ma et al.[122]

A third model which lends intuition to the interaction between the global directional cue and core modules is that of Burak and Shraiman.[132] Key properties of the PCP protein behavior are coded into their model: generic complex formation across cell boundaries, with a simple model of bistability across cell boundaries and inhibition within the cell. Their model also incorporates and evaluates the effects of noise (stochasticity) on polarization. A global cue exerted as either a boundary signal or a weak distributed signal was examined in the context of noise. The weak distributed orienting signal but not the boundary signal was shown to compete with local noise to produce correct polarization within a realistic time frame, suggesting that whatever the nature of the global directional signal, it is likely to be distributed across the tissue. A gradient of the bulk global cue of 0.1% between cells is enough to produce normal polarity, overcoming the tendency to produce a swirling pattern. The authors suggest that imposition of an artificial gradient of defined steepness to compete with the endogenous signal would be a way to measure the gradient of the endogenous global directional cue.

These three models have captured in similar ways an abstract relationship between the global cue and the core module: in essence, the cooperation and competition between a weak global signal, and a strong signal coming from a robust local feedback loop. As such, they are useful in providing a compact intuitive understanding of the relationship, and even more in their ability to suggest quantifiable values whose experimental validation could lead to favoring one model hypothesis over another. We believe that it is the use of such models in refining the molecular-based mathematical models discussed earlier that will lead to continued progress in the understanding of PCP.

An Analytical Model of PCP Signaling

Owen et al. have worked on a class of models representing juxtacrine signaling in an epithelial sheet with a single ligand and receptor, and feedback coupling levels of each on adjacent cells.[133] In exploring the properties of a model for lateral inhibition, they relaxed a prior assumption of homogeneous ligand and receptor distribution around the cell surface to allow for cell polarization, and included diffusion. With these features, the authors identified conditions in which a wild-type planar polarization occurred. In a cell grid, domineering nonautonomy such as that seen near a *fz* or *Vang* clone could be observed.

These properties were explored further in Ref 134 using a mathematical model which abstracts PCP activity to two variables per cell while capturing the mechanisms of feedback and diffusion in a compelling way. Ligand and receptor are equivalent to proximal and distal PCP complexes, and are coupled by negative feedback between adjacent surfaces of neighboring cells. Contributions of diffusion, the strength of feedback, and the strength and distribution of the initial directional cue were explored. The authors concluded that polarization occurs for all parameter sets in which there is sufficiently strong feedback and sufficiently weak diffusion. A weak, distributed, initial polarization signal is sufficient to initiate polarization, and the system can overcome variations in initial conditions across the cell array, as is likely to be necessary *in vivo*. Providing global directional information from a boundary source gave traveling wave models that showed a broader range of behaviors more difficult to reconcile with experimental results. Thus, the model favors the diffuse distribution of a global directional cue over more localized sources, and makes quantitative predictions about the core module that would be interesting to test, but would require defining experimental interventions that modify strength of feedback or diffusion rates.

As with the abstract models of swirling pattern formation discussed in the previous section, these simple models could help us to understand the general behavior of PCP signaling and how certain properties of the pattern formation result. How do the molecular models presented earlier compare in utility with these simpler models? The level of abstraction of a model, and the signals represented therein, should be chosen based on the questions being asked about the system. The molecular models presented earlier were developed to test the hypotheses about molecular interactions, and so it was necessary to include a corresponding level of detail. Otherwise, the model would not be able to reproduce all wild-type and mutant behaviors, and a leap of faith would still be needed to trust the plausibility of the model as a representation of PCP. The simpler models are particularly useful to gain intuition. Ultimately, the ability to experimentally test a model depends on the ability to manipulate

the variables incorporated by the model. Thus, the molecular level models are necessary to answer the questions that originally motivated their design.

PCP Signaling and Primitive Streak Formation

One final, interesting, model does not aim to understand PCP mechanisms, but rather invokes PCP to explain the stereotypical cell migration pattern seen during primitive streak formation in the chick epiblast.[135] Using a grid-free cell-based model in which cells behave as ferromagnets, Newman showed that an initial pattern of PCP based on known patterns of gene expression, together with a coupling of cell velocity and direction to the average PCP vector of cells in the neighborhood, is sufficient to reproduce the observed pattern of cell migration. This appealing model awaits experimental confirmation.

CONCLUSION

While research efforts integrating biological experimentation and mathematical modeling have demonstrated that feedback based on asymmetric protein localization at cell boundaries is a feasible mechanism for core module function in PCP, our understanding of the specific molecular mechanisms is still rudimentary. Furthermore, our understanding of how the global, core, and effector modules are connected together is lacking. Future efforts should be aimed at probing the PCP system from its molecular level interactions, through its multicellular behavior, to the tissue-level effects. More detailed models of each of the modules will allow us to better understand how mutation of specific components compromises function, and how signals from module to module may be received and interpreted. Thus the best strategies will likely be those that employ a hierarchy of mathematical models, from detailed mechanistic models to representations that abstract the function of each module to just a few variables.

Finally, what about the future of mathematical modeling methods in biology? A commonly asked question is 'How have the equations improved our intuitive understanding of how the system works?' Intuition not only allows us to describe in words the behavior of the system, it is necessary in generating likely hypotheses for the function of connected or related biological mechanisms. Simple models comprised of a few parameterized equations, or more complex models that can be represented as simple interconnections of such, win out in this respect. Seeking simplifications in biological modeling can be compared to engineering design, in which mathematical analyses are almost always performed on simple abstracted models, prior to the numerical simulations that validate the design on large scale, complex models more closely representing the true system dynamics. It seems likely that the best modeling efforts will be those that develop hierarchies of equations, which, for example, connect the low level detailed dynamics of molecular interaction, with a higher-level abstract view of the basic functionality involved, such as amplification, feedforward, and feedback.

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