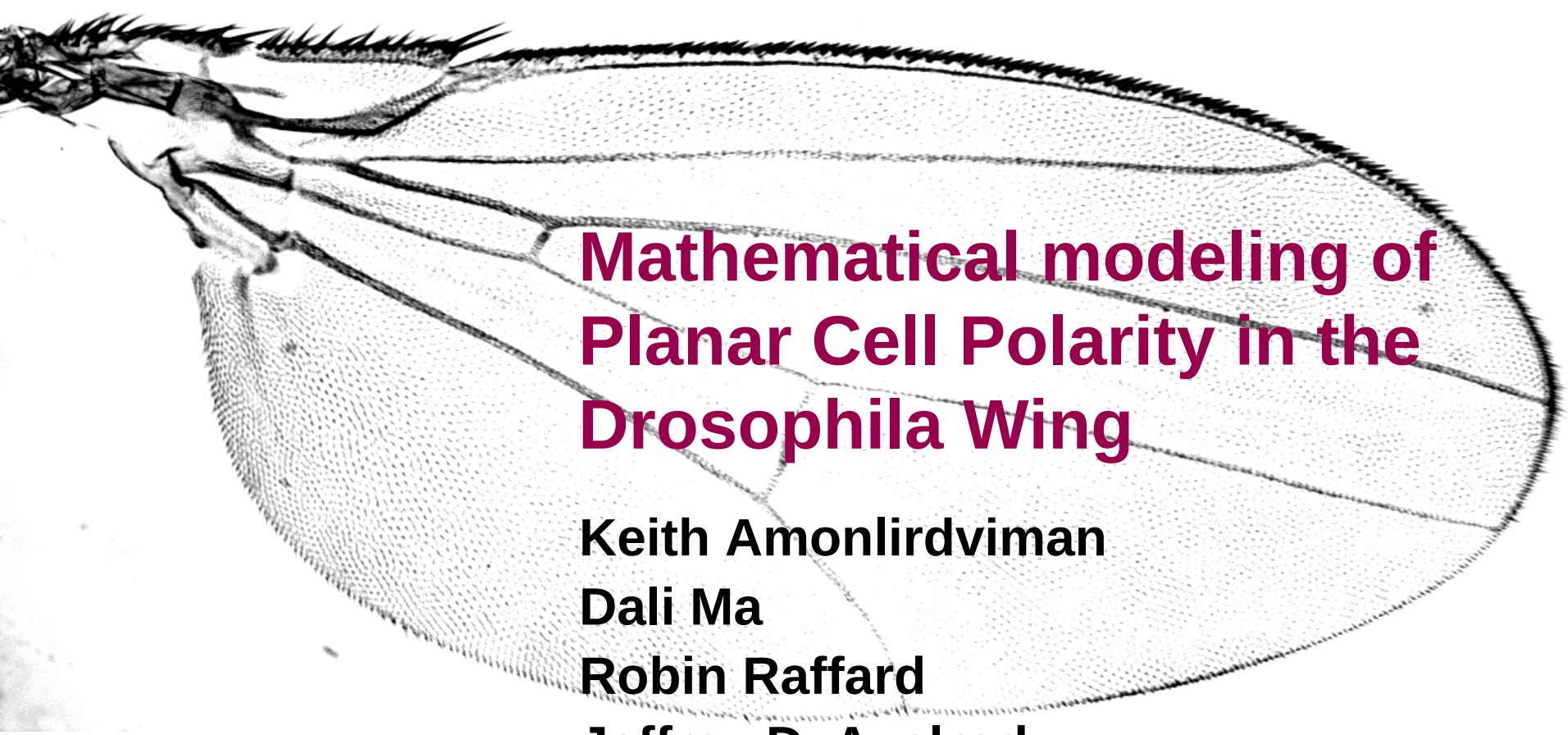


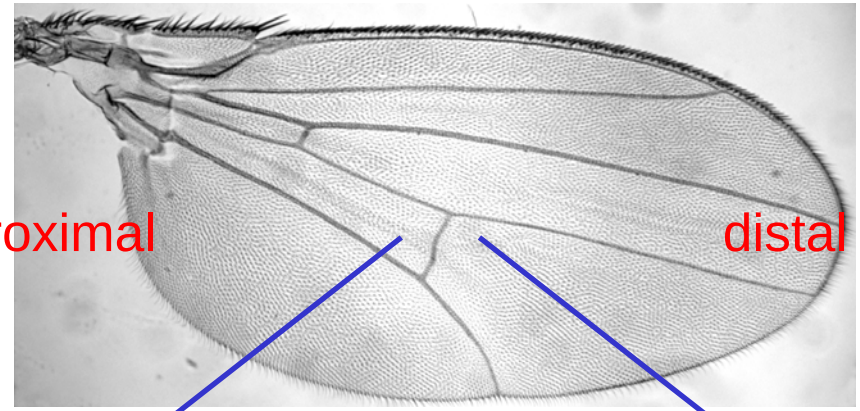
Mathematical modeling of Planar Cell Polarity in the Drosophila Wing

Keith Amonlirdviman
Dali Ma
Robin Raffard
Jeffrey D. Axelrod
Claire J. Tomlin

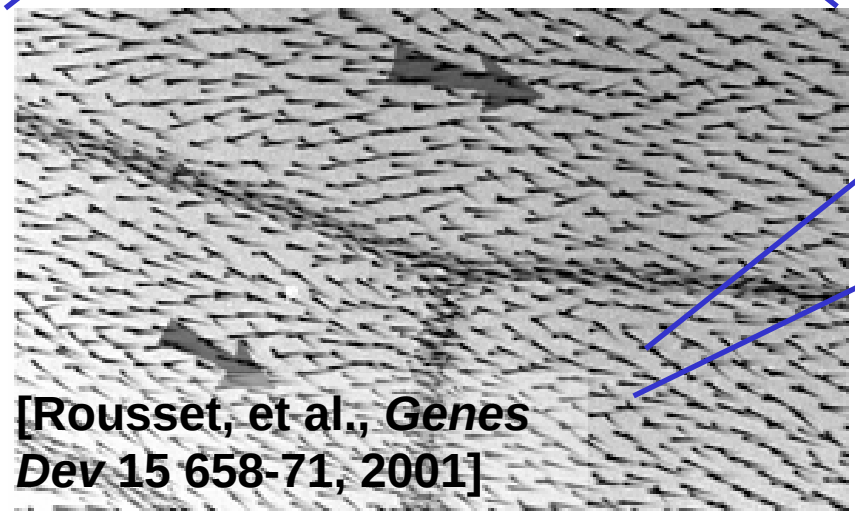
Electrical Engineering and Computer Sciences
UC Berkeley

Aeronautics and Astronautics / Pathology
Stanford University

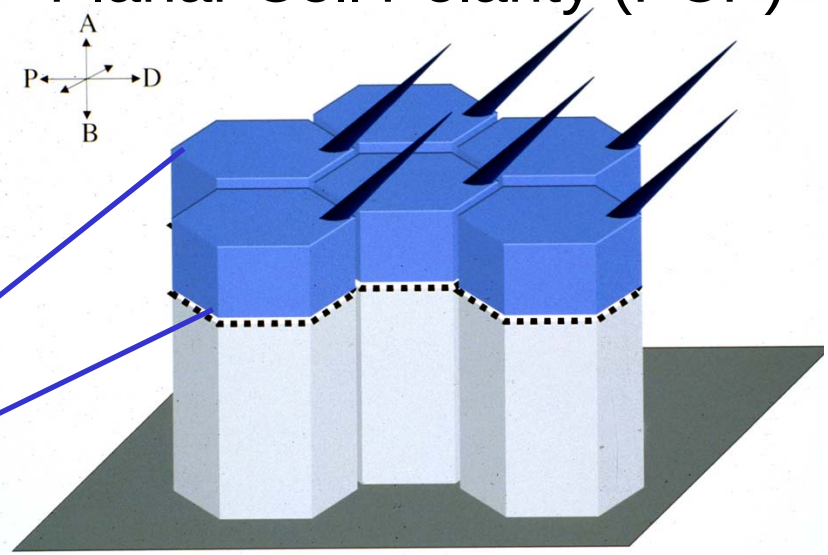
Planar Cell Polarity in *Drosophila* wings



- *Drosophila* wing hairs point distally, virtually error free
- Hexagonally packed, planar
- Planar Cell Polarity (PCP)



[Rousset, et al., *Genes Dev* 15 658-71, 2001]

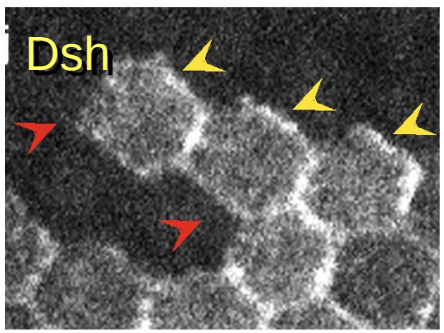


Human pathology:
- cochlear hair cells
- spina bifida
- oncogenic Wnt pathway

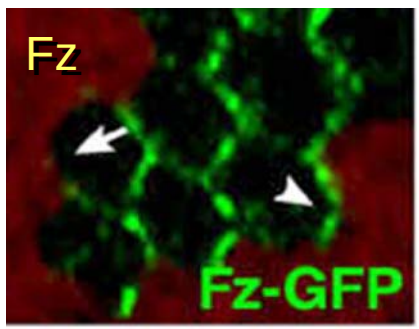
Axelrod Lab

Signaling Molecules

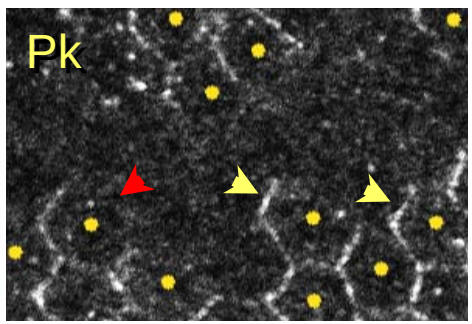
- System amplifies some global directional cue and propagates polarity from cell to cell
- Includes *Frizzled* (Fz), *Dishevelled* (Dsh), *Prickle* (Pk), *Flamingo* (Fmi) and *Van Gogh* (Vang)
- Dsh and Fz localize on the distal portion of each cell
- Pk and Vang localize on the proximal portion of each cell
- Hair grows from the distal portion of each cell



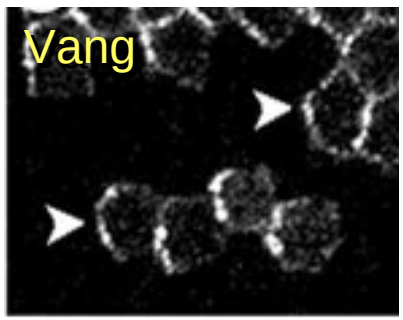
[Axelrod, *Genes Dev* **15**, 1182-7, 2001]



[Strutt, *Molecular Cell* **7**, 367-75, 2001]



[Tree, et al., *Cell* **109**, 371-81, 2002]



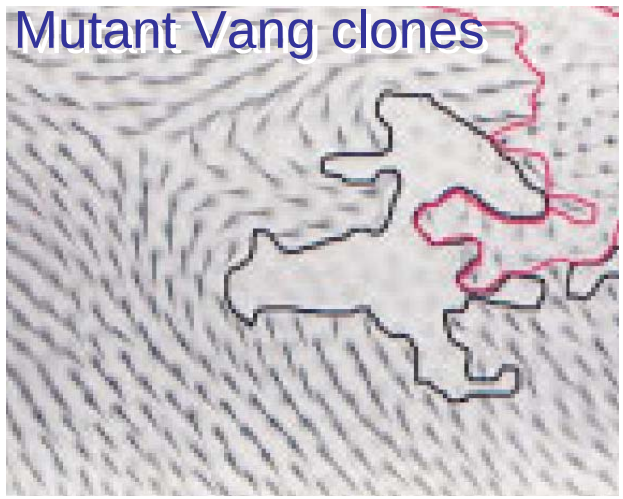
[Bastock, et. al., *Development* **130**, 3007-3014, 2003]

Mutant Wings: Domineering non-autonomy

- Loss of Fz disrupts polarity in distal non-mutant cells
- Loss of Vang disrupts polarity in proximal non-mutant cells

Disruption of signaling molecules is propagated to neighboring cells – Suggests diffusible Factor X?

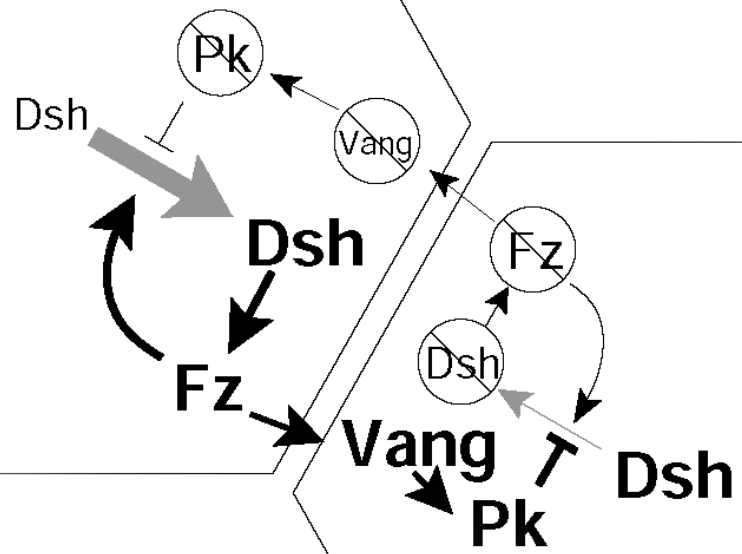
[Vinson and Adler, *Nature* **329**, 549-51, 1987]



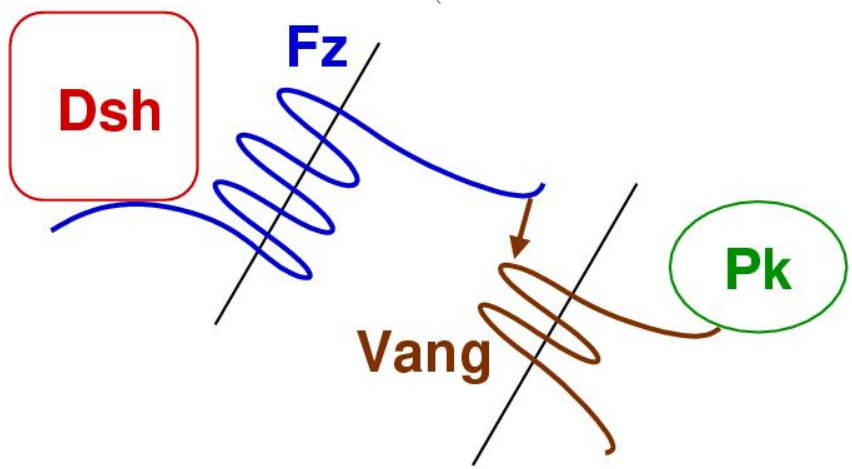
[Taylor, et al., *Genetics* **150**, 199-210, 1998]

Biological Model

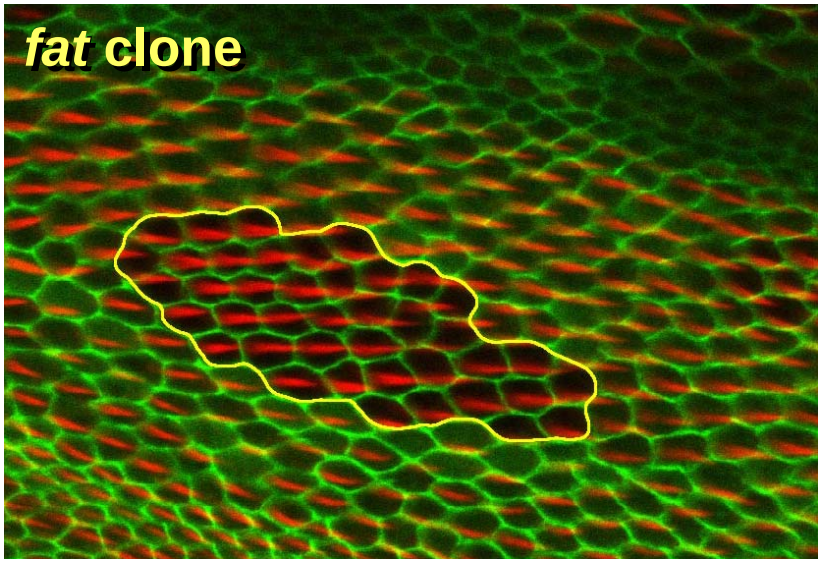
- Fz promotes recruitment of Dsh to a membrane
- Dsh stabilizes Fz localization
- Fz promotes the localization of Vang and Pk on the membrane of a neighboring cell
- Pk and Vang inhibit the recruitment of Dsh to a membrane
- Network amplifies unknown directional cue



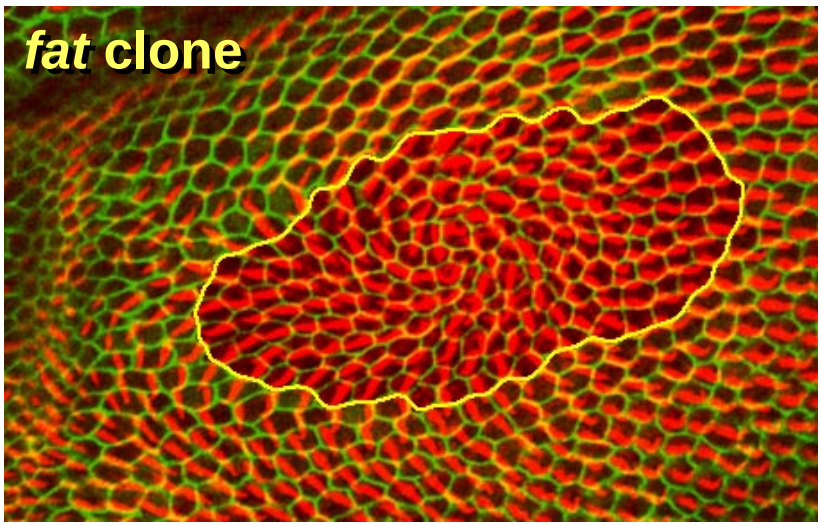
[Tree *et al.*, *Cell* **109**, 371, 2002]



Directional cue: evidence from fat clones?



- In the absence of fat (ft), the feedback loop amplifies and propagates polarity across the clone



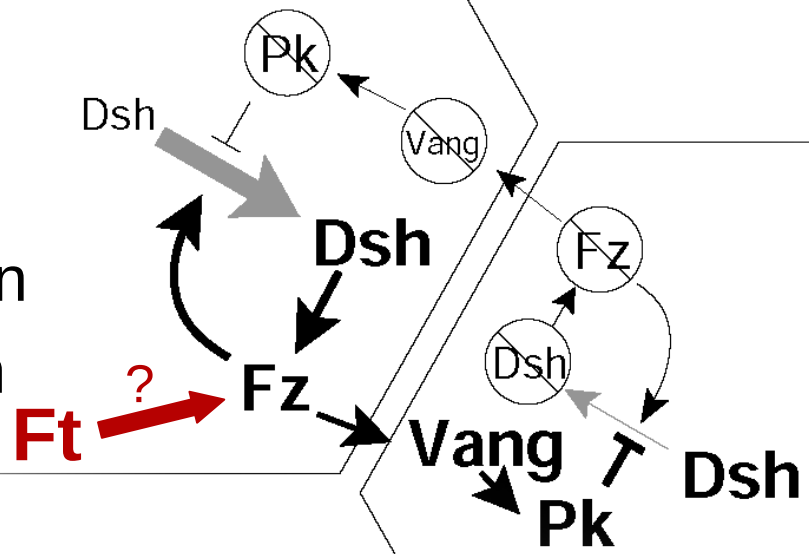
- Polarity does not always propagate correctly, resulting in swirled hair patterns

How do we account for the variability of polarity defects in ft clones?

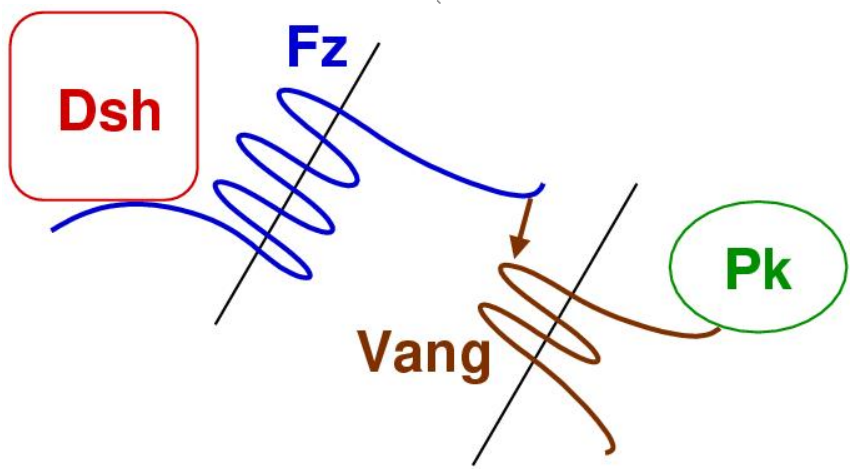
[Courtesy Dali Ma]

Biological Model

- Fz promotes recruitment of Dsh to a membrane
- Dsh stabilizes Fz localization
- Fz promotes the localization of Vang and Pk on the membrane of a neighboring cell
- Pk and Vang inhibit the recruitment of Dsh to a membrane
- Network amplifies unknown directional cue – **Ft?**



[Tree *et al.*, *Cell* **109**, 371, 2002]

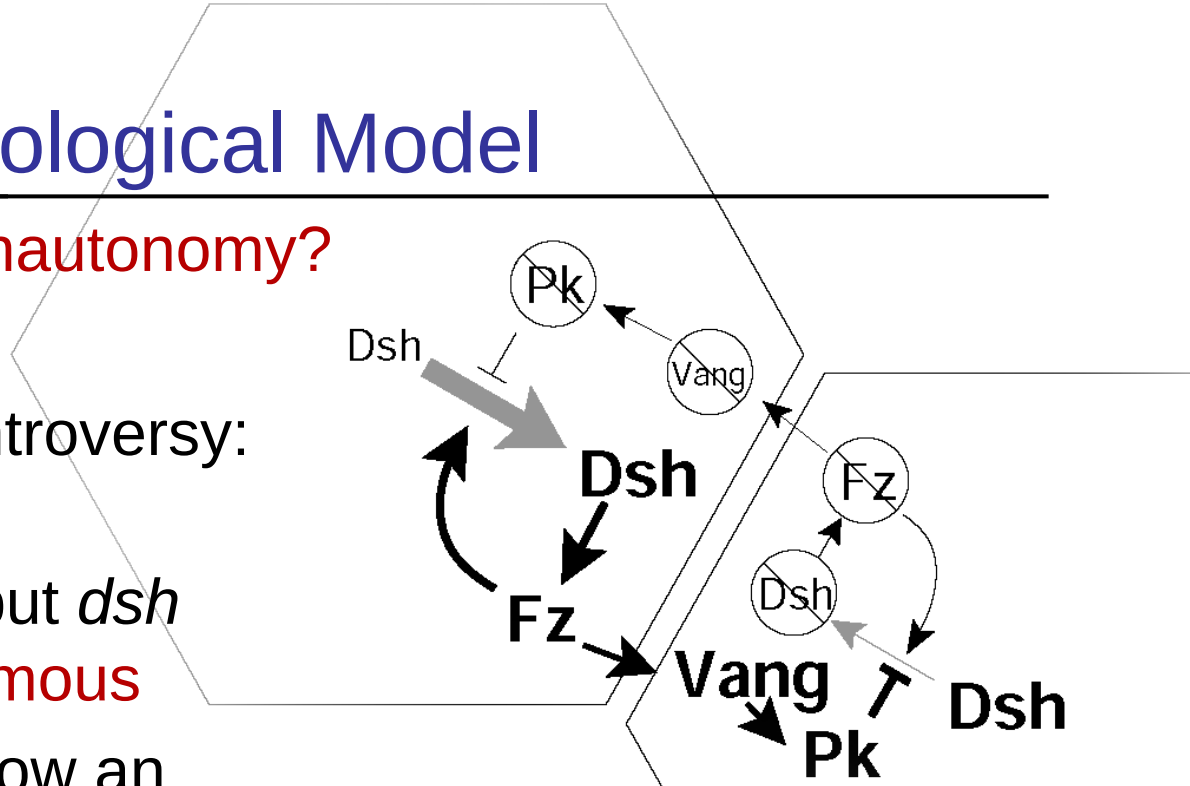


Biological Model

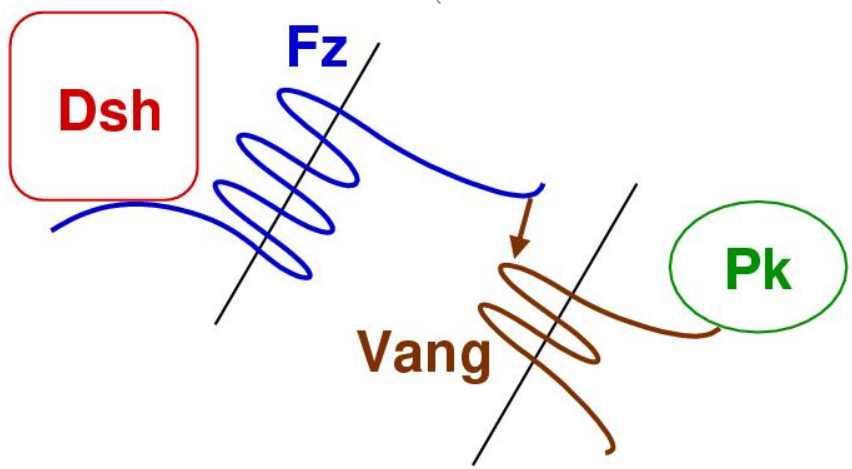
Does this explain nonautonomy?

Some sources of controversy:

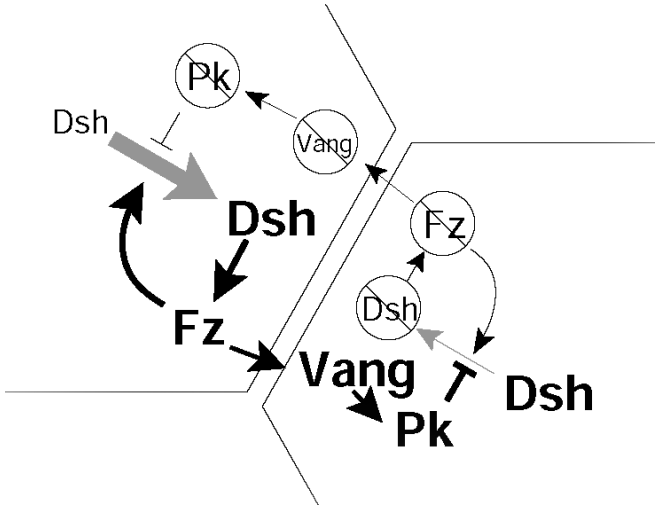
- Null *fz* clones are nonautonomous, but *dsh* clones are autonomous
- Some *fz* alleles show an autonomous polarity phenotype
- Increasing Pk actually increases Dsh and Fz accumulation

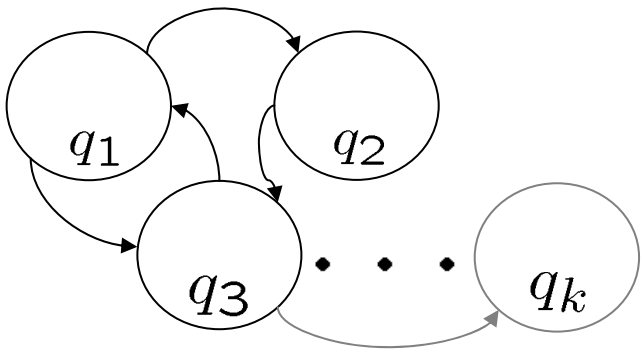


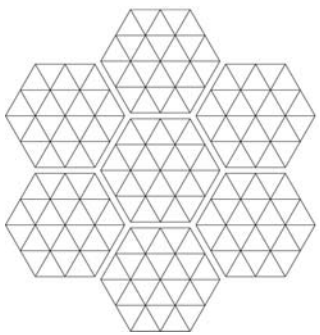
[Tree *et al.*, *Cell* **109**, 371, 2002]



Modeling Planar Cell Polarity







Discrete model

$$\text{if } \mathrm{Dsh}_{\mathrm{distal}} \text{ then } \mathrm{Fz}_{\mathrm{distal}}$$
$$\text{if } \mathrm{Fz}_{\mathrm{distal}} \text{ then } \mathrm{Pk}^{\dagger}_{\mathrm{proximal}}$$
$$\vdots$$

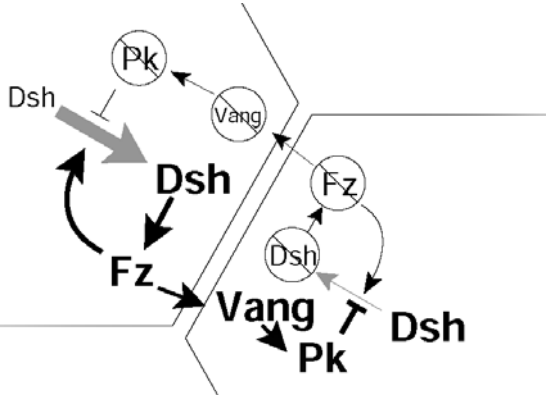
Hybrid model

$$q_1 : \mathrm{Dsh}_{\mathrm{distal}} > \mathrm{thresh}_{\mathrm{Fz}}$$
$$\frac{d[\mathrm{Fz}_{\mathrm{distal}}]}{dt} = \lambda_{\mathrm{Fz}}[\mathrm{Fz}_{\mathrm{distal}}] + R_{\mathrm{Fz}}$$
$$\vdots$$

Continuous model

$$\frac{\partial[\mathrm{DshFz}]}{\partial t} = P_1 - P_4^{\dagger} - P_9^{\dagger} + \mu_{\mathrm{DshFz}}\nabla^2[\mathrm{DshFz}]_D$$
$$\vdots$$
$$P_1 = R_1[\mathrm{Dsh}][\mathrm{Fz}] - A_1 B \lambda_1[\mathrm{DshFz}]$$
$$P_4 = R_4[\mathrm{DshFz}]^{\dagger}[\mathrm{Vang}] - \lambda_4[\mathrm{DshFzVang}]$$
$$P_9 = R_9[\mathrm{DshFz}]^{\dagger}[\mathrm{VangPk}] - \lambda_9[\mathrm{DshFzVangPk}]$$

Hybrid Model for PCP



Example: Dsh in proximal compartment

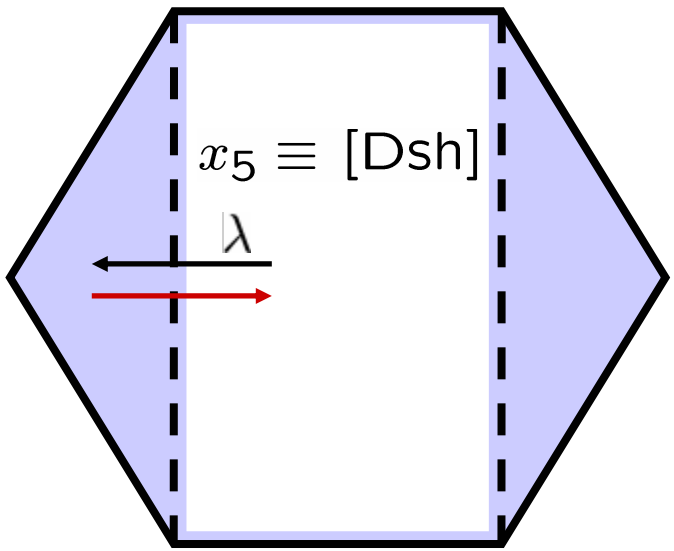
$$\frac{dx_1}{dt} = \lambda_1 f(x_3 - h_{\mathrm{Dsh},1})x_4 - \lambda_2 f(x_2 - h_{\mathrm{Dsh},2})x_1$$

Time rate of change of proximal Dsh concentration =

Transport *from* center compartment if [Fz] higher than threshold

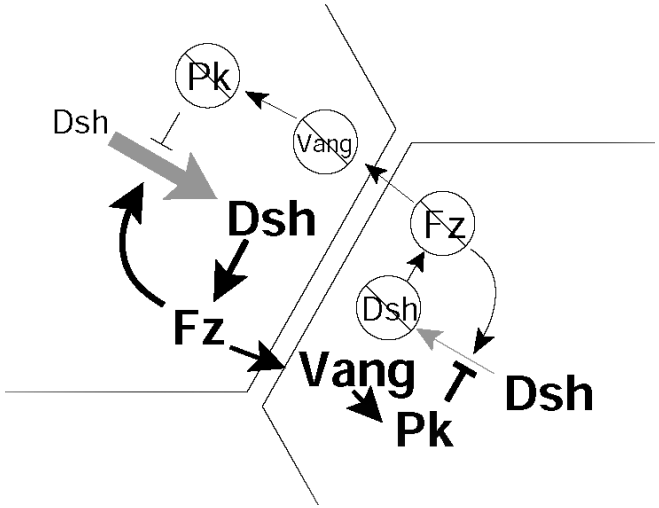
-Transport *to* center compartment if [Pk] is higher than threshold

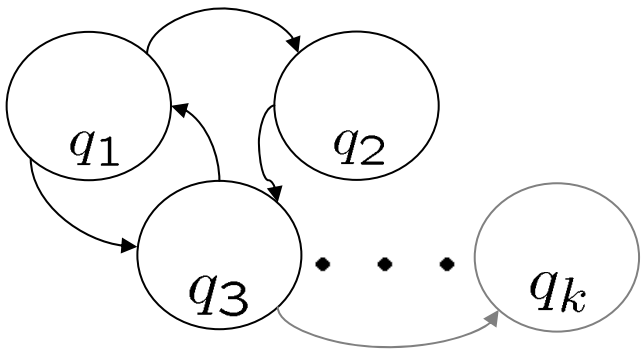
$x_1 \equiv$ [Dsh]
$x_2 \equiv$ [Pk]
$x_3 \equiv$ [Fz]
$x_4 \equiv$ [Vang]

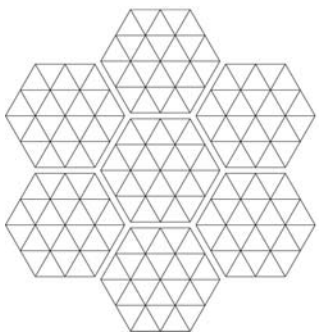


f is a discrete switching function that depends on the Fz / Pk concentrations and on a switching threshold

Modeling Planar Cell Polarity







Discrete model

$$\text{if } \mathrm{Dsh}_{\mathrm{distal}} \text{ then } \mathrm{Fz}_{\mathrm{distal}}$$
$$\text{if } \mathrm{Fz}_{\mathrm{distal}} \text{ then } \mathrm{Pk}^{\dagger}_{\mathrm{proximal}}$$
$$\vdots$$

Hybrid model

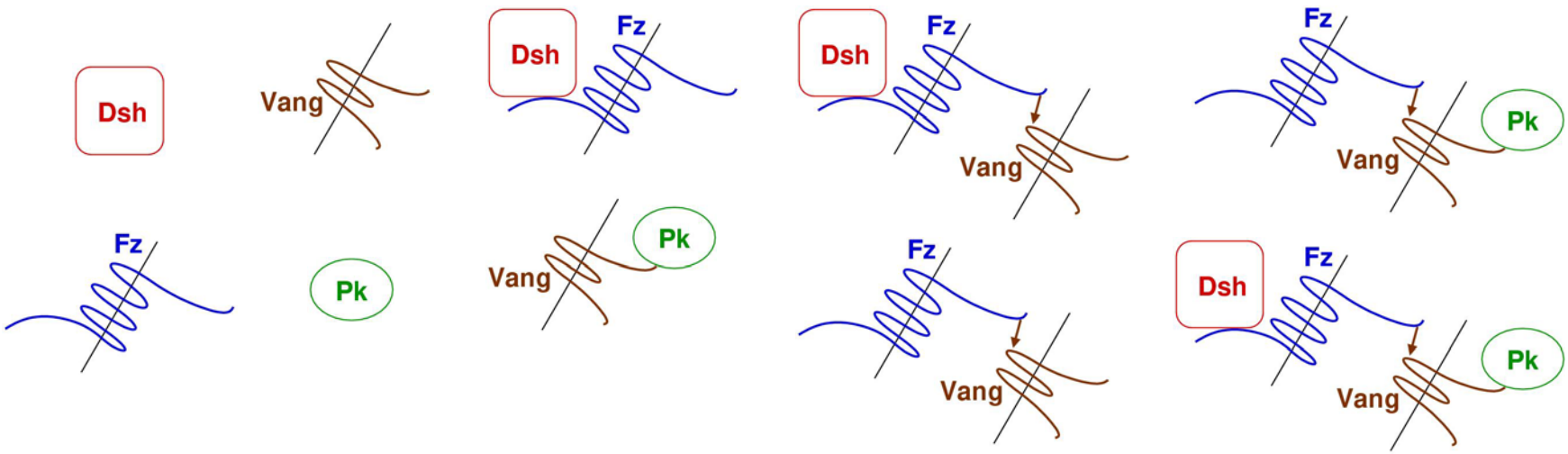
$$q_1 : \mathrm{Dsh}_{\mathrm{distal}} > \mathrm{thresh}_{\mathrm{Fz}}$$
$$\frac{d[\mathrm{Fz}_{\mathrm{distal}}]}{dt} = \lambda_{\mathrm{Fz}}[\mathrm{Fz}_{\mathrm{distal}}] + R_{\mathrm{Fz}}$$
$$\vdots$$

Continuous model

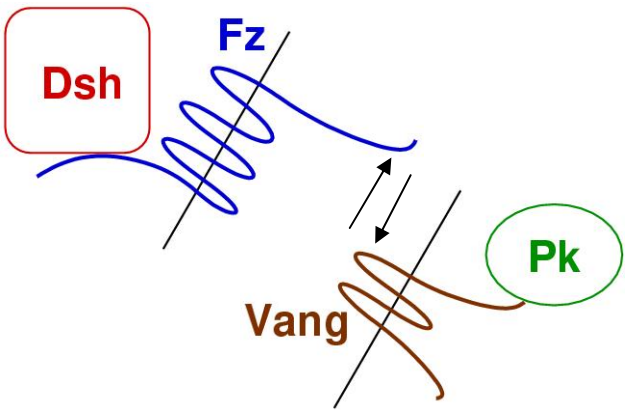
$$\frac{\partial[\mathrm{DshFz}]}{\partial t} = P_1 - P_4^{\dagger} - P_9^{\dagger} + \mu_{\mathrm{DshFz}}\nabla^2[\mathrm{DshFz}]_D$$
$$\vdots$$
$$P_1 = R_1[\mathrm{Dsh}][\mathrm{Fz}] - A_1 B \lambda_1[\mathrm{DshFz}]$$
$$P_4 = R_4[\mathrm{DshFz}]^{\dagger}[\mathrm{Vang}] - \lambda_4[\mathrm{DshFzVang}]$$
$$P_9 = R_9[\mathrm{DshFz}]^{\dagger}[\mathrm{VangPk}] - \lambda_9[\mathrm{DshFzVangPk}]$$

A Continuous Model for PCP

Model 4 proteins and introduce 6 complexes:



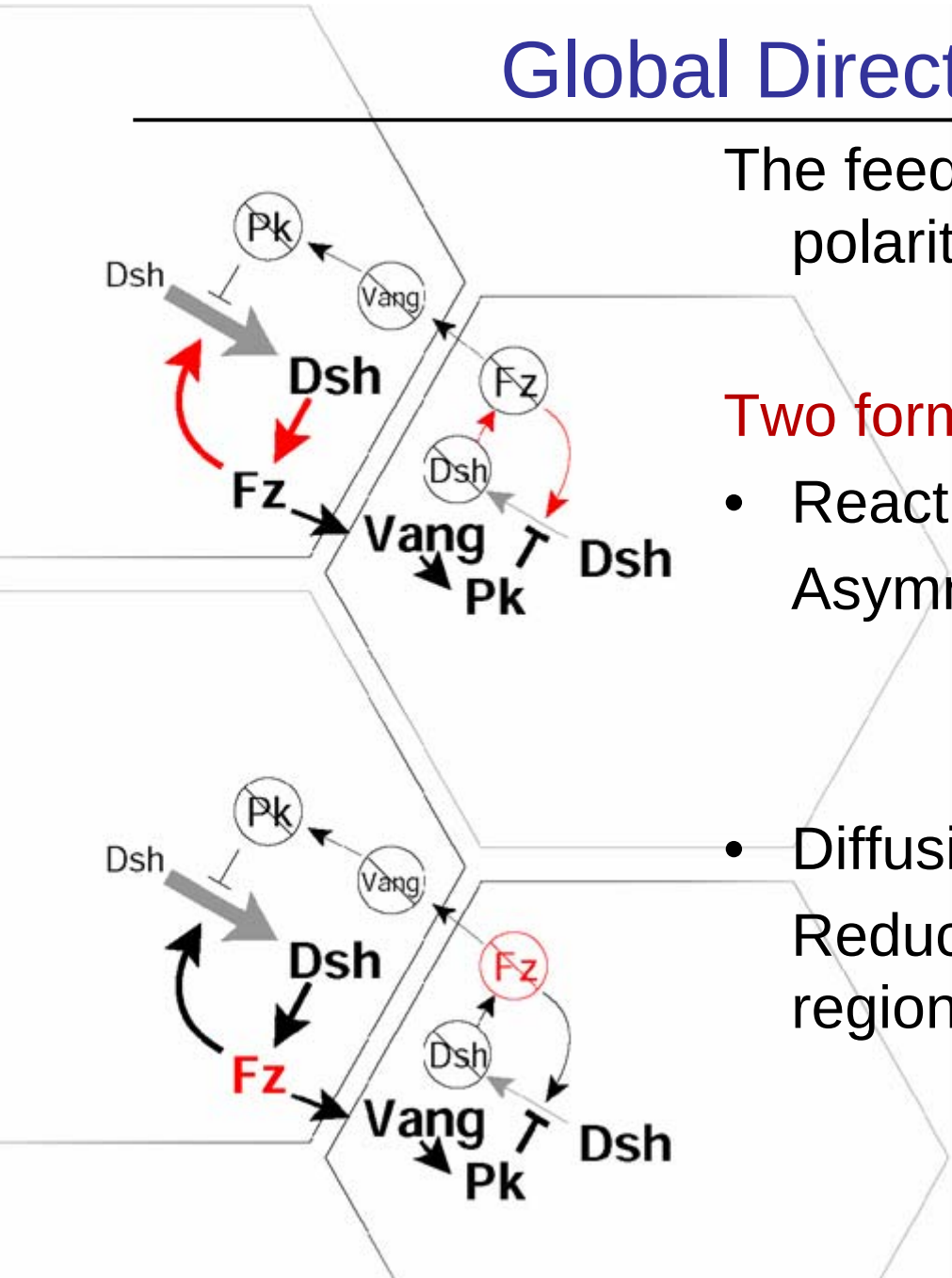
Protein interactions modeled as binding/unbinding reactions



$$\mathrm{DshFz}^{\dagger} + \mathrm{VangPk} \underset{\lambda_9}{\overset{R_9}{\rightleftarrows}} \mathrm{DshFzVangPk}$$

Dagger denotes a component
in a neighboring cell

Global Directional Cue



The feedback loop amplifies a global polarity asymmetry signal

Two forms of the input asymmetry:

- Reaction-based –

  Asymmetric Fz-Dsh interaction

- Diffusion-based –

  Reduced Fz diffusion in the distal region

Reaction Equations

There are 10 such reaction equations:

$$\mathrm{Dsh} + \mathrm{Fz} \underset{A_1 B \lambda_1}{\overset{R_1}{\rightleftarrows}} \mathrm{DshFz}$$

$$\mathrm{Fz}^{\dagger} + \mathrm{Vang} \underset{\lambda_2}{\overset{R_2}{\rightleftarrows}} \mathrm{FzVang}$$

$$\mathrm{Vang} + \mathrm{Pk} \underset{\lambda_3}{\overset{R_3}{\rightleftarrows}} \mathrm{VangPk}$$

$$\mathrm{DshFz}^{\dagger} + \mathrm{Vang} \underset{\lambda_4}{\overset{R_4}{\rightleftarrows}} \mathrm{DshFzVang}$$

$$\mathrm{Dsh}^{\dagger} + \mathrm{FzVang} \underset{A_1^{\dagger} B^{\dagger} \lambda_5}{\overset{R_5}{\rightleftarrows}} \mathrm{DshFzVang}$$

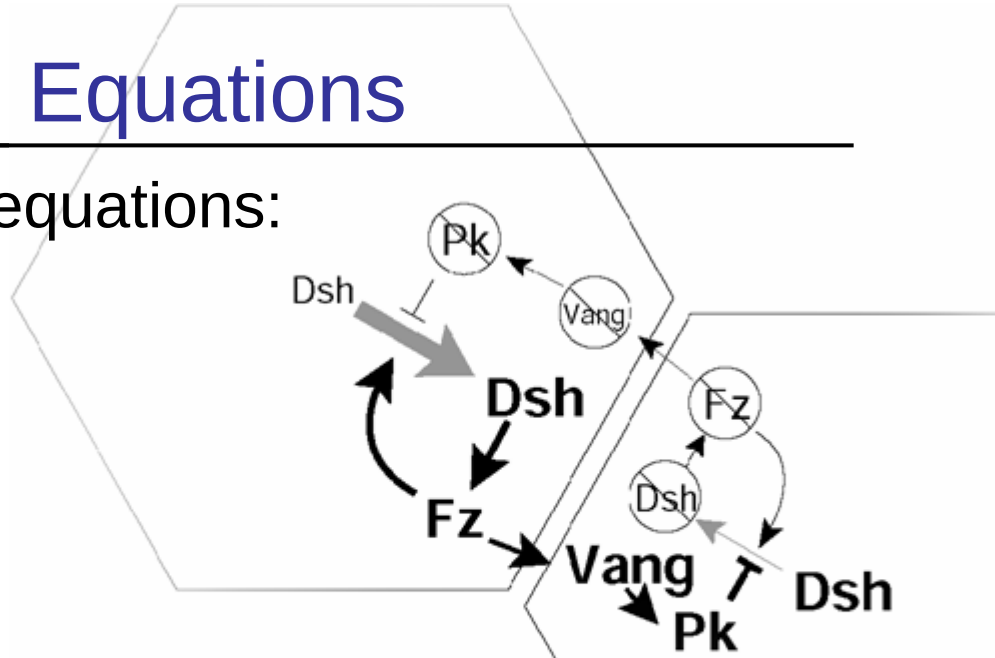
$$\mathrm{Fz}^{\dagger} + \mathrm{VangPk} \underset{\lambda_6}{\overset{R_6}{\rightleftarrows}} \mathrm{FzVangPk}$$

$$\mathrm{FzVang} + \mathrm{Pk} \underset{\lambda_7}{\overset{R_7}{\rightleftarrows}} \mathrm{FzVangPk}$$

$$\mathrm{Dsh}^{\dagger} + \mathrm{FzVangPk} \underset{A_1^{\dagger} B^{\dagger} \lambda_8}{\overset{R_8}{\rightleftarrows}} \mathrm{DshFzVangPk}$$

$$\mathrm{DshFz}^{\dagger} + \mathrm{VangPk} \underset{\lambda_9}{\overset{R_9}{\rightleftarrows}} \mathrm{DshFzVangPk}$$

$$\mathrm{DshFzVang} + \mathrm{Pk} \underset{\lambda_{10}}{\overset{R_{10}}{\rightleftarrows}} \mathrm{DshFzVangPk}$$



Reaction-based direct asymmetry signal:

$$A_1 = \begin{cases} M_1 < 1 & \text{distal vertex} \\ 1 & \text{otherwise} \end{cases}$$

Pk and Vang dependent inhibition of Dsh recruitment:

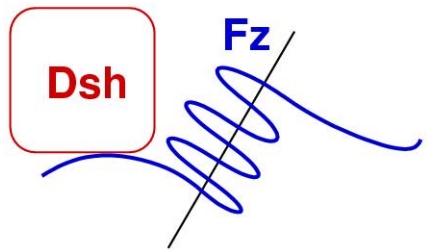
$$B = (1 + K_b(K_{\mathrm{Pk}}[\mathrm{Pk}] + [\mathrm{VangPk}] + [\mathrm{FzVangPk}] + [\mathrm{DshFzVangPk}] + K_{\mathrm{Vang}}([\mathrm{Vang}] + [\mathrm{FzVang}] + [\mathrm{DshFzVang}]))^{K_p})$$

Model Development

$$\mathrm{Dsh} + \mathrm{Fz} \underset{A_1 B \lambda_1}{\overset{R_1}{\rightleftarrows}} \mathrm{DshFz}$$

$$\mathrm{DshFz}^{\dagger} + \mathrm{Vang} \underset{\lambda_4}{\overset{R_4}{\rightleftarrows}} \mathrm{DshFzVang}$$

$$\mathrm{DshFz}^{\dagger} + \mathrm{VangPk} \underset{\lambda_9}{\overset{R_9}{\rightleftarrows}} \mathrm{DshFzVangPk}$$



Net production rate = Forward reaction rate – Backward reaction rate

$$P_1 = R_1[\mathrm{Dsh}][\mathrm{Fz}] - A_1 B \lambda_1[\mathrm{DshFz}]$$
$$P_4 = R_4[\mathrm{DshFz}]^{\dagger}[\mathrm{Vang}] - \lambda_4[\mathrm{DshFzVang}]$$
$$P_9 = R_9[\mathrm{DshFz}]^{\dagger}[\mathrm{VangPk}] - \lambda_9[\mathrm{DshFzVangPk}]$$

Local time rate of change of complex concentration =
Reaction production rate + Diffusion rate

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

Parameter Selection

All of the model parameters are unknown and not measurable from the available data

- Express PCP phenotypes as feature constraints
- Search for a feasible solution by adjusting model parameters using an optimization algorithm

Optimizer

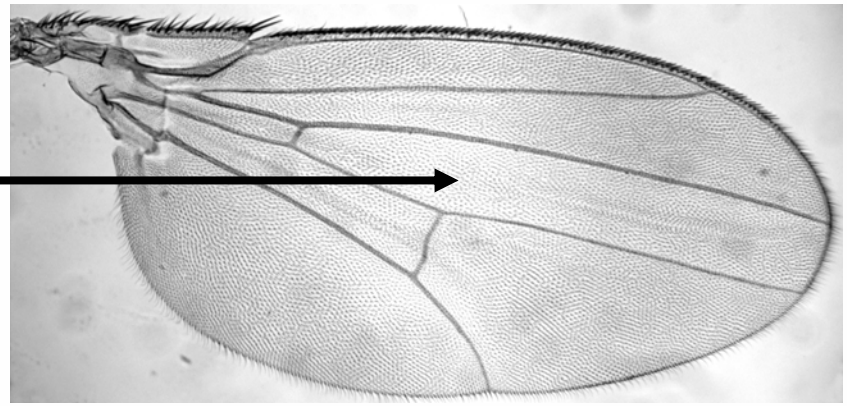
Minimize sum of quadratic penalty functions enforcing feature constraints

Objective Feature Constraints

Case	Obj	Constraint description
Wild-type	J_{wt}	Dsh and Fz accumulation distally Vang and Pk accumulation proximally
dsh clone	J_{dsh}	Autonomous phenotype
fz clone	J_{fz}	Distal polarity reversal
Vang clone	J_{Vang}	Proximal polarity reversal
pk clone	J_{pk}	No polarity reversal
>>*dsh* clone	$J_{>>dsh}$	Proximal polarity reversal
>>*fz* clone	$J_{>>fz}$	Proximal polarity reversal
>>*Vang* clone	$J_{>>Vang}$	Distal polarity reversal
>>*pk* clone	$J_{>>pk}$	Distal polarity reversal
$fz^{autonomous}$ clone	J_{fza}	Autonomous phenotype
>>$fz^{autonomous}$ clone	$J_{>>fza}$	Proximal polarity reversal
>>*pk-en*	$J_{pk\text{-}en}$	*pk* overexpression results in accumulation greater than or equal to wild-type

Governing model

$$X(t,s) = \begin{pmatrix} [\text{Dsh}](t,s) \\ [\text{Pk}](t,s) \\ [\text{Fz}](t,s) \\ [\text{Vang}](t,s) \\ [\text{DshFz}](t,s) \\ [\text{VangPk}](t,s) \\ [\text{FzVang}](t,s) \\ [\text{DshFzVang}](t,s) \\ [\text{FzVangPk}](t,s) \\ [\text{DshFzVangPk}](t,s) \end{pmatrix}$$



$$\underbrace{\frac{\partial X(t,s)}{\partial t}}_{\substack{\text{rate of change of} \\ \text{protein concentration}}} = \underbrace{P(X(t,s),\theta)}_{\text{rate of reaction}} + \underbrace{\mu(\theta)\,.\,\nabla_s^2 X(t,s)}_{\text{rate of diffusion}}$$

$\theta \in \mathbb{R}^{37}$ is the parameter vector to estimate:
reaction, diffusion rates, initial concentrations, asymmetry parameters

Parameter ID via Optimization

- Hair polarity $= f(X(T,.))$
  (Hair polarity is a function of Dsh concentration in each cell)

- Parameter ID problem:

$$\begin{aligned} &\text{minimize} && J(\theta) = ||f(X(T,.)) - f^{\text{observed}}||_2^2 \\ &\text{subject to} && \frac{\partial X(t,s)}{\partial t} = P(X(t,s),\theta) + \mu(\theta)\,.\,\nabla_s^2 X(t,s) \end{aligned}$$

- Find the best model in this class

Adjoint-based Algorithm

Gradient of the cost function:

$$\nabla J = - < q \, , \, \nabla_\theta P(X, \theta) + \nabla_s^2 X \, . \, \nabla \mu(\theta) >$$

provided that costate $q(s,t)$ satisfies

$$\frac{\partial \, q(t,s)}{\partial t} = -\nabla_x P(X,\theta)^T q(s,t) - \mu(\theta) \, . \, \nabla_s^2 \, q(s,t) \quad \text{(adjoint PDE)}$$

$$q(T,s) = 2(f(X(T,s)) - f^{observed})^T \nabla f(X(t,s)) \quad \text{(boundary conditions)}$$

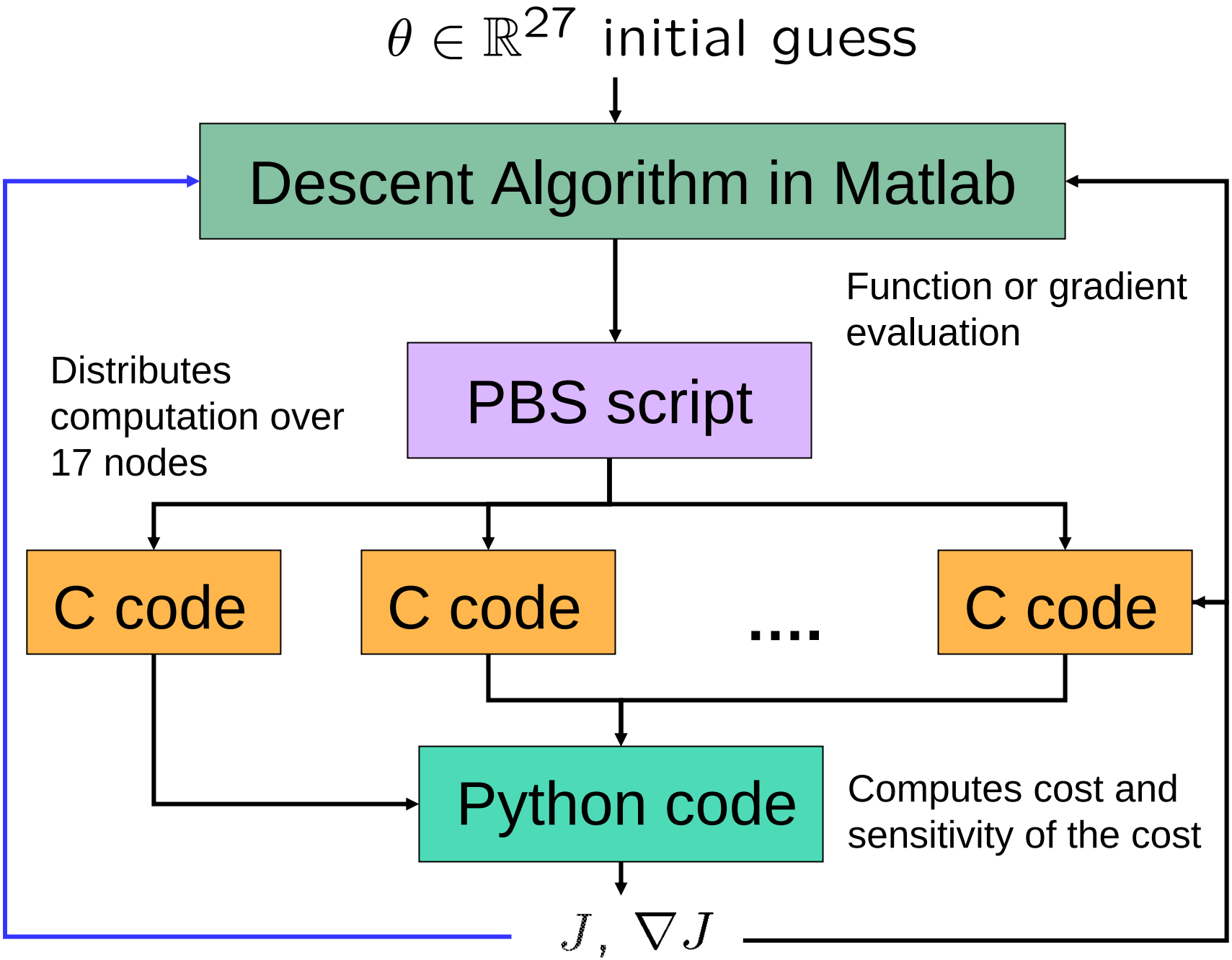
Algorithm 1 *:*

(1) Guess parameter θ

(2) Solve PDE numerically

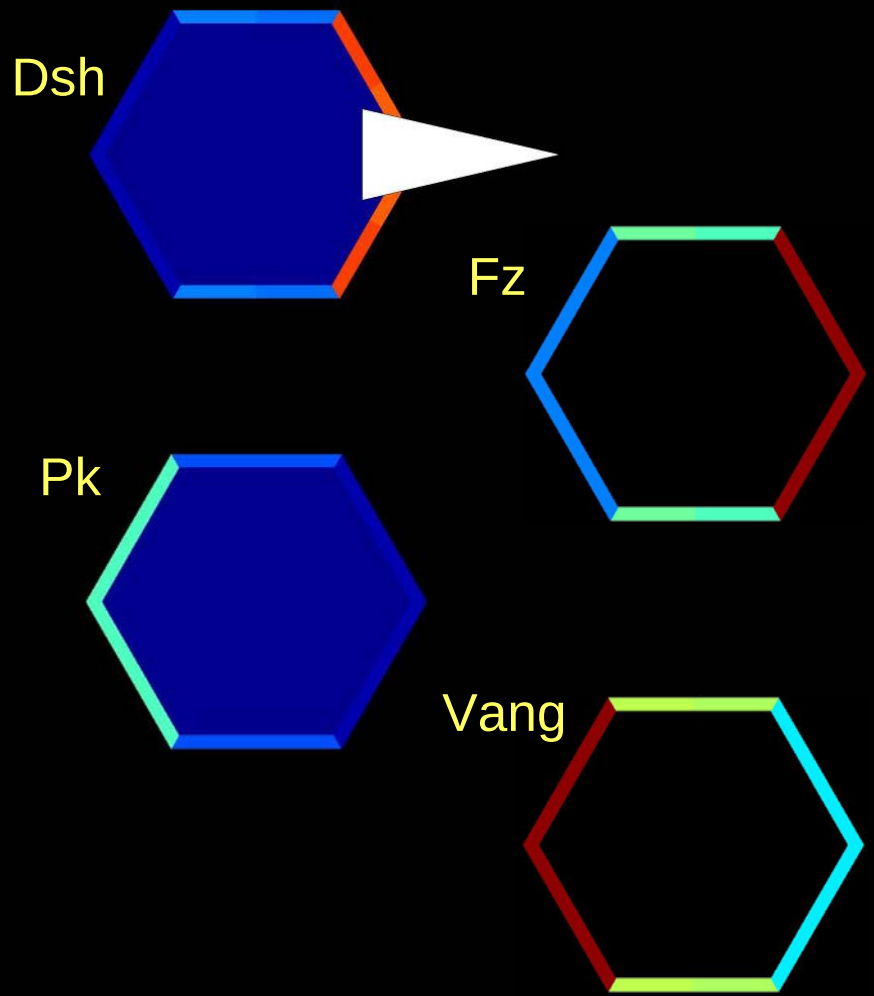
(3) Solve Adjoint PDE numerically

(4) Update parameter $\theta := \theta - \alpha \nabla J$

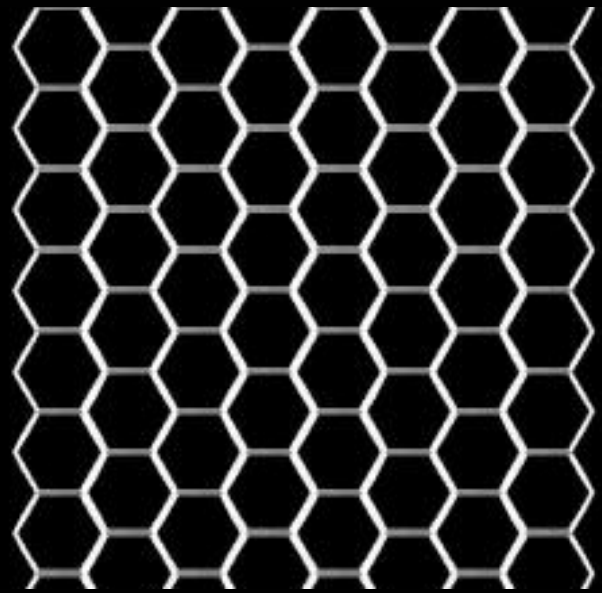

$\theta \in \mathbb{R}^{27}$ initial guess
Descent Algorithm in Matlab
Function or gradient evaluation
Distributes computation over 17 nodes
PBS script
C code
C code
....
C code
Python code
Computes cost and sensitivity of the cost
$J, \nabla J$

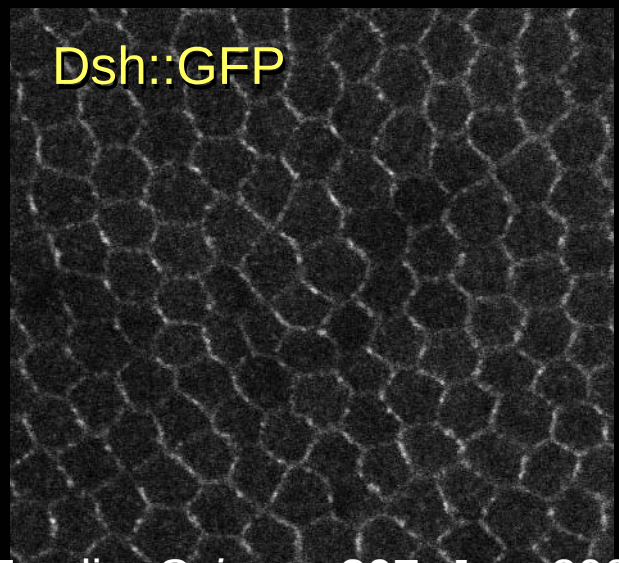
Wild-type Numerical Results

Periodic “infinite” array of cells



Dsh



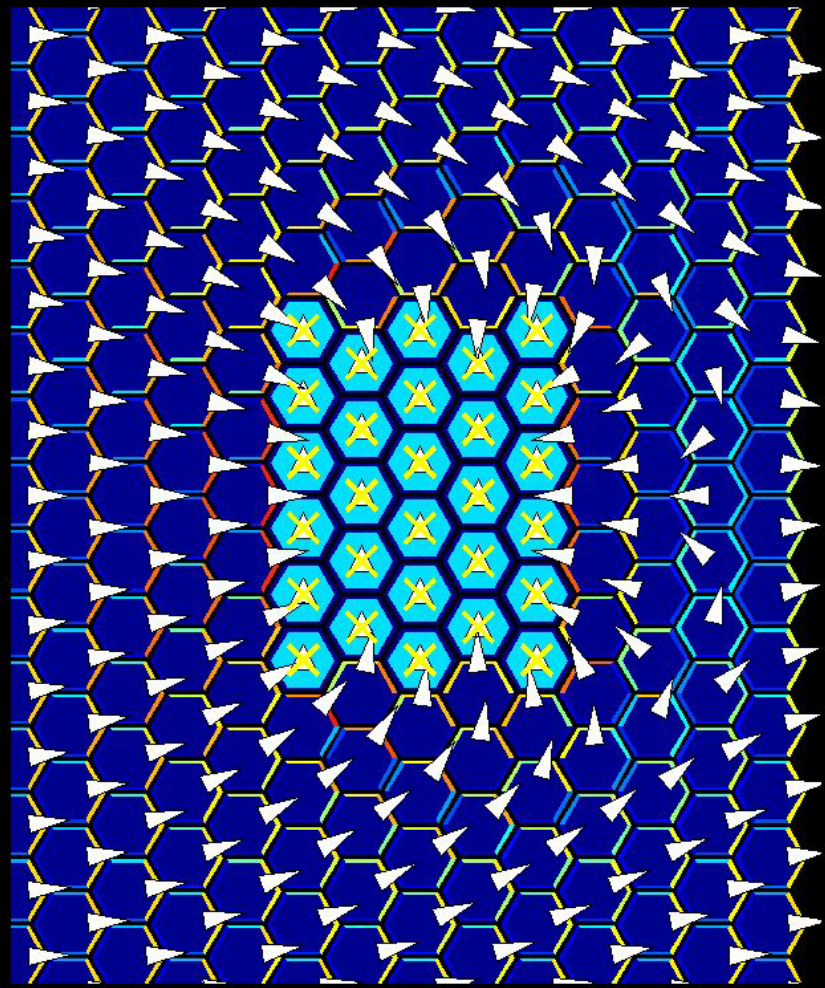


[Amonlirdviman, Khare, Tree, Chen, Axelrod, Tomlin, *Science* **307**, Jan. 2005]

Loss-of-Fz Numerical Results

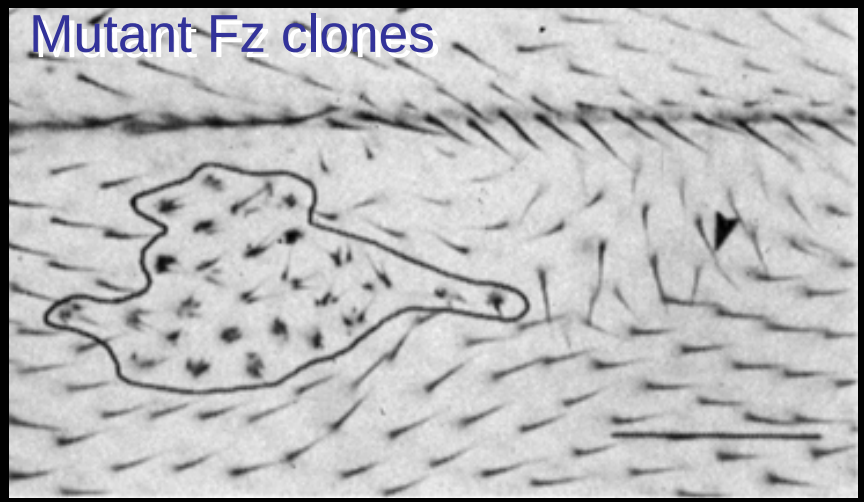
Dsh Distribution w/ Resulting Hair Pattern, 14 x 20 periodic cell array

fz clones



[Amonlirdviman et al, *Science* **307**, Jan. 2005]

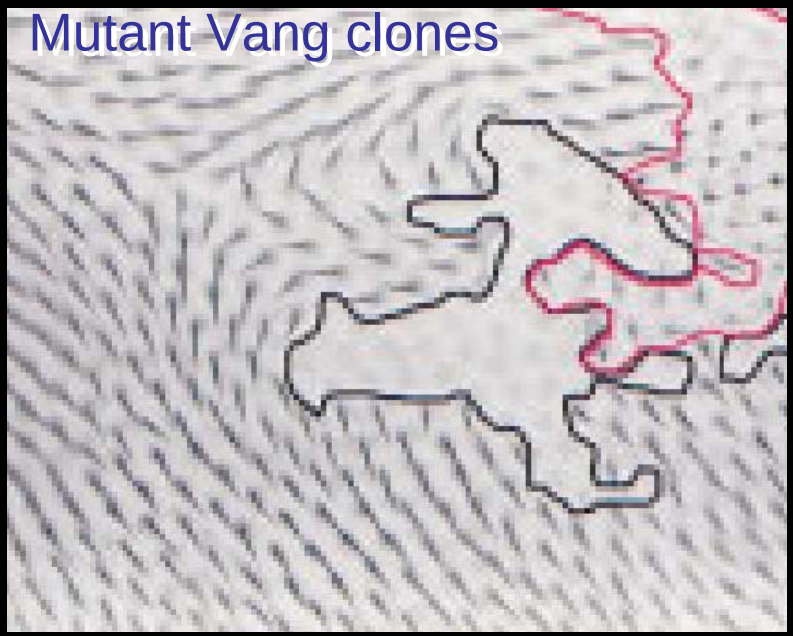
Domineering non-autonomy distal of cloned mutant cells



[Vinson and Adler, *Nature* **329**, 549-51, 1987]

Loss-of-Vang Numerical Results

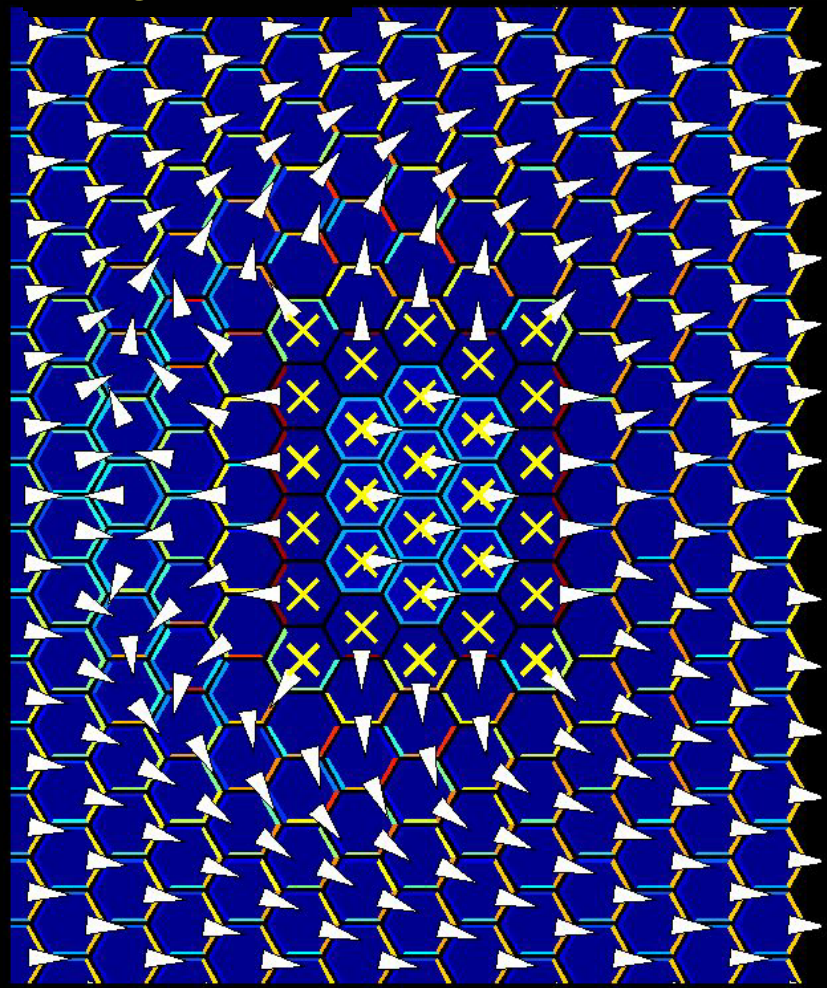
Domineering non-autonomy proximal of cloned mutant cells



[Taylor, et al., *Genetics* **150**, 199-210, 1998]

Dsh Distribution w/ Resulting Hair Pattern. 14 x 20 periodic cell array

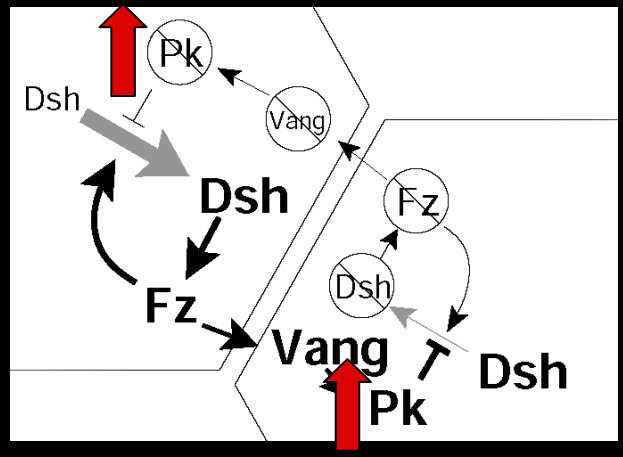
vang clones



[Amonlirdviman et al, *Science* **307**, Jan. 2005]

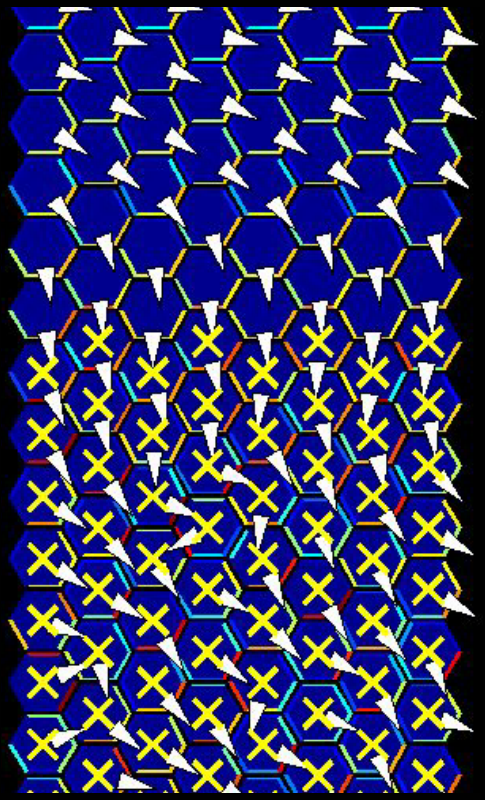
Biological Insights

- Demonstrates sufficiency
- Explains even non-intuitive results

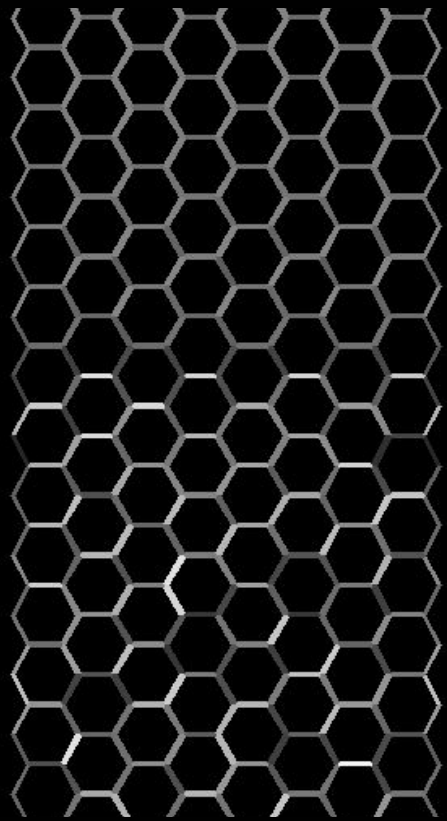


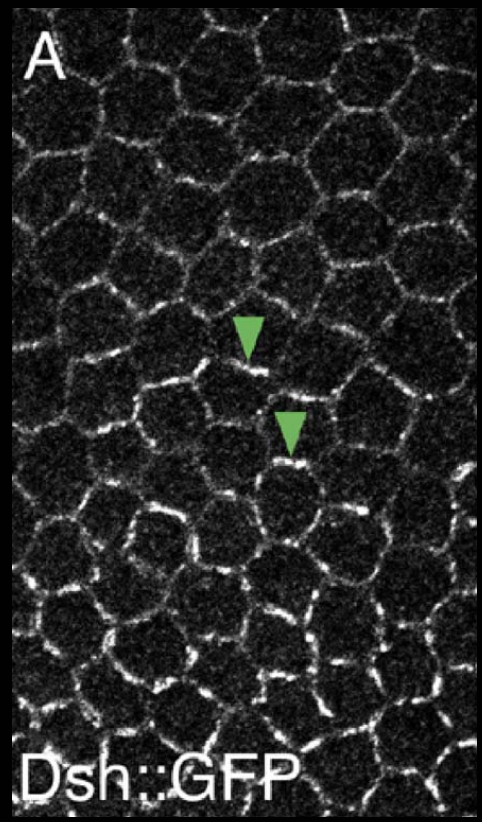
Suppose you overexpress Pk in part of the wing:

Dsh



x Pk

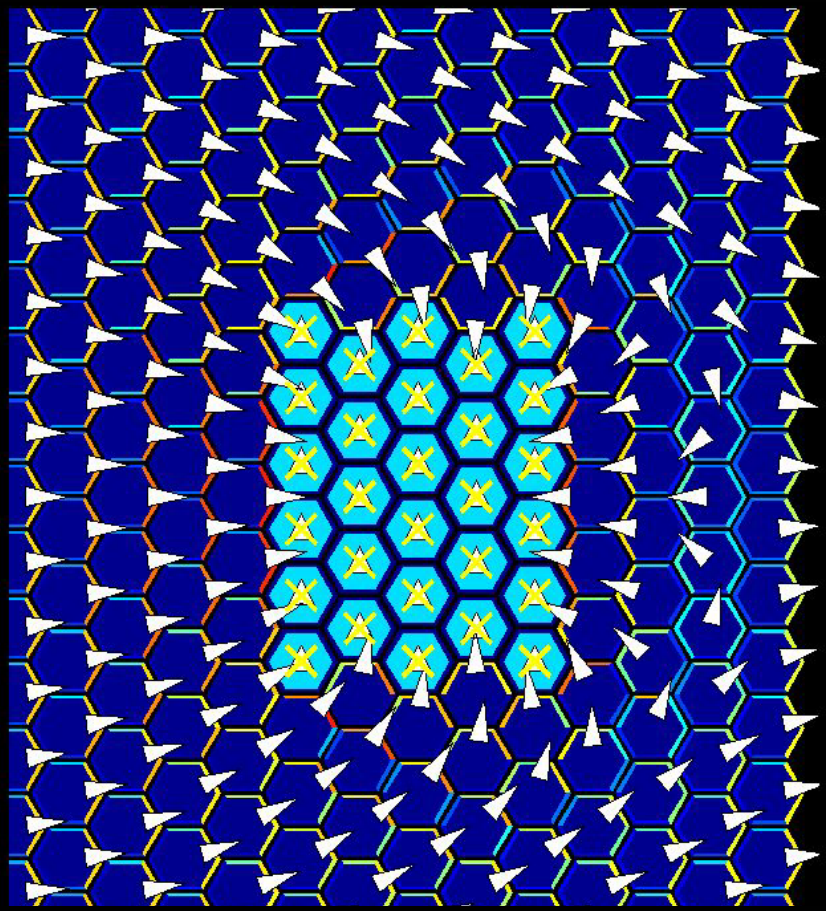




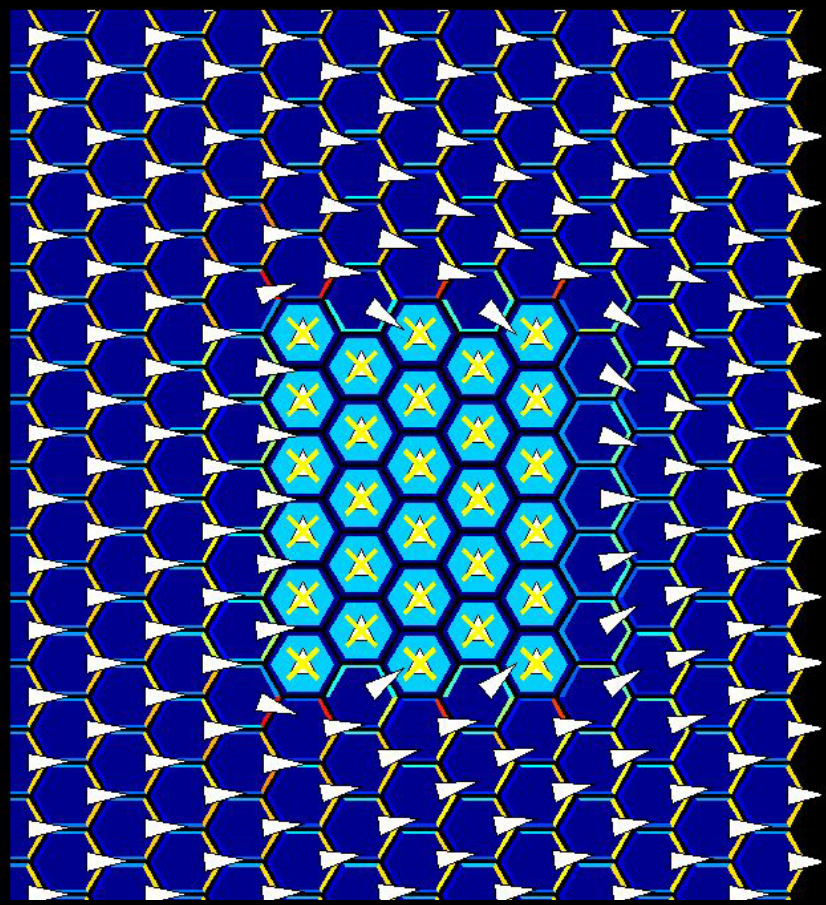
Understanding *fz* Autonomous Alleles

- Suggests mechanism for explaining phenotypes of different mutant Fz alleles

fz nonautonomous allele –
All Fz function removed



fz autonomous allele –
Fz–Dsh interaction reduced to 0.01%



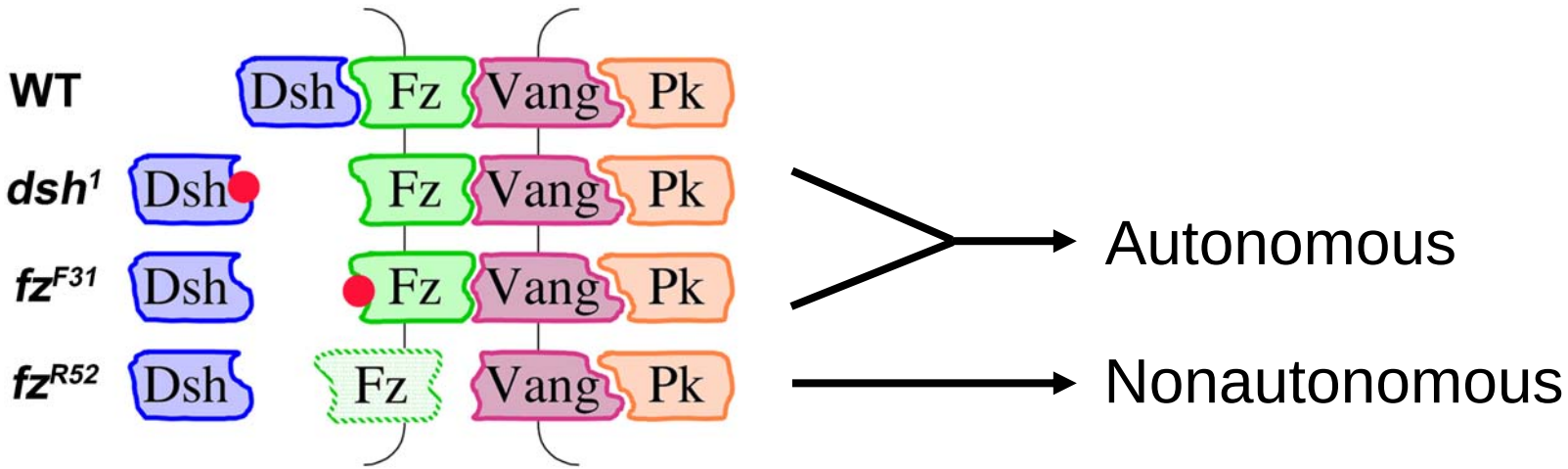
Understanding *fz* Autonomous Alleles

- Propose that Fz autonomous proteins are deficient in complexing with Dsh, but retain Vang interaction
- Nonautonomous *fz* alleles lose both interactions

Hypothesis makes two predictions:

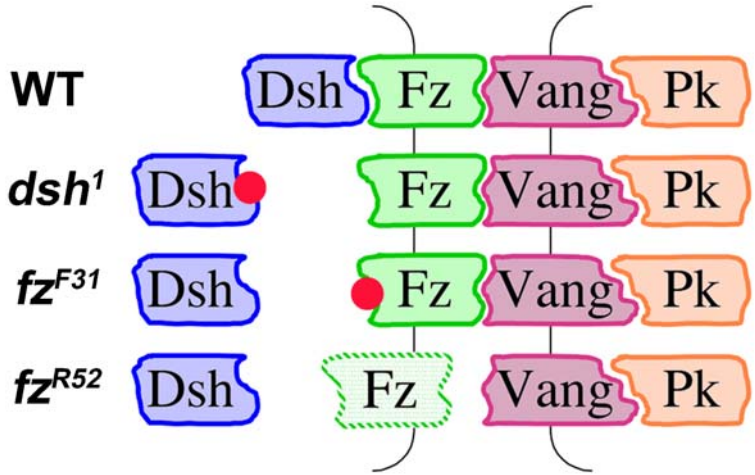
- Autonomous Fz protein recruits Vang to neighboring membranes;

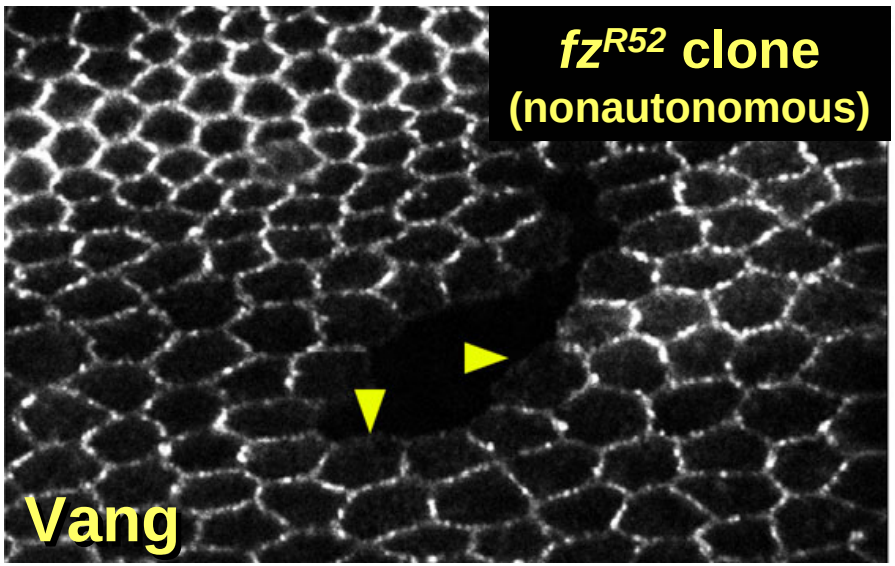
  nonautonomous Fz protein should not
- Both proteins should fail to recruit Dsh

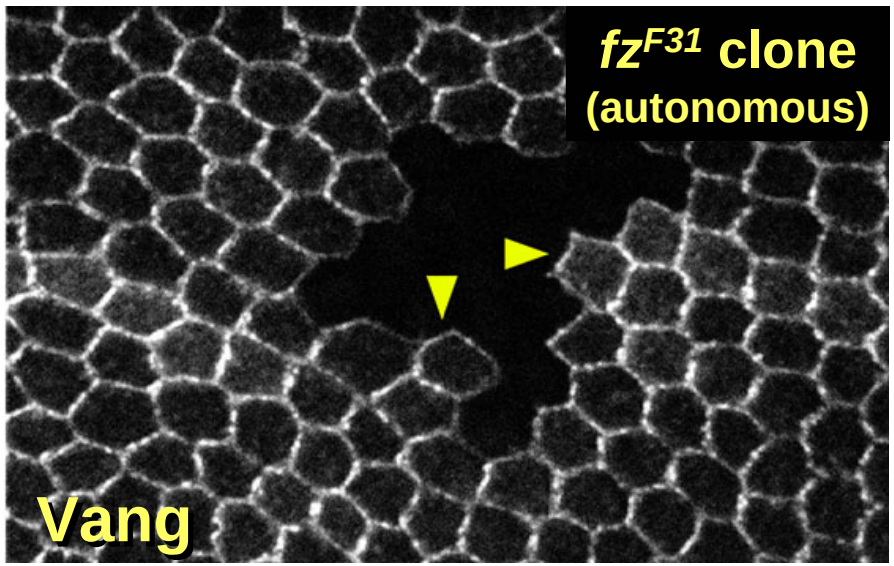


Understanding *fz* Autonomous Alleles

- Vang::YFP does not accumulate at boundaries of fz^{R52} (nonautonomous) clones
- Vang::YFP accumulates at boundaries of fz^{F31} (autonomous) clones



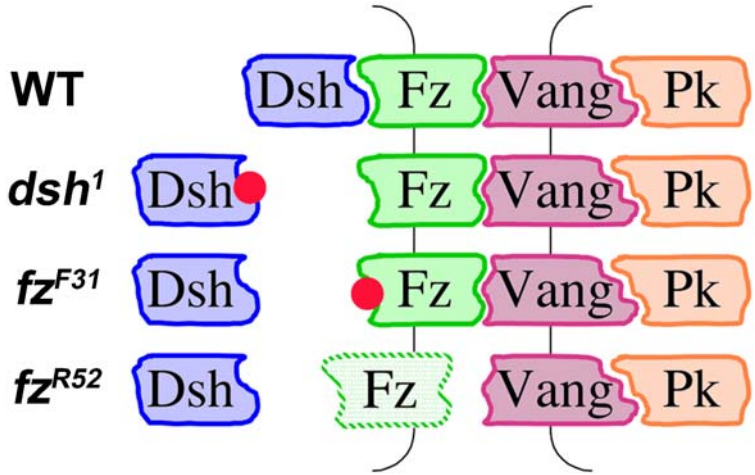


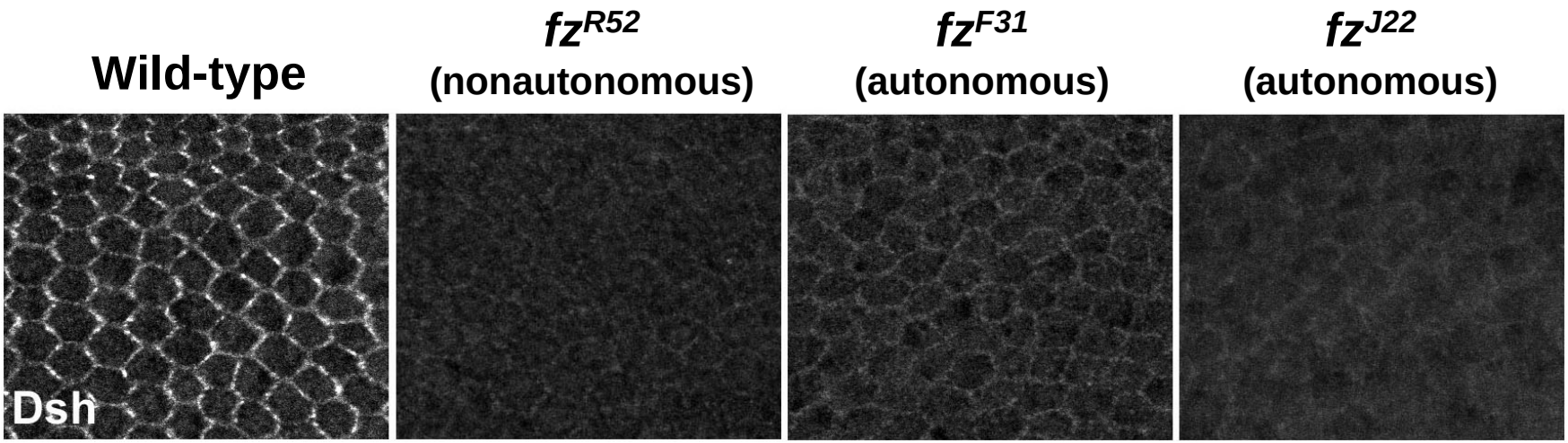


[Amonlirdviman et al, *Science* **307**, Jan. 2005]

Understanding *fz* Autonomous Alleles

- Dsh::GFP is not recruited by fz^{R52} (nonautonomous)
- Dsh::GFP is poorly recruited by fz^{F31} and more poorly recruited by fz^{J22} (autonomous)



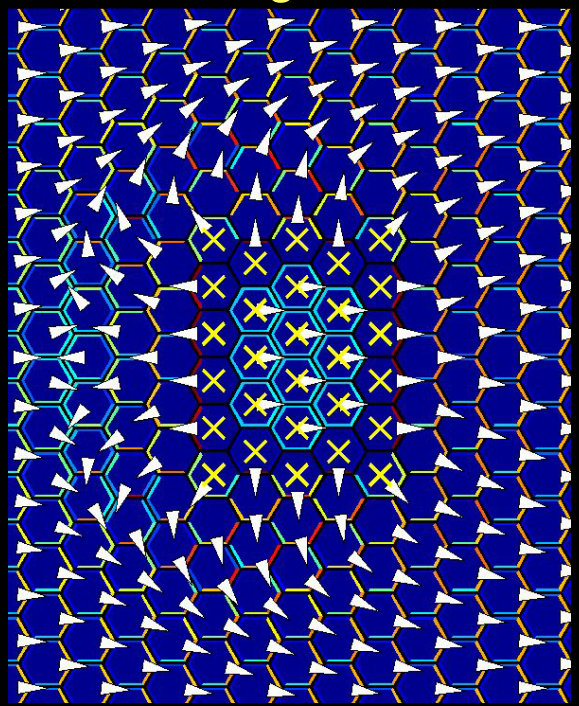


[Amonlirdviman et al, *Science* **307**, Jan. 2005]

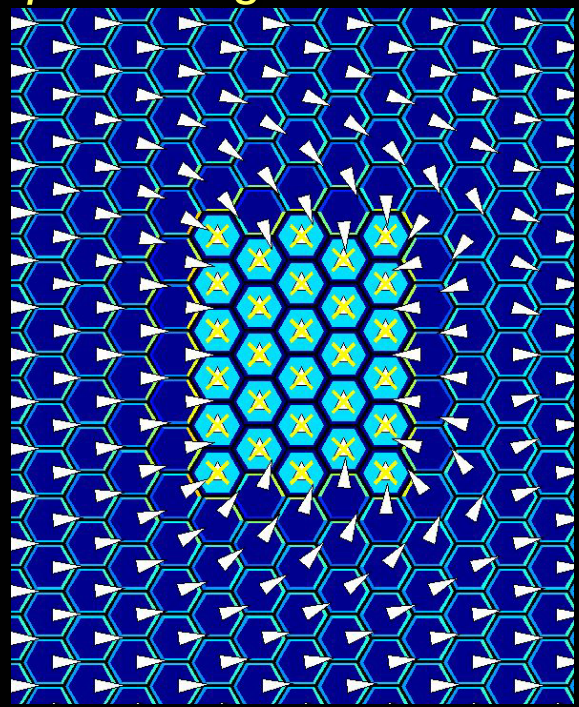
“Lawrence Challenge”

- Conditions proposed by Lawrence, Casal, and Struhl prior to publication of results from the *Drosophila* abdomen

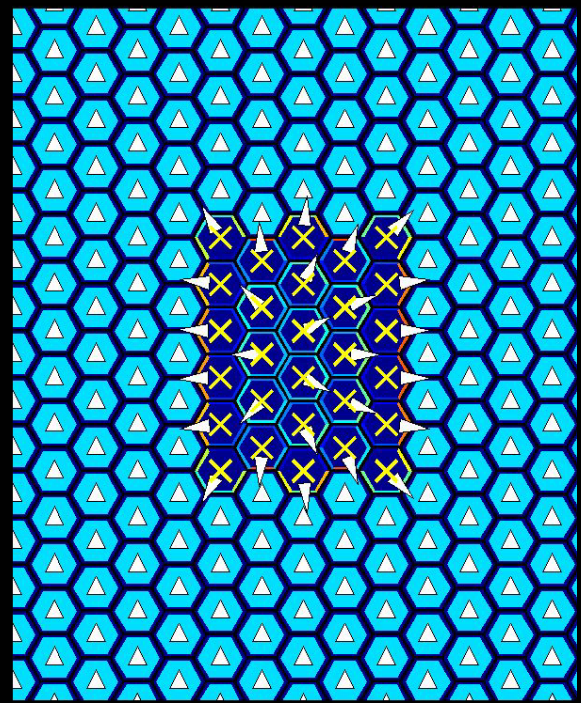
>>fz / *Vang*$^{-}$ clone



fz^{-} clone / *pk*$^{-}$ background



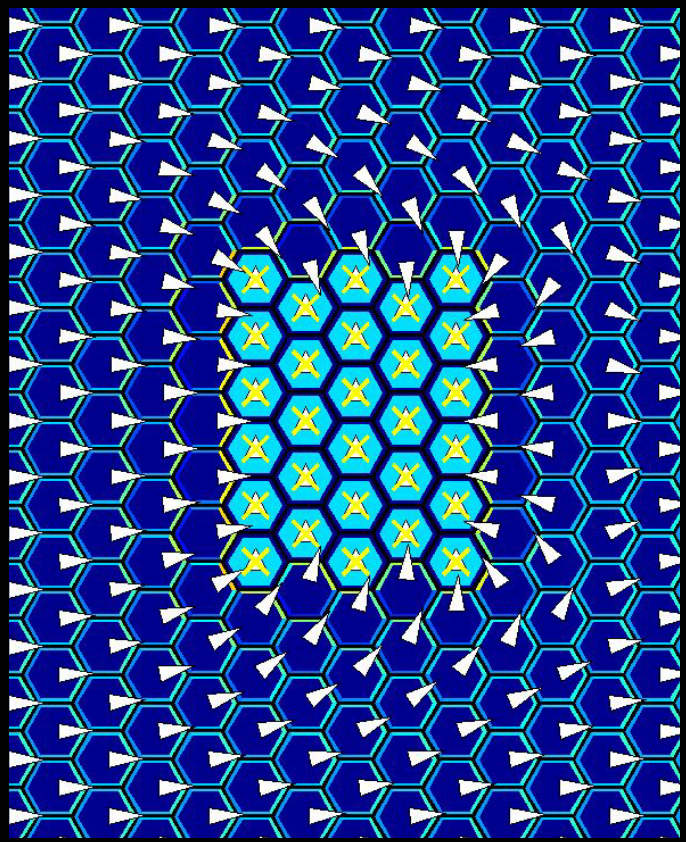
>>fz clone / *fz*$^{-}$ background

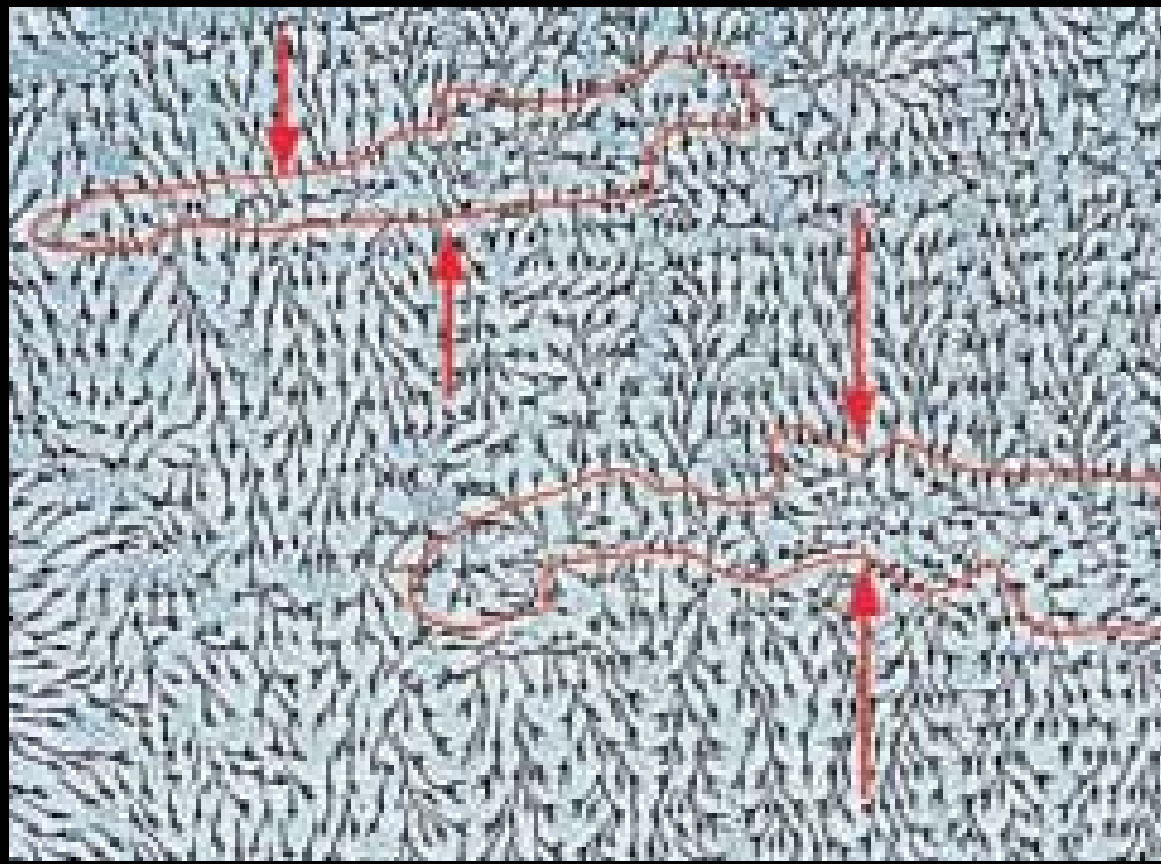


“Lawrence Challenge”

- Example of nonautonomy in the absence of a core polarity component, *pk*

fz^{-} clone / *pk*$^{-}$ background





[Lawrence *et al.*, *Development* **131**, 4651, 2004]

Insights into Nonautonomy

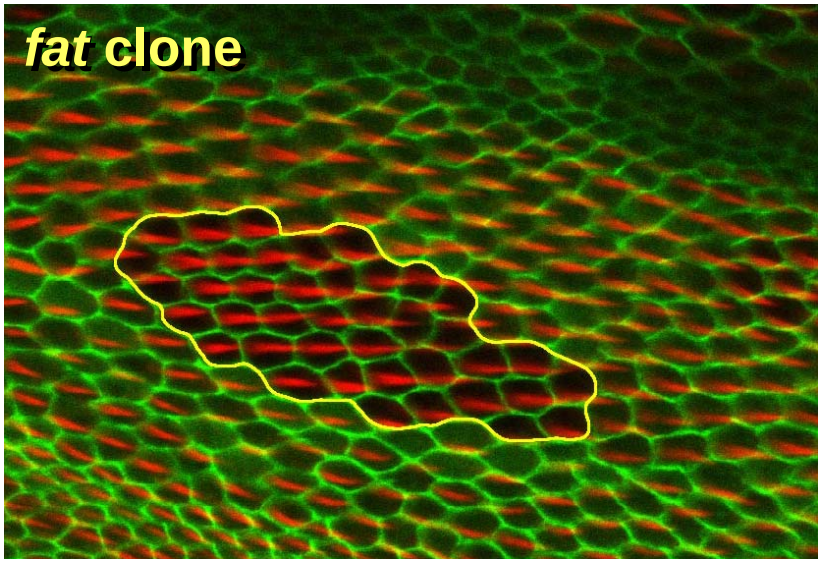
- Demonstrated that the feedback loop can fully reproduce characteristic PCP phenotypes – Unidentified diffusible factors unnecessary

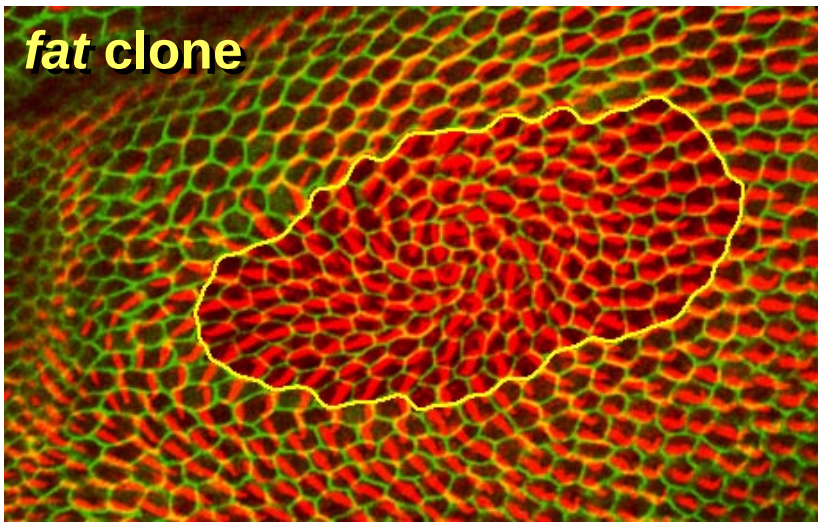
- Showed that the feedback loop model more readily accounts for slight nonautonomy of clones of *dsh* and autonomous *fz* alleles

- Proposed a mechanistic explanation for the difference between autonomous and nonautonomous *fz* alleles, motivating experiments supporting this hypothesis

- Predicted other phenotypes not used to train the model

Understanding *fat* clones





[Courtesy Dali Ma]

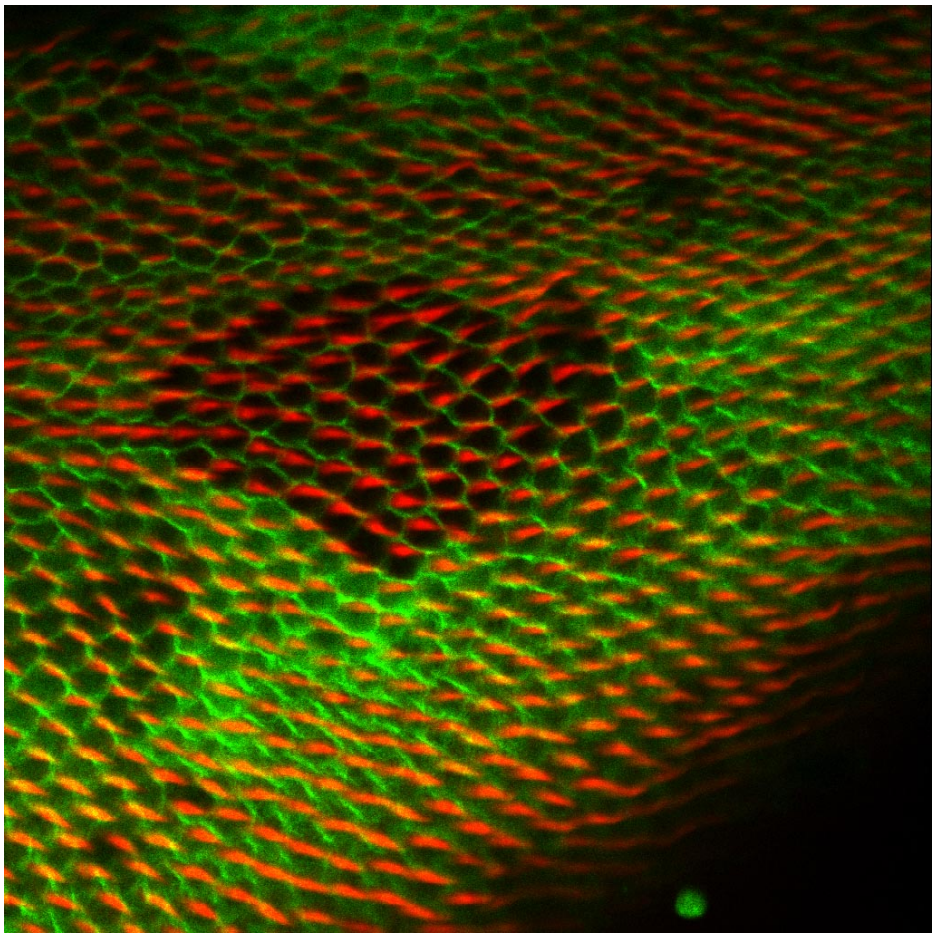
The role of cell geometry

- Polarity defects correlate to irregular cell geometry
- Frequency of polarity defects can be modified by altering cell shape

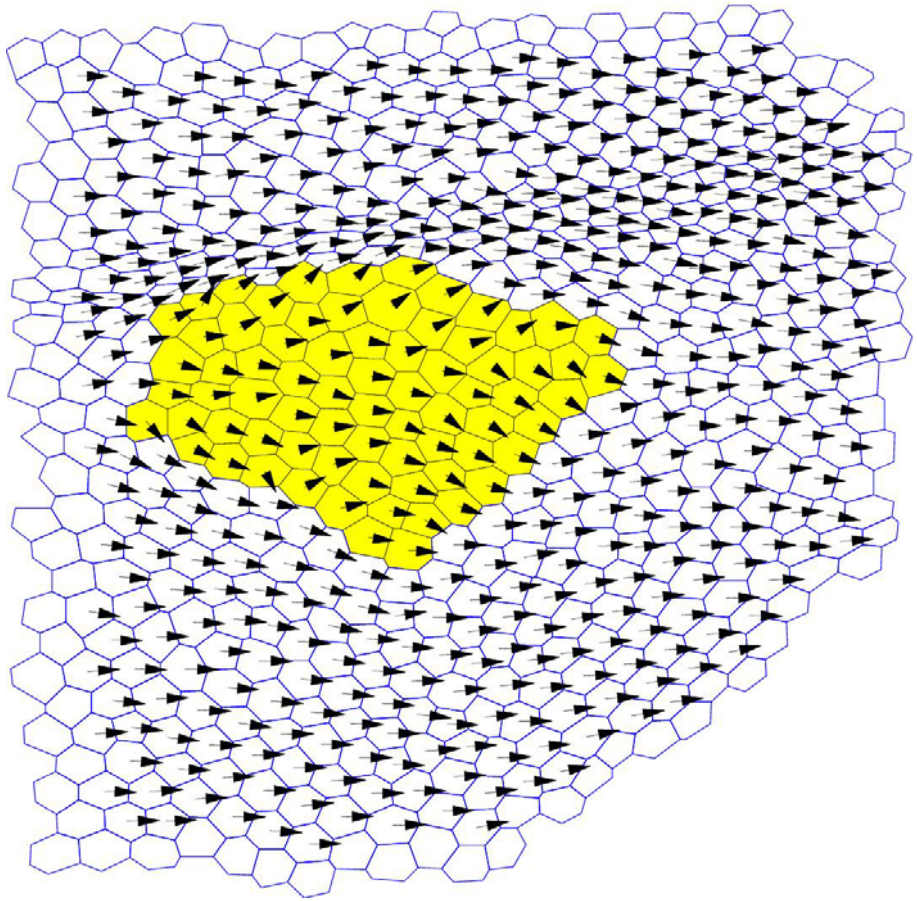
Are polarity defects a consequence of the Fz feedback loop when confronted with irregular cell geometries?

Understanding *fat* clones

ft clone



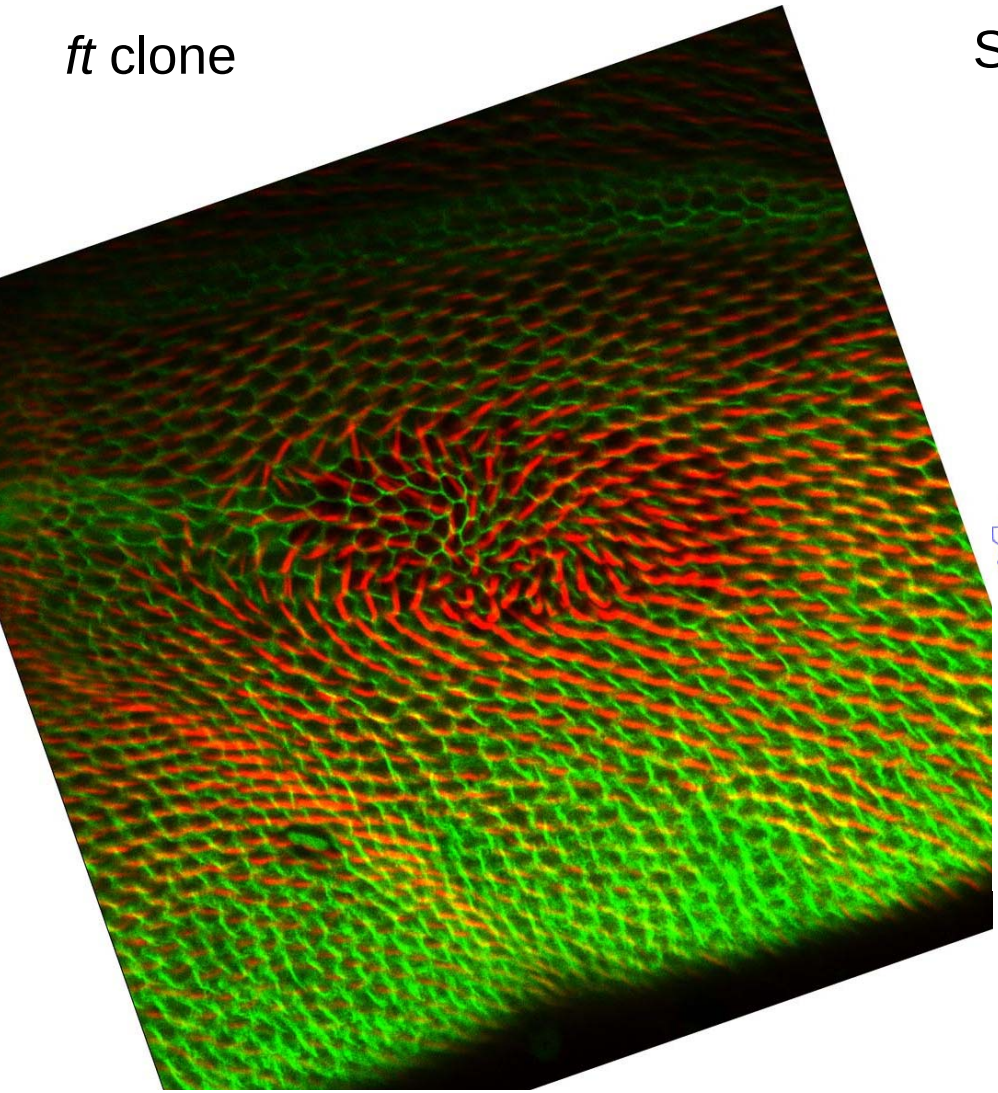
Simulated polarity



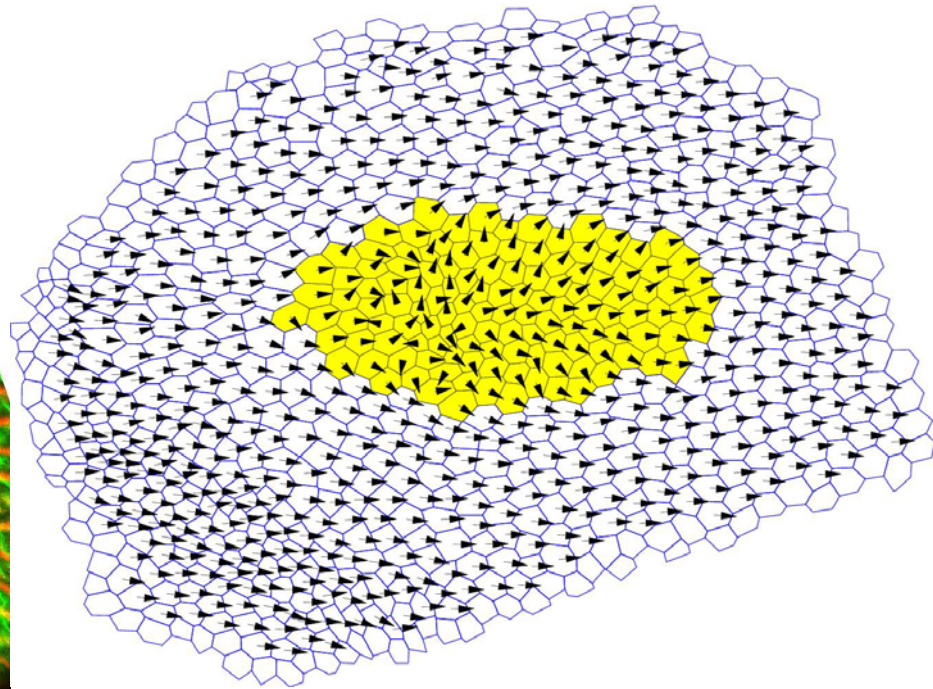
[Ma et al., *Submitted, 2006*]

Understanding *fat* clones

ft clone



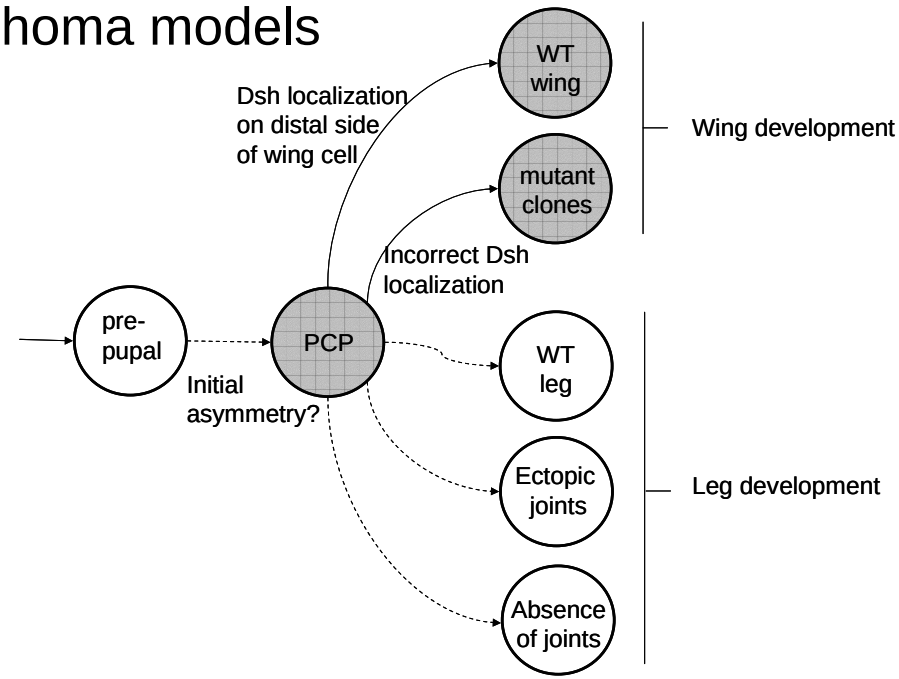
Simulated polarity

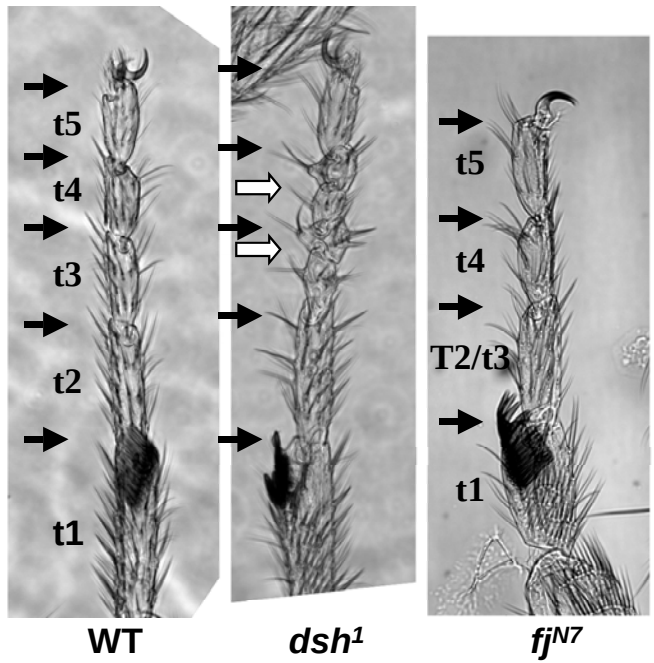


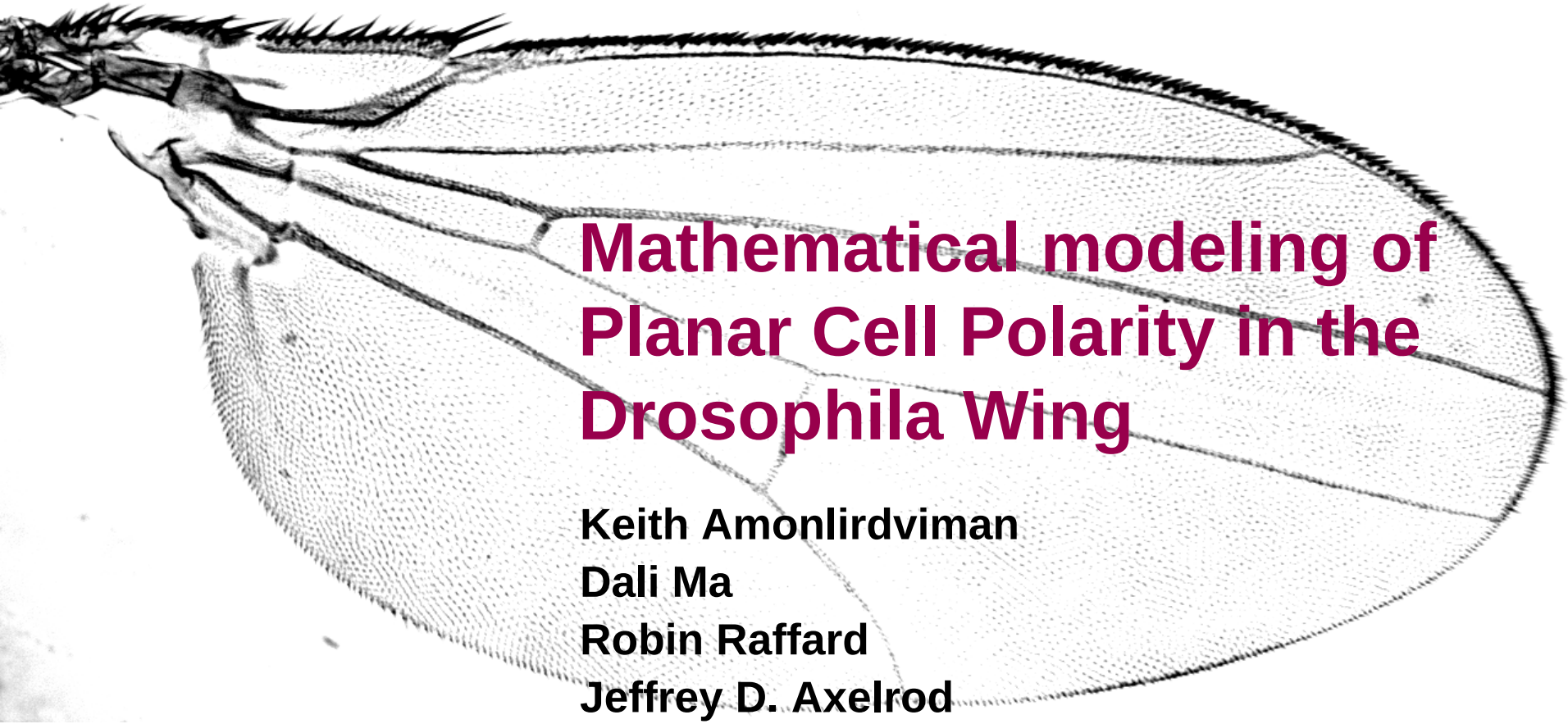
[Ma et al., *Submitted, 2006*]

Summary and current work

- Demonstrated the sufficiency of the model [Factor X unnecessary]
- Begun to derive insights into the nature of domineering non-autonomy
- Proposed and conducted experiments exploring the interaction of Dsh with different Fz alleles
- Developing analytical tools for parameter identification based on hybrid systems methodology and adjoint method
- Interaction of PCP with other protein networks
- Lymphoma models







Mathematical modeling of Planar Cell Polarity in the Drosophila Wing

Keith Amonlirdviman
Dali Ma
Robin Raffard
Jeffrey D. Axelrod
Claire J. Tomlin

NIH, DARPA, Stanford's Bio-X program, Honeywell Labs