Mathematical modeling of planar cell polarity to understand domineering non-autonomy

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Summary

A reaction-diffusion, partial differential equation model of planar cell polarity signaling in the Drosophila fly wing is designed and used to help understand domineering non-autonomy.
Abstract

Planar cell polarity (PCP) signaling generates subcellular asymmetry along an axis orthogonal to the epithelial apical-basal axis. Through a poorly understood mechanism, cell clones mutant for some PCP signaling components, including some, but not all alleles of the receptor frizzled, cause polarity disruptions of neighboring, wild-type cells, a phenomenon referred to as domineering non-autonomy. Here, a contact dependent signaling hypothesis, derived from experimental results, is shown by reaction-diffusion, partial differential equation modeling and simulation to fully reproduce PCP phenotypes, including domineering non-autonomy, in the Drosophila wing. The sufficiency of this model, and the experimental validation of model predictions, reveal how specific protein-protein interactions produce autonomy or domineering non-autonomy.

As the understanding of cellular regulatory networks grows, system dynamics and behaviors resulting from feedback effects of such systems have proven to be sufficiently complex as to prevent intuitive understanding. Mathematical modeling has sought to extrapolate from existing information and underlying principles, and has been successful in increasing understanding of some biological systems(1-3). However, more often, only incomplete abstracted hypotheses exist to explain observed complex patterning and functions. The challenge has become to show that enough of a network is understood to explain the behavior of the network. We have used mathematical modeling to show the
sufficiency and to study properties of a proposed model for PCP signaling, explicitly
demonstrating that it can explain the often counter-intuitive, complex behaviors of the
system, obviating the need for additional factors to explain these behaviors, and
providing insight into the system dynamics that govern them.

Many epithelia are polarized along an axis orthogonal to the apical-basal axis (Fig. 1A). On the *Drosophila* adult cuticle, each hexagonally packed cell elaborates an actin-rich trichome, or hair, that develops from the distal vertex and points distally. Similarly, the fly eye is comprised of about 800 ommatidia – clusters of chirally organized photoreceptor and other cell types – that are arranged in mirror image patterns reflected across the equator of the eye. Genetic analyses have identified a group of PCP proteins whose activities are required to correctly polarize these arrays(4-6). The domineering non-autonomy adjacent to cell clones mutant for some PCP genes has not yet been adequately explained(4). For example, in the *Drosophila* wing, *Van Gogh/strabismus* (*Vang*)(7, 8) clones disrupt polarity proximal to the mutant tissue(7), whereas null *frizzled* (*fz*) alleles disrupt polarity on the distal side of the clone(9, 10). In the eye, domineering non-autonomy near mutant clones that disrupt Wingless (*Wg*) signaling, such as *arrow* mutant cells, is seen on the equatorial side of the clone(11), while clones disrupting *fz* produce domineering non-autonomy on the polar side of clones(12).

To explain how cells mutant for PCP genes can affect the polarity of neighboring, wild-type cells, a class of models has been proposed in which cells respond to an initial cue by producing and secreting a diffusable second factor whose graded distribution
determines polarity(4, 11, 13-19) (Fig. 1B). In the eye, Wg signals in a gradient that is highest at the poles and lowest at the equator. It was hypothesized that Wg signaling induces the dose dependent secretion of “factor X,” which diffuses, and in turn regulates Fz signaling(11). Clonal disruption of the response to Wg would perturb the factor X gradient in both the mutant and the neighboring wild-type tissue, thereby producing non-autonomy. Similarly, in response to a graded upstream signal, Fz and Vang have been hypothesized to regulate production of a diffusible “factor Z” that is required for polarity readout(4, 15).

Interestingly, while for most fz alleles, including null alleles, clones of mutant cells produce domineering non-autonomy, clones of cells mutant for some fz alleles produce an almost cell autonomous polarity phenotype(9, 10, 20). Clones of cells mutant for dishevelled (dsh), a component of the Fz signaling pathway(21), also produce nearly cell autonomous polarity disruptions(22, 23). Diffusible factor models explain these observations with the hypothesis that Fz mediates two separately mutable signaling functions, transducing a cell autonomous signal through Dsh, and a Dsh independent non-autonomous signal(7, 24-26). The non-autonomous signal was proposed to be mediated by factor X, or a second, similar, factor Z, in this case produced in response to Fz signaling, and feeding back through Fz, while the autonomous signal was proposed to initiate cell polarization (4, 15). Non-autonomous Fz signaling has been proposed to temporally precede autonomous signaling in both the eye and wing(26). However, despite the ability of these models to explain domineering non-autonomy, diffusible
factors X or Z have not been identified(17), and no molecular level understanding of the autonomous and non-autonomous signaling functions of Fz has been obtained(4, 26).

**Biological Model**

Recently, it has been demonstrated that Fz and other PCP signaling components accumulate asymmetrically in cells, selectively on the distal or proximal side of wing cells (Fig. 1A) or on the equatorial or polar sides of the R3 and R4 photoreceptor cells in ommatidia, thereby defining their polarity(27-36). Evidence has been provided that these proteins function in a feedback loop that amplifies an asymmetry cue, converting uniform distributions of PCP proteins into highly polarized distributions(16, 28, 30, 31) (Fig. 1A and C). The feedback mechanism depends on several functional relationships(31). First, Fz recruits Dsh to the cell membrane(28, 37). In addition, Fz promotes the recruitment of Prickle-spiny-legs (Pk) (9, 31, 38) and Vang(33) to the cell membrane of the adjacent cell. Feedback is provided by the ability of Pk (and Vang; as described with new supporting data below) to cell-autonomously block Fz dependent recruitment of Dsh to the membrane(31). The result is an evolution from a symmetric to a highly asymmetric protein distribution, with Fz and Dsh on one side of the intercellular boundary, and Vang and Pk on the opposite side. This feedback loop functions strictly locally, between adjacent cells. Global directionality is imposed through the opposing expression gradients of the novel transmembrane protein Four-jointed and the cadherin Dachsous, which are thought to asymmetrically activate the uniformly expressed cadherin Fat (Ft) (34, 39, 40). Through an unknown mechanism, Ft, which associates with the intracellular
protein Atrophin (18), biases the direction in which the Fz feedback loop operates. The PP2A regulatory subunit Widerborst, also biases the direction of Fz accumulation, though its relationship to Ft is unknown, and its substrate has not been identified (41).

We propose that the experimentally identified local feedback loop and global directional cue comprise the central components of a PCP signaling network, and that examination of their properties may yield testable insights into the mechanisms underlying domineering non-autonomy. However, intuition alone is insufficient to deduce the behavior of this regulatory network. Indeed, progress in understanding PCP signaling has been severely hampered by an inability to deduce, given a particular signaling network hypothesis, definitive links between molecular genetic interventions and tissue level patterning effects. Thus, it is not readily apparent that this network is sufficient to explain the complex patterns observed in fields of cells containing mutant clones, and others have argued that it cannot account for some of the observed phenotypes (4, 26). For example, while it is apparent that removing Dsh or Fz would disrupt the feedback loop, it is not obvious how the feedback loop in adjacent wild-type cells responds, such that dsh mutant clones behave autonomously, while fz clones behave non-autonomously. Furthermore, Pk overexpression promotes the asymmetric accumulation of Dsh and Fz (31), while the role of Pk in the feedback loop is to inhibit the membrane recruitment of Dsh. If the model is correct, then this amplification must be a non-obvious consequence of the feedback loop system dynamics. Finally, the design and interpretation of additional experiments will require a clear prediction of expected outcomes.
We have developed a mathematical model based solely on the described feedback loop and global directional cue (31, 34, 39) in order to demonstrate the possible dynamics of the system, and to predict testable features of the mechanism. While mathematical modeling cannot prove the correctness of the underlying biological model, the ability of the mathematical model to capture the known behaviors of the system proves the feasibility of the biological model. From this result, we can conclude that it is unnecessary to invoke diffusable factors X or Z. Furthermore, the model led us to test and verify hypothesized differences in function between the autonomous and non-autonomous fz alleles. Our results provide an overall insight into the factors contributing to autonomy and non-autonomy.

**Mathematical Model**

We have represented the features of the biological feedback loop model as a mathematical reaction-diffusion model that describes the concentrations of Dsh, Fz, Vang and Pk throughout a network of cells. We have assumed that the quantities of proteins present in the system are sufficiently large so that protein concentrations may be treated as continuous, deterministic variables. We have also assumed that Dsh and Pk move freely and isotropically within the cell interior, while Vang and Fz move only in the membrane. These movements have been modeled as diffusion, though more complex mechanisms may be involved. For simplicity, we have disregarded the depth of the cells and have modeled them in two dimensions. While the underlying mechanisms for the interactions in the local feedback loop are not yet fully understood, the essential logic of
this feedback loop is preserved by representing these interactions as binding to form protein complexes (Fig. 1D). For example, Fz interacts with Dsh to form a DshFz complex, and Vang interacts with Pk to form VangPk. For the reaction between cells, Fz on the membrane of one cell reacts with Vang on the membrane of a neighboring cell to form a complex denoted FzVang. FzVang is then restricted to diffusing only along the shared edge of the cells. FzVang can further react with Dsh or Pk to form larger complexes. Backward reactions separate the complexes back into their constituent proteins. The six protein complexes included in this model are DshFz, VangPk, FzVang, DshFzVang, FzVangPk, and DshFzVangPk.

Direct biochemical evidence exists for the VangPk complex(33, 36). It has been observed that Fz induces the recruitment of Dsh to the cell membrane in vivo and in heterologous systems, and evidence of direct, albeit weak, binding exists for Fz and the PDZ domain of Dsh (28, 37). It is also observed that Fz and Vang accumulate across intercellular interfaces, but no evidence for a direct interaction exists. Whether these proteins directly interact, or if they interact through one or a series of intermediary proteins is not expected to alter the nature of the results of this model. Flamingo (Fmi)(25, 27) and Diego (Dgo)(32), other proteins known to be involved in PCP signaling, were not included in the model. Fmi is required for the membrane localization of the other proteins (33), and both are required to generate asymmetry, but otherwise, the influences of Fmi and Dgo on other proteins are not understood.
Both genetic and cell culture data previously led us to propose that Pk inhibits Fz dependent membrane recruitment of Dsh\(^{(31)}\). In a cell culture assay, we showed that Pk cell-autonomously antagonizes Dsh recruitment\(^{(31)}\). This result has been replicated by some, but not others\(^{(33, 42-44)}\). Here, we demonstrate a dose dependent inhibition of Dsh membrane recruitment by both Pk and Vang, and a combinatorial effect when both are expressed (Fig. S1). This is consistent with the hypothesis that Pk and Vang work together on the proximal side of the cell, and with the observation that, like Pk, Vang binds Dsh \(^{(31, 33, 45)}\). The differences between our results and those of others might therefore depend on differences in the uncontrolled levels of host cell Vang, or other uncontrolled aspects of the experiment.

We used these observations to develop the representation of inhibition of DshFz complex formation in the reaction-diffusion model (Fig. 1E). Inhibition of Dsh membrane recruitment by Pk and Vang is represented in the reaction-diffusion model as an increase in the backward reaction rate of reactions in which Dsh binds Fz (or Fz complexes) by a factor dependent on the local concentration of Pk and Vang. This implementation does not require a detailed mechanistic understanding of the inhibition.

A global asymmetry cue is mediated by the proteins Four-jointed (Fj), Dachsous (Ds) and Fat (Ft)\(^{(34, 39)}\). The influence of this upstream signal is to somehow bias the feedback loop, such that the local alignment of cells occurs in the proper direction with respect to the tissue axes. The specific mechanism for the introduction of this bias into the feedback loop network is not known. Two forms of a direct, global biasing signal
were therefore implemented. In a reaction-based mechanism, which might reflect a post-translational modification of Dsh (a phosphoprotein) or Fz, for example, the reverse reaction rates of Dsh binding with Fz and Fz complexes were decreased in the distal region of each cell. In an alternative, diffusion-based mechanism, which might reflect the interaction of Fz with another protein in the distal membrane in response to Ft activity, only a fraction of Fz and Fz complexes in the distal region of each cell were free to diffuse to the rest of the cell, reflecting a bias for Fz to collect on the distal membrane of these cells. The results using either of these biasing models were similar (Figs. S5-S8 and S10-S14).

Based on these representations and assumptions, a mathematical model for PCP signaling was derived from a set of 10 reaction equations. For example, the three reactions involving DshFz are

\[
\text{Dsh} + \text{Fz} \xrightarrow{R_1} \xrightarrow{A_1 B_1} \text{DshFz}
\]

\[
\text{DshFz} \xrightarrow{R_4} \xrightarrow{A_4} \text{DshFzVang}
\]

\[
\text{DshFz} \xrightarrow{R_0} \xrightarrow{A_0} \text{DshFzVangPk}
\]

Adding a diffusion term yields the equation describing the dynamics of the concentration of DshFz.
where the net reaction rate terms are given by

\[
\frac{\partial[DshFz]}{\partial t} = P_1 - P_4 + P_9 + \mu_{DshFz} \nabla^2[DshFz]_D
\]

and where inhibition of Dsh recruitment to the membrane by Pk and Vang is introduced through

\[
P_1 = R_1[Dsh][Fz] - A_1 B \lambda_1[DshFz]
\]

\[
P_4 = R_4[DshFz]^\dagger[Vang] - \lambda_4[DshFzVang]
\]

\[
P_9 = R_9[DshFz]^\dagger[VangPk] - \lambda_9[DshFzVangPk]
\]

Brackets are used to denote local protein and protein complex concentration values. The \( R \) and \( \mathbb{I} \) terms are the forward and backward reaction rates for each of the reaction equations. The factor \( A_1 \) is less than 1 only in the distal region of the cell, reflecting the reaction-based global biasing signal (A discussion of this implementation appears in the supporting online text). \( B \) acts to promote the backward reaction rate in proportion to a function that depends on the local concentration of Pk and Vang, with constant of proportionality \( K_b \). This function, which may be nonlinear, is approximated through the exponent, \( K_p \). \( K_{Pk} \) is a constant which multiplies only the unbound Pk, and likewise \( K_{Vang} \) is a constant which multiplies only the Vang not bound to Pk. \( K_{Pk} \) and \( K_{Vang} \) were set to 0.5, so that Vang and Pk had equal and additive inhibitory effects. This equation describes the time rate of change of the DshFz concentration at a particular location in the
cell in response to local reactions, and the diffusion of DshFz in response to the local gradient of the DshFz concentration, with coefficient of diffusion \( \square^{DshFz} \). The subscript \( D \) is used to indicate that the diffusion term acts only on the diffusible DshFz concentration, which is less than the actual DshFz concentration on the distal cell membrane when the diffusion-based global biasing signal is used. The dagger is used to denote a component or reaction situated in a neighboring cell. Similarly, equations were constructed to describe the dynamics of Dsh, Fz, Vang, Pk and their remaining complexes, resulting in a system of 10 reaction-diffusion nonlinear partial differential equations. The full development of these equations can be found in the supporting online text. These equations were discretized using standard finite volume methods on a two-dimensional computational grid representation of a cell. With a given set of model parameters, an array of such cells could then be simulated from an initial state with all of the proteins uniformly distributed in each cell. Periodic boundary conditions were implemented at the edges of the cell network, resulting in an effectively infinite array of cells. A semi-implicit numerical integration method was used to integrate these equations for a fixed length of simulation time (supporting online text; ref. (46)).

Based on the protein concentration distribution at the end of a simulation, the simulator predicts a hair growth direction for each cell in the array. It has been observed that in wings mutant for the core polarity genes \( fz, pk, dsh \) and \( Vang \), hairs tend to emerge from the center of the cell, whereas, in wild-type (and other classes of PCP mutants) hairs arise from the periphery on the side toward which they project(39, 47, 48). Although the
mechanism for how the protein distribution is translated into a location for hair initiation is not known, we have chosen to use the distribution of Dsh to predict the hair growth direction because we observe that hairs emerge consistently from the center of the cell in dsh mutants but not in the other mutants (Fig. S3). Therefore, in the simulations, the hair of a given cell grows in the direction corresponding to the vector sum of Dsh localization in that cell. If the Dsh concentration is not polarized above a specified threshold, then the hair is assigned to the center of the cell.

**Parameter Selection**

The first aim of our experiment was to ask if this mathematical model could reproduce wild-type polarity and phenotypes associated with mutant clones. The initial protein concentrations, the reaction rate constants and the protein and protein complex diffusion constants were not known, and so these parameters were left to be identified by being constrained to result in specific qualitative features of the hair pattern phenotypes, such as the domineering non-autonomy when a certain loss-of-function mutant cell is introduced into an array of wild-type cells. The constants affecting the strength of the Pk and Vang inhibition of Dsh-Fz binding, the strength of the direct global biasing signal and the degree of induced protein overexpression were also left as parameters to be identified. We constructed an objective function consisting of quadratic penalty parameters for each of the qualitative feature constraints observed from the experimental data. We used the Nelder-Mead simplex method(49) to minimize this objective function with respect to the model parameters to find a feasible solution set of parameters that
simultaneously satisfied all of the feature constraints. This nonlinear method produced feasible solutions within a reasonable amount of computational time without relying on gradients of the objective function, which were not readily available from the simulation results.

We studied the sensitivity of the results to the model parameter values by varying the parameters individually and determining the ranges in which each feature constraint continued to be satisfied. We would expect biologically that these feature constraints would be satisfied despite variations in the model parameters. The resulting ranges for each feature constraint were large, often spanning an order of magnitude or more (Fig. S4). This demonstrates that the mathematical model is not highly sensitive to parameter values, and suggests that our conclusions regarding the feasibility of the model are valid for sets of model parameters outside of that which we selected. Furthermore, the parameter ranges for which each of the mutant objective constraints is satisfied gives some indication as to which interactions in the model most directly affect this phenotype and offers insight into the behavior of the system. For example, the relative sensitivity of the feature constraint associated with autonomous dsh clones as compared to the non-autonomous fz and Vang clones might explain occasional polarity defects near dsh clones, and predicts that dsh clones might have a non-autonomous phenotype in other species or in some sensitized backgrounds.

Results
For both the reaction-based and diffusion-based global biasing models, we determined sets of model parameters that simultaneously satisfy all of the feature constraints. The simulation results shown in Figs. 2-5 reflect results from a single set of model parameters for the case in which the direct, global biasing signal was introduced through the reaction rate of the Dsh-Fz interaction. The feature constraint set serves to fit the model parameters to reproduce experimental observations, and consequently results from any set of parameters satisfying all of the feature constraints appear qualitatively similar to the results presented here. However, the extent of non-autonomy observed could vary depending on the specific model parameter values used.

In wild-type cells (Fig. 2), Dsh and Fz localize on the distal membrane, while Vang and Pk localize to the proximal membrane, as is seen in vivo (28, 30, 31, 33). The hair direction indicated by this feedback loop, based on the final distribution of Dsh, results in hairs emerging from the distal vertex of the cell. The removal of Dsh or Fz from all cells disrupts polarity completely, and removing Vang from the network almost completely eliminates the asymmetric localization of the proteins (not shown). Removing Pk does not completely disrupt cell polarity, but the asymmetry at the end of the simulation is substantially reduced (not shown), such that it would be very difficult to detect experimentally, consistent with previous observations (38).

We simulated clones of cells mutant in each of the PCP genes in 20 by 32 cell arrays. Simulated clones of cells lacking fz function disrupt polarity in wild-type cells distal to the clones (Fig. 3E, compare to A,B; ref. (9, 10)), whereas simulated clones of
cells lacking *Vang* function disrupt polarity on the proximal side of the clones, thereby reproducing the observed domineering non-autonomy (Fig. 3F, compare to C,D; ref. (7)). Simulated clones of cells lacking *dsh* function result in the disruption of polarity within the mutant cells, but only show a mild effect on cells outside of the clones (Fig. 3L). The nearly, though not fully cell autonomous phenotype is similar to that which we observed experimentally (Fig. 3G-I, and P.N. Adler, personal communication), despite allusions in the literature to a fully cell autonomous phenotype for *dsh*. Clones of cells lacking all *pk* function show only a very subtle phenotype, consistent with our data and published reports (Fig. 3J,K,M; ref. (38)). Distributions of other proteins are shown in Fig. S5, and the results are highly concordant with the available published experimental observations (Table S1). Similarly, simulated overexpression clones produce results closely mimicking observed experimental results (Fig. S6). For example, Dsh and Fz over-expression disrupt polarity on the proximal side of the clones, whereas *Vang* and *Pk* over-expression disrupt polarity on the distal side of the clones.

When originally formulating the Fz feedback loop model, we found that *Pk* overexpression in the posterior wing domain enhanced the accumulation of Fz and Dsh at cell boundaries, and we proposed that this reflected an enhancement of accumulation at the opposing cell surface through the feedback mechanism (31). However, that the mechanism would show this property is not obvious. To examine this more rigorously, we simulated this experiment in a periodically repeating 10 by 80 cell array with cells variably overexpressing *Pk* in a band 40 cells wide and extending periodically along the proximal-distal axis (Fig. 4D-F). Consistent with experiments overexpressing *pk* in the
posterior of the wing (31), Fig. 4A-C), Dsh and Fz are seen to accumulate to higher levels in the region overexpressing pk than in the wild-type region, and accumulate perpendicular to the wild-type orientation near the anterior-posterior boundary (green arrowheads). Therefore, despite the cell autonomous inhibition of DshFz complex formation, the feedback loop causes the system to respond to excess Pk by maintaining high levels of membrane bound Dsh, Fz, and Pk.

**Insights into autonomy**

Our results suggested a mechanistic explanation for the difference between autonomous and non-autonomous fz alleles. Because the nearly autonomous fz alleles (fzJ22 and fzF31, ref. (20)) have phenotypes similar to dsh clones, we hypothesized that these alleles may be selectively deficient in complexing with Dsh, but normal in their ability to complex with Vang. Simulations of fz clones fully impaired in Dsh interaction but retaining Vang interaction are identical to Dsh clones, and are nearly cell autonomous (Fig. S7). Similarly, clones of cells in which we disrupted the interaction between Dsh and Fz by reducing the corresponding forward reaction rates to between 0-0.02% of their wild-type values also produced nearly cell autonomous polarity phenotypes (Figs. 5C and S7).

This hypothesis makes two easily testable predictions. First, Fz autonomous proteins should be present in the membrane, and should recruit Vang to the adjacent membrane, while Fz non-autonomous protein should not recruit Vang. It has previously
been shown that GFP-tagged \( \text{Fz}^{\text{J22}} \), expressed in a wild-type background, is present at the apical cell cortex, but remains symmetrically distributed (30), a distribution in accordance with our simulation of this condition (Fig. S9). Examining this further, we found that in cells adjoining clones of the autonomous \( \text{fz}^{\text{F31}} \) allele, Vang is recruited to the boundary between wild type and mutant cells, while significantly less Vang is recruited to those boundaries in cells adjoining clones of the non-autonomous \( \text{fz}^{\text{R52}} \) allele (Fig. 5A). This is evident at the distal sides of the clones (blue arrowheads), where polarity is reversed for non-autonomous alleles, and is also observed at anterior and posterior boundaries, where the clones have less impact on the polarity of neighboring cells (yellow arrowheads). Thus, \( \text{Fz}^{\text{autonomous}} \) proteins recruit Vang to the opposing cell surface, while non-autonomous alleles do not. The second prediction is that autonomous \( \text{Fz} \) proteins should fail to recruit Dsh. Indeed, we find that both are substantially impaired in Dsh recruitment, though somewhat less impaired than the very strong, non-autonomous \( \text{fz}^{\text{R52}} \) allele (Fig. 5B). Thus, strong \( \text{fz} \) alleles, many of which fail to accumulate \( \text{Fz} \) protein (20), display no or severely impaired interaction with Dsh and Vang, whereas autonomous alleles have impaired interaction with Dsh, but retain substantial ability to recruit Vang to the adjacent membrane. Notably, simulated overexpression of \( \text{Fz} \) with impaired Dsh interaction also produced the correct polarity disruption in cells proximal to the clones (5) (Fig. S7). In simulations and in wings, relatively small clones of cells lacking a global biasing signal show no phenotype, demonstrating that not all cells need to respond to the global directional signal for the feedback loop to cooperatively align all of the cells (not shown; ref. (39)).
Discussion

The ability of our mathematical model to simultaneously reproduce all of the most characteristic PCP phenotypes (Table 1) demonstrates the feasibility of the underlying biological model as a PCP signaling mechanism. Further, the mathematical model demonstrates how the overall scheme of the model – a local feedback loop between adjacent cells, biased by a global directional cue – can produce not only wild-type polarity, but can explain more complex patterns seen in the autonomous and non-autonomous behavior of PCP mutant clones. Alternative models invoking diffusible factors have not been supported by the identification of such factors \((17)\), and the contact-dependent intercellular signaling model more readily accounts for the slight non-autonomy of \(dsh\) and \(f_{z}^{autonomous}\) clones than do the diffusible factor models. We therefore propose that PCP arises through the behavior of locally signaling cellular automata that exist within an expression gradient of molecules providing a directional cue. While we have simulated this mechanism for the wing, it may also describe the PCP signaling mechanism in eyes and bristles.

A mechanistic explanation for the relative autonomy and non-autonomy of various PCP mutants emerges from the mathematical model and is substantiated by verification of its predictions. Retention of the Fz-Vang interaction and loss of Dsh recruitment results in relatively autonomous clonal phenotypes, whereas loss of both interactions produces non-autonomy. The Dsh\(^1\) protein produces nearly autonomous clones, and it carries a mutation in its DEP domain, which is required for membrane localization\((28,\)
autonomous fz alleles bear point mutants in the first cytoplasmic loop, suggesting these mutations may affect the same interaction. A low affinity interaction between the Dsh PDZ domain and a sequence in the cytoplasmic tail of Fz has been demonstrated. Our data suggest that sequences in the Dsh DEP domain, and in the Fz first intracellular loop, are important for Dsh membrane association, and suggest a model in which a bipartite interaction mediates regulated, high affinity association of Dsh with Fz.

Simulations of cells overexpressing pk offer some insight into why Dsh and Fz can continue to accumulate asymmetrically at the cell membrane despite the increased presence of a factor inhibiting their interaction. Upon pk overexpression, an increased Pk concentration is also available to complex with proteins in neighboring cells, reinforcing the feedback loop to produce a greater asymmetric localization of all of the proteins, and consequently offsetting the reduced interaction between Dsh and Fz that would be expected in isolated cells where the feedback loop could not function. We note that simulation of sufficiently high levels of Pk can block most Dsh membrane association and prevent asymmetry, assuming a non-saturable inhibition (not shown).

In constructing our mathematical model, the lack of complete biological understanding required the inclusion of several assumptions. We modeled a direct Fz-Vang interaction, though this has not been demonstrated. Fz and Vang localize to opposite sides of the cell, and we have shown that Vang accumulation depends on the Fz allele (autonomous or non-autonomous) in the adjacent cell. However, a direct
interaction need not be invoked to preserve the logic of our model. For example, Fz and Vang could be linked through a mutual affinity for a bridging cadherin such as Fmi, which is most likely present in both membranes, and is required for assembly of the other components(27).

Because the mechanism by which the global Ft signal influences the feedback loop has not been determined, we implemented two forms of a direct biasing signal, and found that both models produced essentially similar results. Therefore, while the nature of the asymmetric input is not known, the conclusions we draw with respect to autonomy do not depend on a specific mechanism.

We do not know the mechanism by which Pk and Vang antagonize DshFz complex formation. However, the mathematical model only requires that these proteins inhibit the Dsh-Fz interaction, without relying on a specific molecular mechanism for this activity. We found that the results were not very sensitive to diffusion rate parameters. Since diffusion does not favor asymmetric distributions, should a more complex mechanism for component movement be discovered, such as active transport, for example, the model should easily accommodate this and achieve equally good or better results. Finally, while we have omitted other known proteins from the simulation (Fmi, Dgo, Wdb), these can be accounted for without perturbing the function of the feedback loop, and could provide additional degrees of freedom that may enhance the concordance with experimental data. Therefore, our model should not be sensitive to these assumptions.
Understanding PCP signaling has numerous implications. Since PCP-like signaling regulates both the polarity of cochlear hair cells, and convergent extension in vertebrates, insight into PCP signaling will enhance understanding of these processes, and the human diseases associated with their disruption, including congenital deafness syndromes (e.g., Usher Syndrome), and neural tube closure defects leading to spina bifida and related conditions(51). Furthermore, the PCP signaling mechanism shares a requirement for Fz, Dsh and other components with the oncogenic Wnt signaling pathway, and the mechanistic relationship between these two pathways is still being explored. Beyond this, in developing a mathematical model of PCP, we have also demonstrated the usefulness of this type of modeling to address the class of biological problems for which neither a set of governing equations nor physical parameters are well understood, and which exhibit sufficiently complex behavior to prevent intuitive interpretation alone. (1-3)

42. F. Carreira-Barbosa *et al.*, *Development* **130**, 4037-4046 (Sep 1, 2003).
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Supporting Online Material
www.sciencemag.org
Supporting text
Materials and Methods
Table S1
Figs. S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13 and S14

Figure legends

Figure 1. Diagrams illustrating PCP in Drosophila wings and ommatidia. (A) Hexagonally packed wing cells accumulate Fz and Dsh distally (blue and green) and Pk and Vang proximally (pink and orange). An actin rich trichome, or hair, is elaborated from the distal vertex of each cell, and points distally. (B) Proposed PCP signaling
models invoking a diffusible factor “X” or “Z.” Domineering non-autonomy in regions around mutant clones in which production of the factor is suppressed (shown) or enhanced (not shown) would result from diffusion of the factor, followed by reading of the factor gradient. (C) A local feedback loop model for PCP signaling. Large lettering indicates proteins accumulating at the designated location. Smaller lettering and circles with slashes indicate proteins whose concentrations are decreased at that location. (D) Representation of the feedback loop model as a series of binding interactions to form complexes at the cell membrane. (E) Inhibition model representing disruption of the interaction between Dsh and Fz by Pk and Vang.

Figure 2. Wild-type simulation results showing the concentration distributions of Dsh (A), Fz (B), Pk (C) and Vang (D) at the end of the simulation. Overlaid on the Dsh distribution (A) is the predicted hair growth direction. The same color scale is used in all simulation result figures, where blue indicates the absence of protein, 1 is scaled to the initial uniform concentration of Dsh ([Dsh]₀) and the scale is truncated so that concentrations greater than 3 times [Dsh]₀ are shown in red. (E) Simulated distribution of Dsh displayed as an intensity representing the sum of Dsh concentrations in the membranes of adjacent cells, corresponding to the appearance of Dsh::GFP in wild-type experiments.
Figure 3. Images of adult wings showing PCP phenotypes (A-D and G-K) and corresponding simulation results showing the concentration distribution of Dsh at the end of the simulation with predicted hair growth directions (E,F and L,M). Mutant cells are outlined in yellow in wing images and are marked with yellow crosses in simulation results. Throughout all simulation result figures, greater Dsh asymmetry is represented by hair placement at increasing distances from the center toward the periphery of the cell. When the Dsh asymmetry does not exceed the threshold value, the hair is depicted as a triangle at the center of the cell. (A,B and E) \( f_z^{R52} \) null clones. (C,D and F) Vang mutant clones. (G-I and L) \( dsh \) mutant clones. (J,K and M) \( pk \) mutant clones. Asterisks mark non-autonomous effects near \( dsh \) clones.

Figure 4. Overexpression of \( pk \). Pk (A), Fz::GFP (B) and Dsh::GFP (C) near the anterior-posterior boundary of pupal wings overexpressing \( pk \) in the posterior \textit{engrailed} domain. (B) Simulation of a periodic horizontal band of random \( pk \) 1x-11.58x overexpression resembling the overexpression patterns observed in the posterior \textit{engrailed} domain showing Pk (C), Fz (D) and Dsh (E). Reorientation of Fz accumulation is denoted with green arrowheads.

Figure 5. Cell autonomous \( f_z \) allele function. (A) \( f_z^{R52} \) (non-autonomous, strong allele) and \( f_z^{F31} \) (autonomous allele) clones with Vang::YFP in all non-mutant cells. Note that
Vang accumulates at all boundaries of the $f_\xi^{F31}$ but not the $f_\xi^{R52}$ clones. (B) Dsh::GFP and Fmi localization in wild-type, $f_\xi^{R52}$ (null), $f_\xi^{F31}$ and $f_\xi^{J22}$ (cell autonomous) alleles, viewed on the apical surface (top) or in edge cells (bottom three rows), showing that Dsh::GFP is not recruited to the membrane by $F_\xi^{R52}$ and is poorly recruited by $F_\xi^{F31}$ and more poorly by the stronger $F_\xi^{J22}$. Fmi staining was used to identify the correct focal plain. Dsh::GFP in $f_\xi^{F31}$ and $f_\xi^{J22}$ resembles Dsh$^1$::GFP localization (ref. (28)). (C) Simulation of a Fz autonomous clone in which Fz interacts with Vang but has only 0.01% of wild-type interaction with Dsh, showing nearly autonomous effect. (D) Schematic showing interactions for wild-type, Dsh$^1$, Fz autonomous ($f_\xi^{F31}$) and Fz non-autonomous ($f_\xi^{R52}$) alleles. Red dots indicate point mutations and the faded Fz indicates reduced or absent protein in some null alleles. Spaces between proteins indicate loss of interaction.
Table 1. Feature constraint functions representing the characteristic PCP phenotypes reproduced by the mathematical model.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Objective</th>
<th>Constraint description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>$J_{wt}$</td>
<td>Asymmetric accumulation of Dsh and Fz on the distal cell membrane. Asymmetric accumulation of Pk and Vang on the proximal cell membrane (28, 30, 31, 33).</td>
</tr>
<tr>
<td>$dsh$</td>
<td>$J_{dsh}$</td>
<td>Polarity disruption inside of the mutant clone. Autonomous phenotype (22, 23).</td>
</tr>
<tr>
<td>$fz$</td>
<td>$J_{fz}$</td>
<td>Distal domineering non-autonomy (9).</td>
</tr>
<tr>
<td>Vang</td>
<td>$J_{Vang}$</td>
<td>Proximal domineering non-autonomy (7).</td>
</tr>
<tr>
<td>pk</td>
<td>$J_{pk}$</td>
<td>No polarity reversal. (this paper)</td>
</tr>
<tr>
<td>$&gt;&gt;dsh$</td>
<td>$J_{&gt;&gt;dsh}$</td>
<td>Proximal domineering non-autonomy. (our unpublished observations)</td>
</tr>
<tr>
<td>$&gt;&gt;fz$</td>
<td>$J_{&gt;&gt;fz}$</td>
<td>Proximal domineering non-autonomy (30).</td>
</tr>
<tr>
<td>$&gt;&gt;Vang$</td>
<td>$J_{&gt;&gt;Vang}$</td>
<td>Distal domineering non-autonomy. (our unpublished observations)</td>
</tr>
<tr>
<td>$&gt;&gt;pk$</td>
<td>$J_{&gt;&gt;pk}$</td>
<td>Distal domineering non-autonomy.</td>
</tr>
<tr>
<td>$fz_{autonomous}$</td>
<td>$J_{fza}$</td>
<td>Polarity disruption inside of the mutant clone. Autonomous phenotype (20).</td>
</tr>
<tr>
<td>$&gt;&gt;fz_{autonomous}$</td>
<td>$J_{&gt;&gt;fza}$</td>
<td>Proximal domineering non-autonomy (30).</td>
</tr>
<tr>
<td>EnGAL4, UASpk</td>
<td>$J_{&gt;&gt;pk-en}$</td>
<td>Overexpression of pk results in protein accumulation to a degree greater than or equal to that for wild-type results (31).</td>
</tr>
</tbody>
</table>
Fig. 1