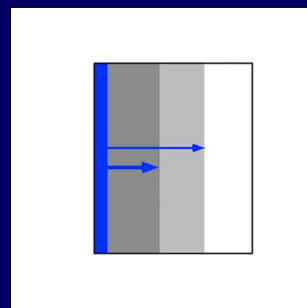


Morphogen Gradients

Morphogens

- secreted signaling proteins
- localized source
- act directly at a distance
- concentration-dependent
- instruct cells about their prospective fate



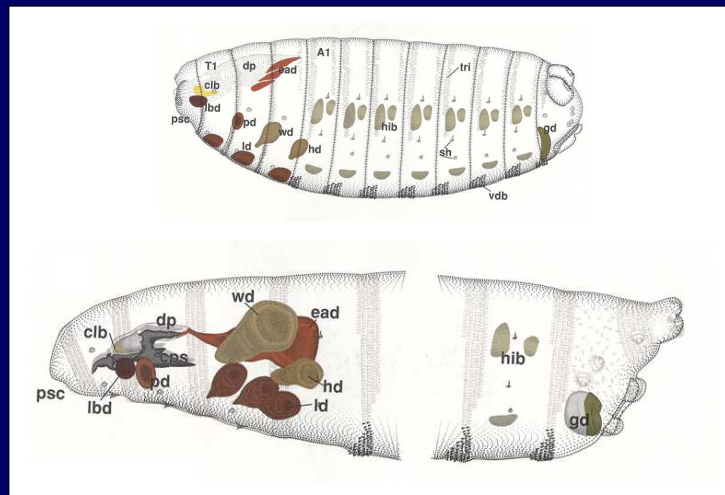
Agenda

Introduction to the system

'Recent' developments

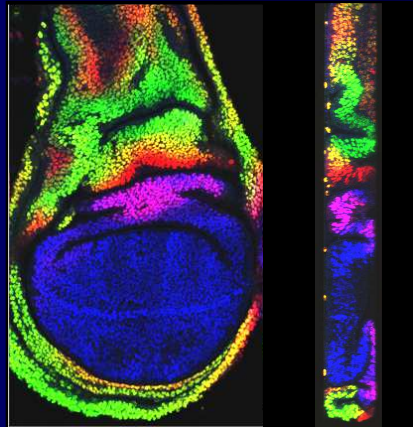
Modeling & Open Questions

Imaginal discs

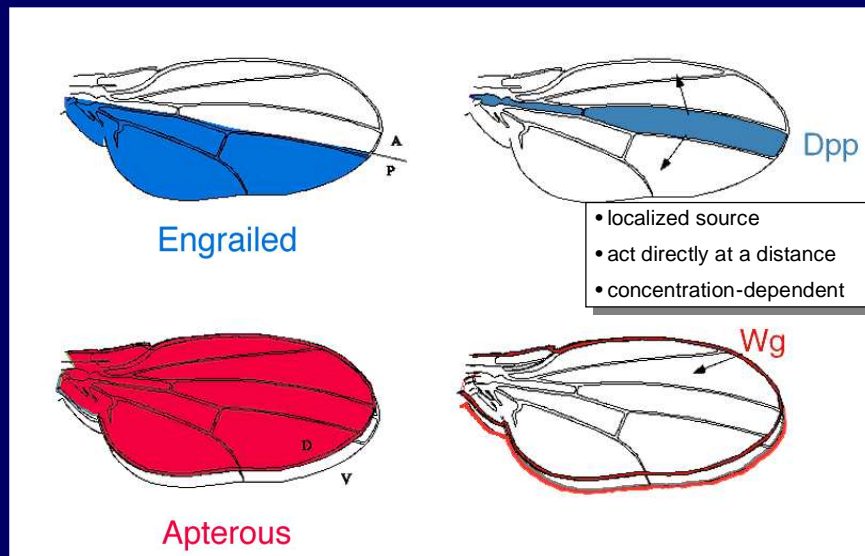


grow from 50 to 50,000 cells

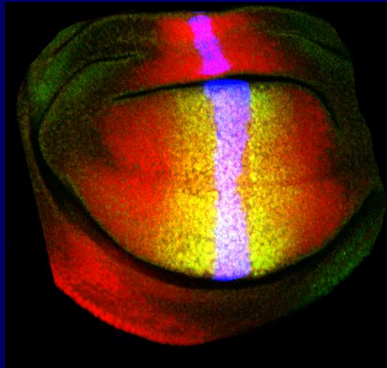
Wing imaginal disc



Signaling across compartment boundaries induces localized expression of morphogens

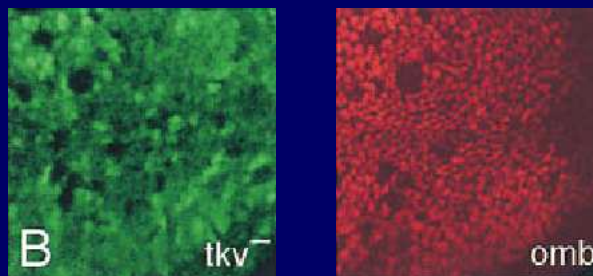


Dpp induces Spalt & Omb at different threshold levels



dpp-lacZ reporter
Spalt omb

Dpp signaling is directly required at a distance



Source: Nellen et. al., Cell 85, 357-368

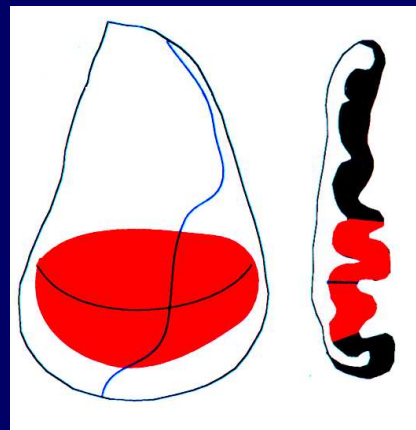
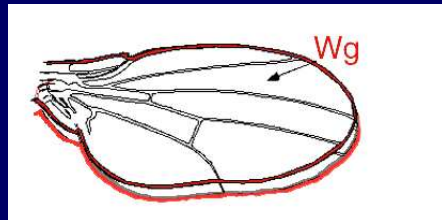
Agenda

Introduction to the system

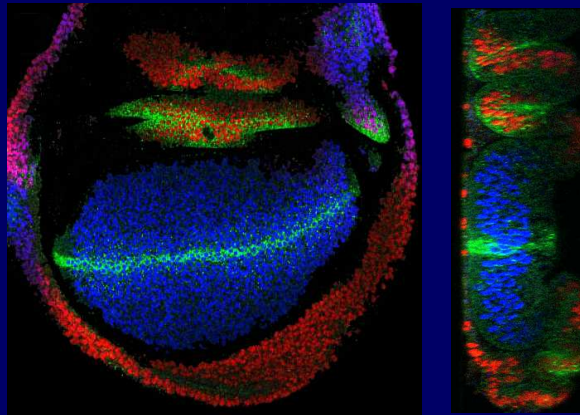
'Recent' developments

Modeling & Open Questions

Detection of wingless protein...

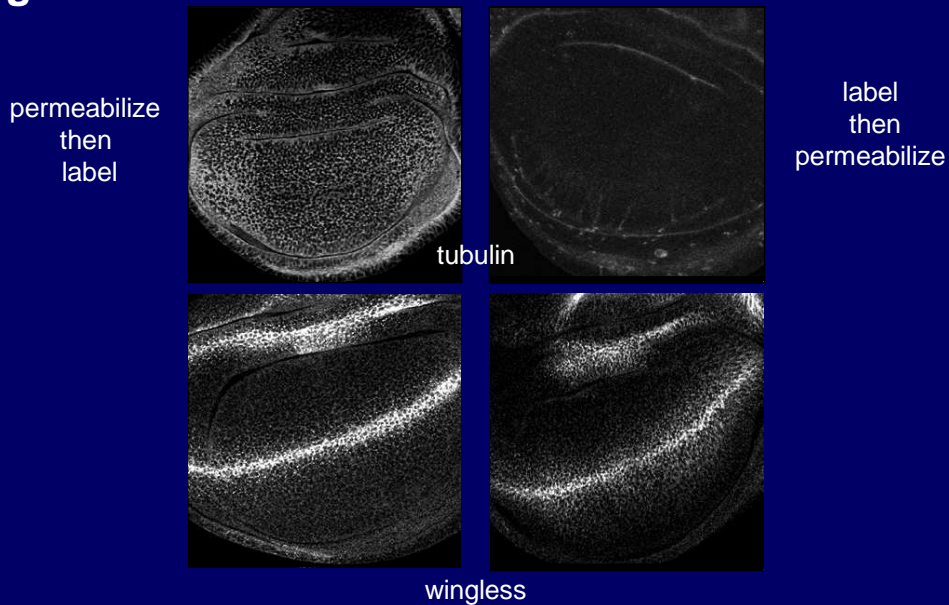


... by antibody staining

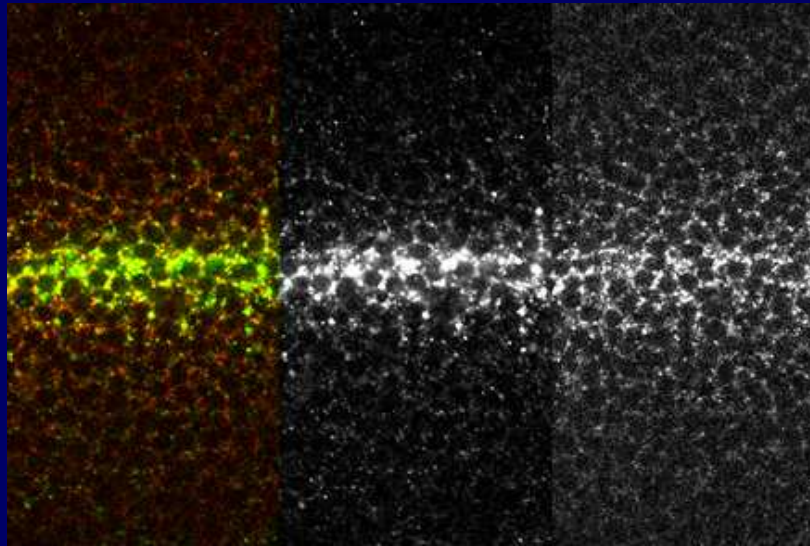


Wingless

Intracellular and extracellular wingless protein gradients



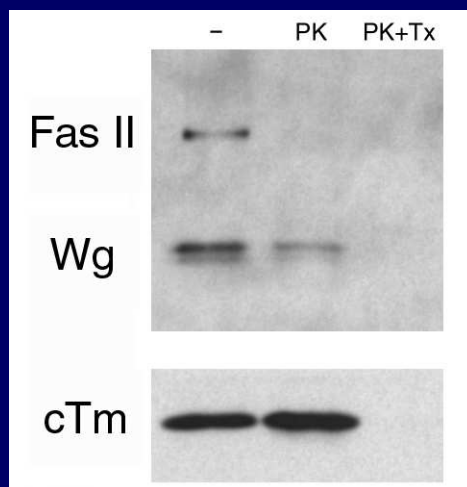
Intracellular and extracellular wingless protein gradients



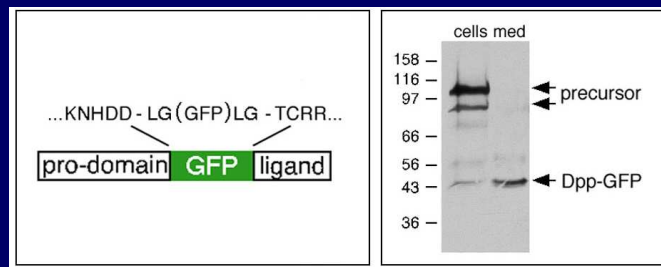
intracellular

extracellular

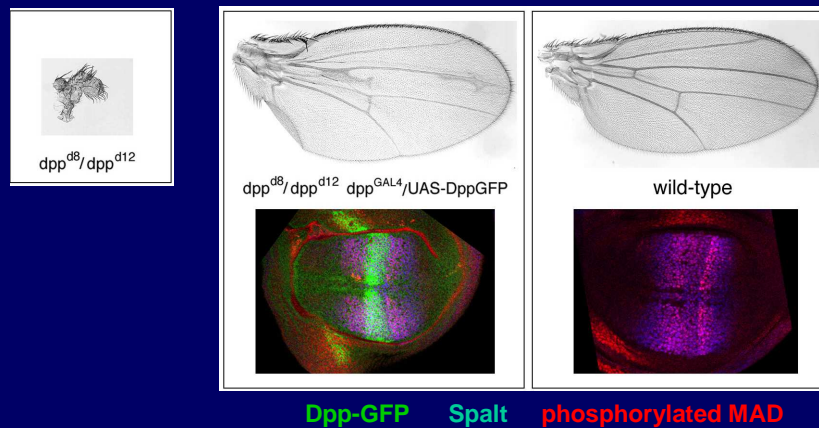
Intracellular and extracellular wingless



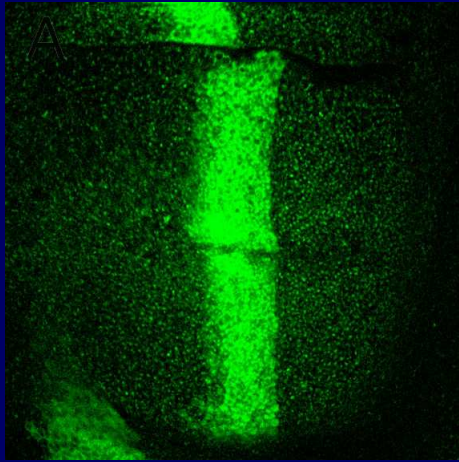
Visualization of Dpp gradient via GFP fusion protein



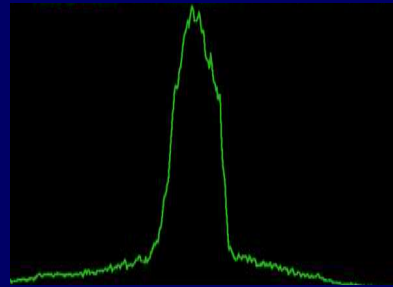
Dpp-GFP can replace endogenous Dpp and support wing development



Dpp-GFP forms a long-range gradient



live disc image



intensity profile

Morphogen Summary

Wingless & Dpp

- Made at localized source
- Form a concentration gradient
- Secreted, and large % found extracellularly
- Act directly at a distance
- Specify cells as function of conc.

Agenda

Introduction to the system

'Recent' developments

Modeling & Open Questions

- • Mechanism of spreading?
- Size accommodation
- Ligand vs. activity gradients

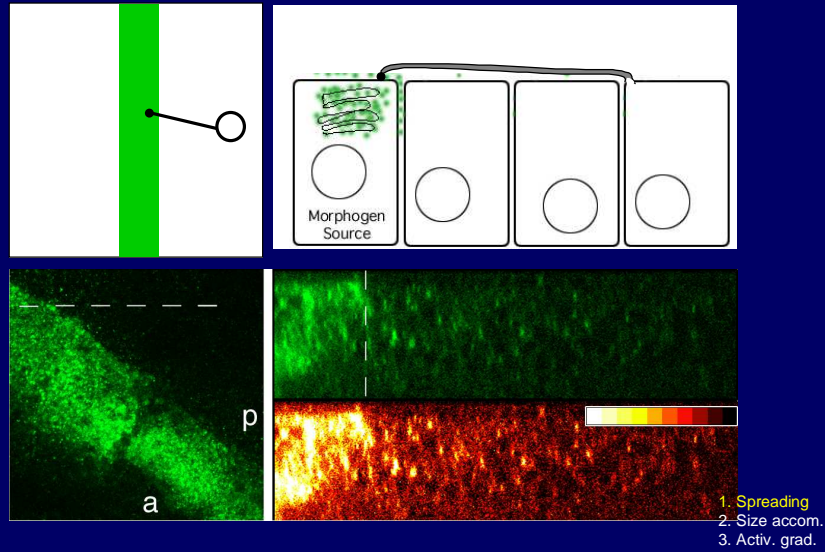
1. Spreading
2. Size accom.
3. Activ. grad.

Five mechanisms of gradient formation have been proposed for Dpp

1. Cell growth
2. Diffusion
- passive - - - 3. Bucket-brigade
4. Trans-cytosis
- 5. Cytoneme transport

1. Spreading
2. Size accom.
3. Activ. grad.

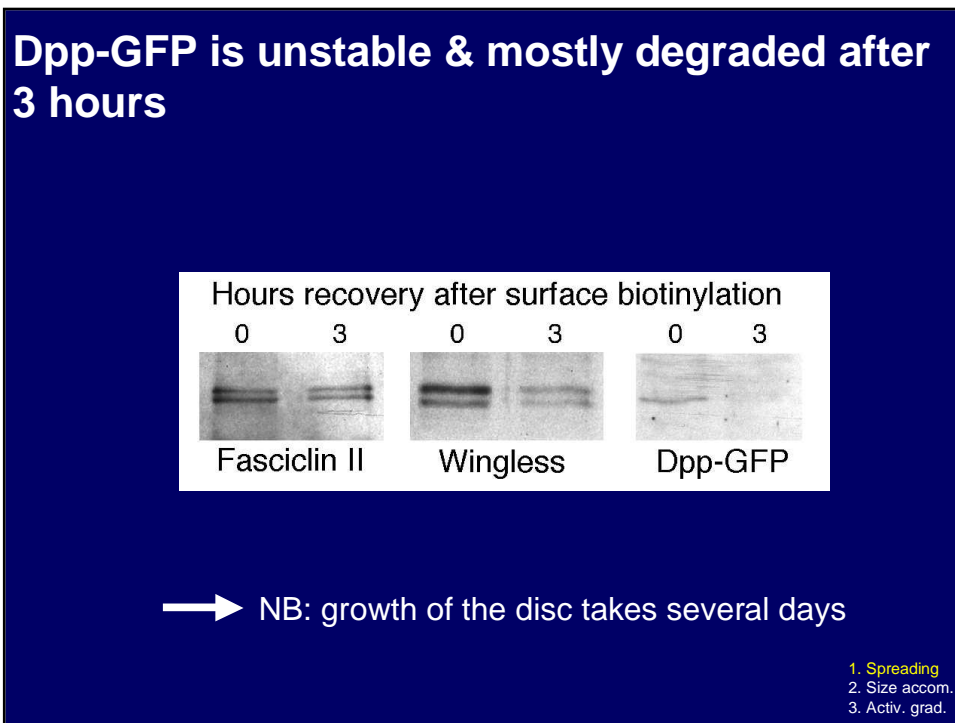
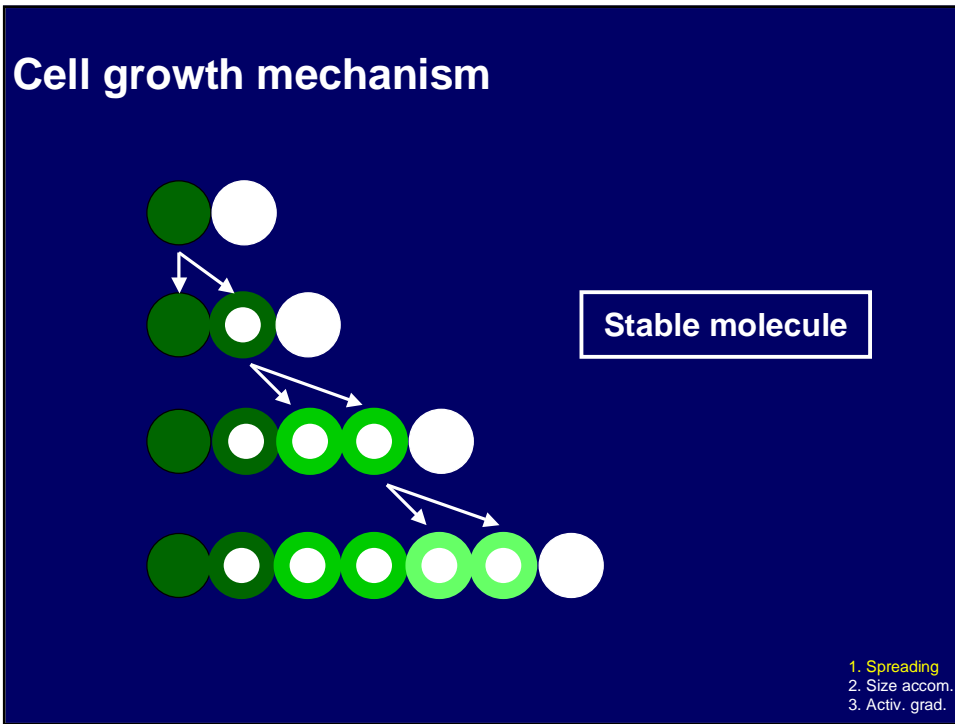
Cytoneme transport unlikely



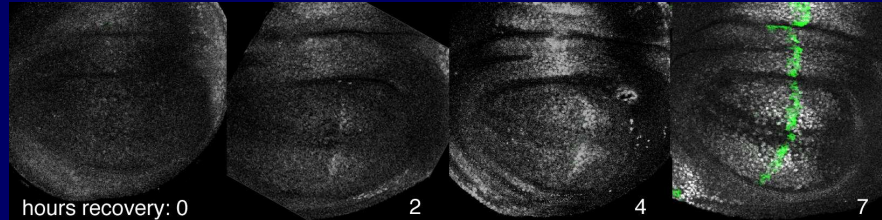
Five mechanisms of gradient formation have been proposed for Dpp

- 1. Cell growth
- 2. Diffusion
- 3. Bucket-brigade
- 4. Trans-cytosis
- X** 5. Cytoneme transport

1. Spreading
2. Size accom.
3. Activ. grad.



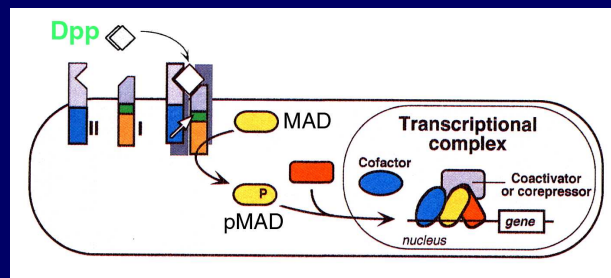
Dpp-GFP gradients forms rapidly



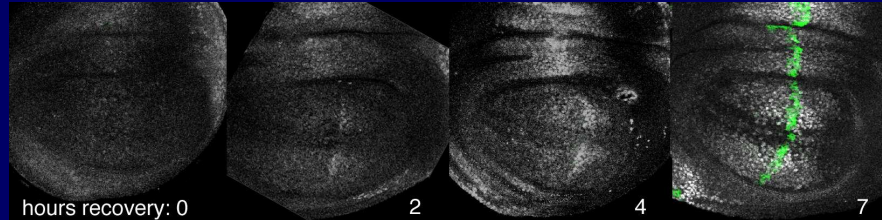
pMAD
dpp-lacZ reporter

1. Spreading
2. Size accom.
3. Activ. grad.

Detecting Dpp signaling via pMAD levels



Dpp-GFP gradients forms rapidly



pMAD
dpp-lacZ reporter

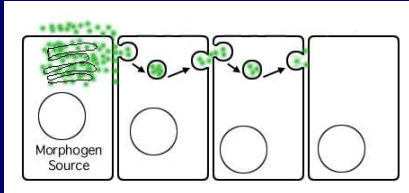
1. Spreading
2. Size accom.
3. Activ. grad.

Five mechanisms of gradient formation have been proposed for Dpp

- X** 1. Cell growth
2. Diffusion
3. Bucket-brigade
- 4. Trans-cytosis
- X** 5. Cytoneme transport

1. Spreading
2. Size accom.
3. Activ. grad.

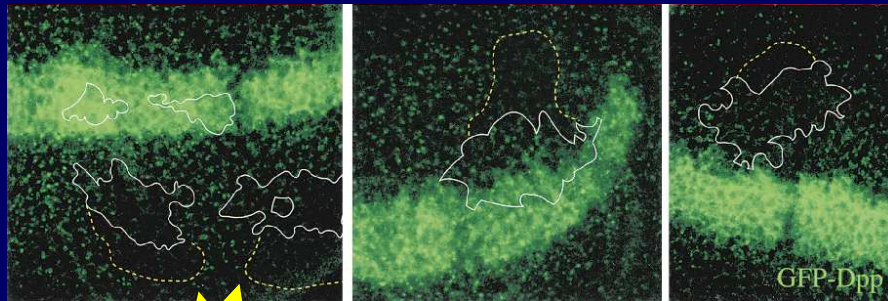
Conceptual diagram - transcytosis



→ Increase in effective diffusivity

- 1. Spreading
- 2. Size accom.
- 3. Activ. grad.

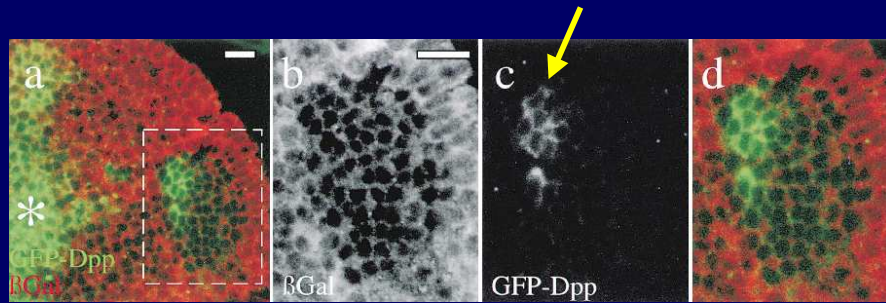
Effect of blocking endocytosis on Dpp gradient



Source: Entchev, Schwabedissen & Gonzalez-Gaitan, Cell 103, 981-991

- 1. Spreading
- 2. Size accom.
- 3. Activ. grad.

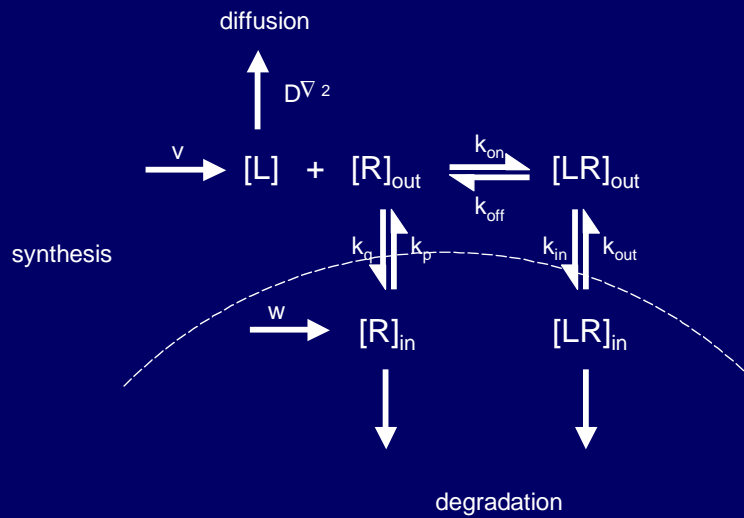
Dpp-GFP in clones lacking receptor (Tkv)



Source: Entchev, Schwabedissen & Gonzalez-Gaitan, Cell 103, 981-991

1. Spreading
2. Size accom.
3. Activ. grad.

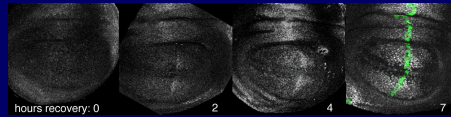
Modeling



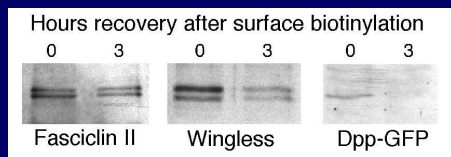
Source: Lander, Nie & Wan, Dev. Cell 2, 785-796

1. Spreading
2. Size accom.
3. Activ. grad.

Determining parameters



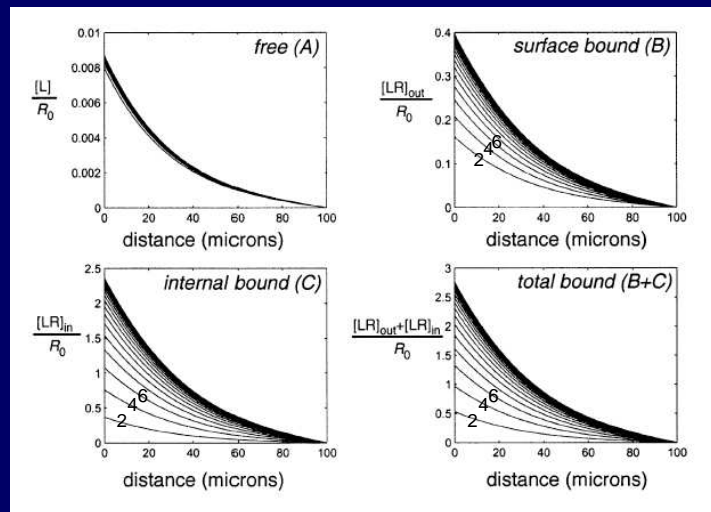
→ Rate of gradient formation



→ Rate of Dpp degradation

1. Spreading
2. Size accom.
3. Activ. grad.

Dpp-GFP model of diffusion also predicts gradient formation



Source: Lander, Nie & Wan, Dev. Cell 2, 785-796

1. Spreading
2. Size accom.
3. Activ. grad.

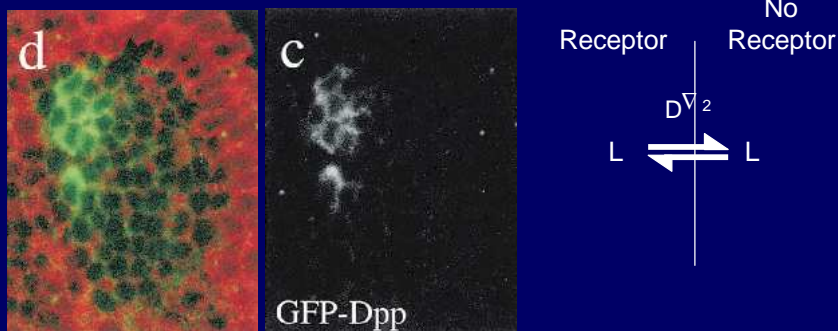
Kinetics suggest diffusion is most likely mechanism for Dpp spreading

Mechanism	Data	Diffusivity
Diffusion	Dpp biochemical & biophys. properties	$10^{-7} \text{ cm}^2 \text{ s}^{-1}$
Bucket-brigade	transmembrane planar diffusion	$10^{-8} - 10^{-10} \text{ cm}^2 \text{ s}^{-1}$
Trans-cytosis	endocytosis rates 2-20 min	$10^{-9} - 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ Too Slow?

Source: Lander, Nie & Wan, Dev. Cell 2, 785-796

1. Spreading
2. Size accom.
3. Activ. grad.

Local accumulation hard to explain



→ Neither diffusion nor trans-cytosis models can explain local accumulation

1. Spreading
2. Size accom.
3. Activ. grad.

Binding to proteoglycans could explain phenomenon



Source: Baeg et. al., Development 128, 87-94

1. Spreading
2. Size accom.
3. Activ. grad.

Five mechanisms of gradient formation have been proposed for Dpp

- X 1. Cell growth
- ? 2. Diffusion
- ? 3. Bucket-brigade
- ? 4. Trans-cytosis
- X 5. Cytoneme transport

1. Spreading
2. Size accom.
3. Activ. grad.

Agenda

Introduction to the system

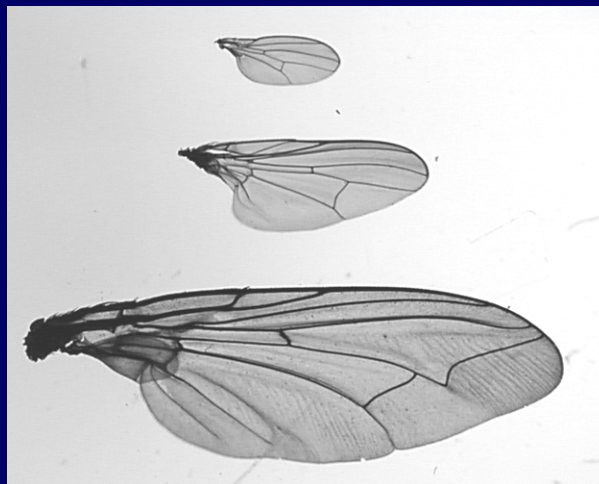
'Recent' developments

Modeling & Open Questions

- Mechanism of spreading?
- • Size accomodation
- Ligand vs. activity gradients

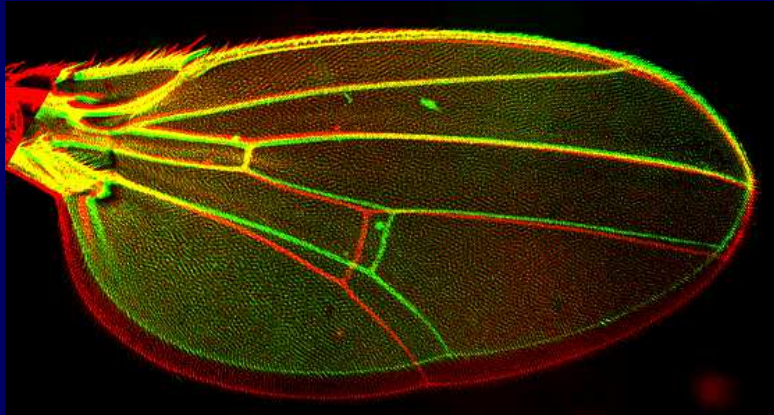
1. Spreading
2. Size accom.
3. Activ. grad.

Size and pattern



1. Spreading
2. Size accom.
3. Activ. grad.

Overgrowth of the posterior compartment



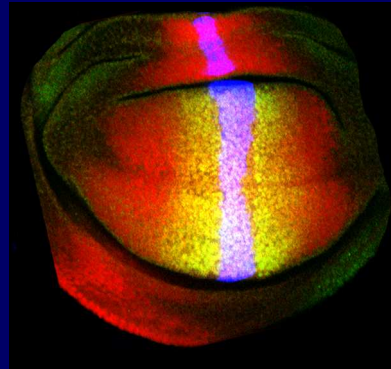
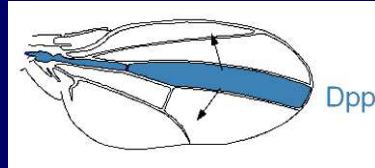
1. Spreading
2. Size accom.
3. Activ. grad.

Size accommodation



1. Spreading
2. Size accom.
3. Activ. grad.

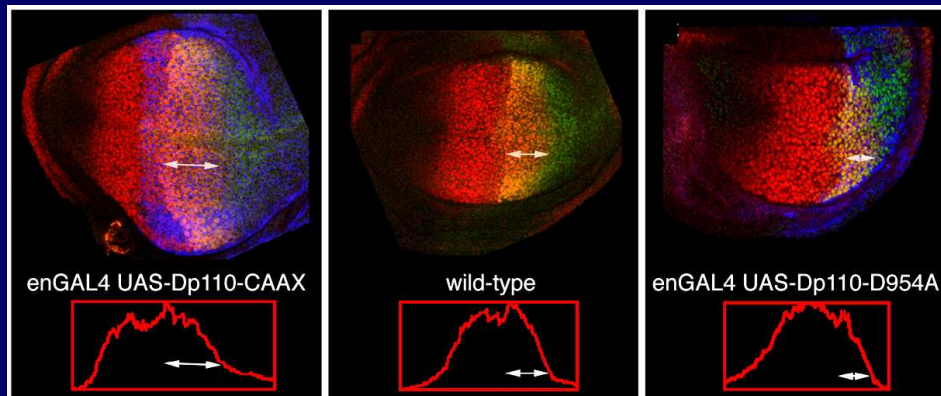
A/P axis patterned by Dpp



dpp-lacZ reporter
Spalt omb

1. Spreading
2. Size accom.
3. Activ. grad.

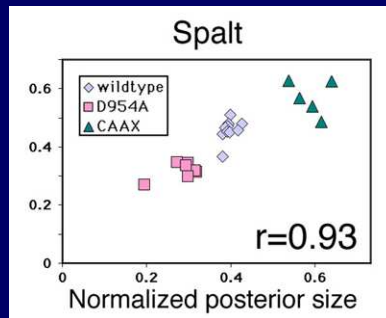
Dpp target gene domains accommodate to compartment size



En Spalt myc-PI3K

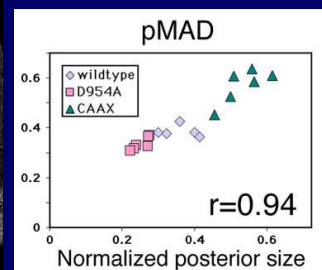
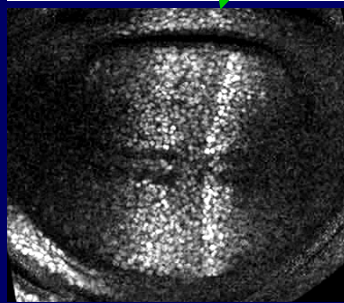
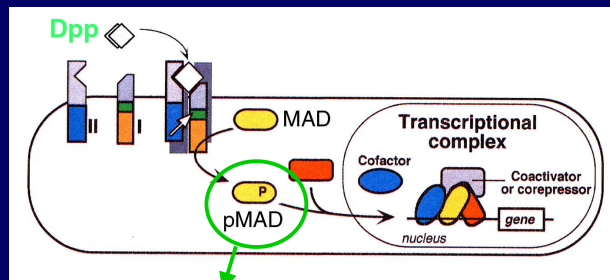
1. Spreading
2. Size accom.
3. Activ. grad.

Spalt domain size accommodation - quantification



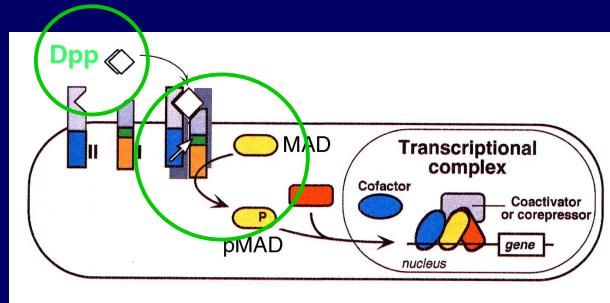
1. Spreading
2. Size accom.
3. Activ. grad.

Size accommodation at level of Mad phosphorylation



1. Spreading
2. Size accom.
3. Activ. grad.

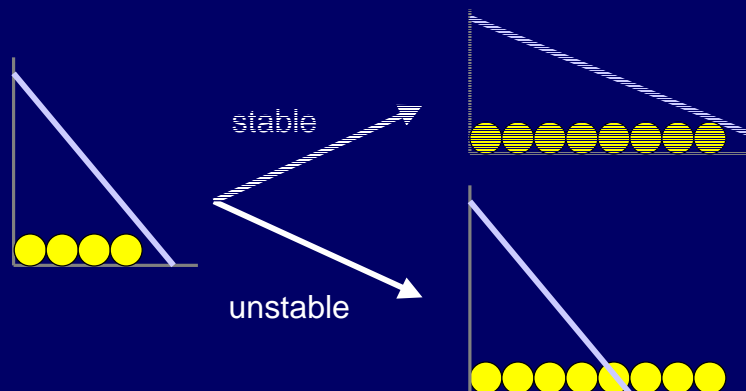
Size accommodation can occur at two levels



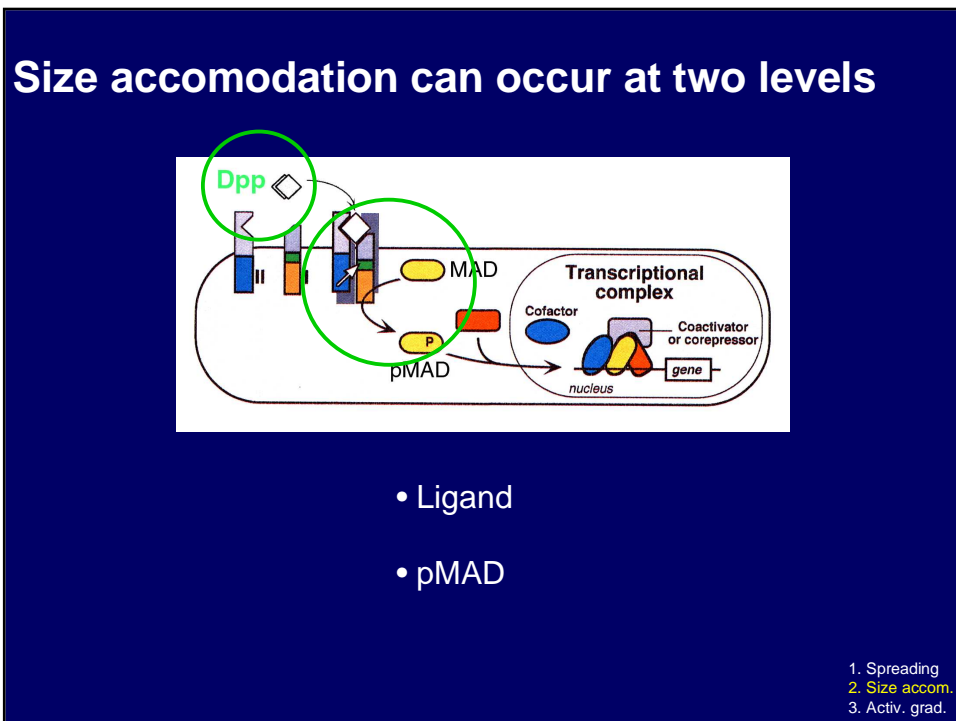
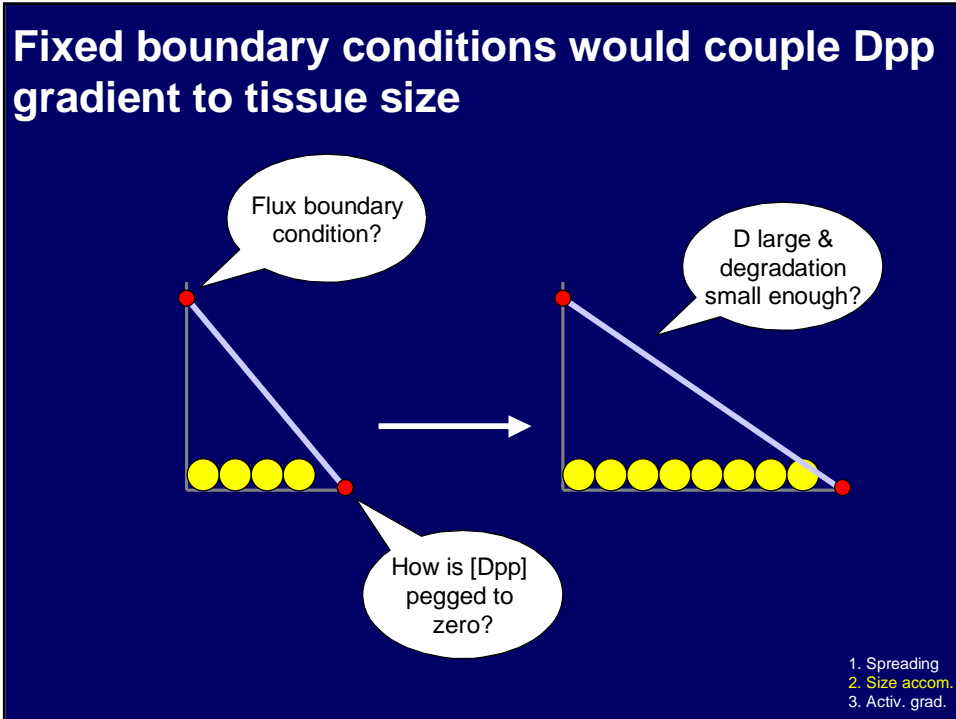
- Ligand
- pMAD

1. Spreading
2. Size accom.
3. Activ. grad.

Dpp gradient & size would be inherently coupled if Dpp spread by tissue growth



1. Spreading
2. Size accom.
3. Activ. grad.



Agenda

Introduction to the system

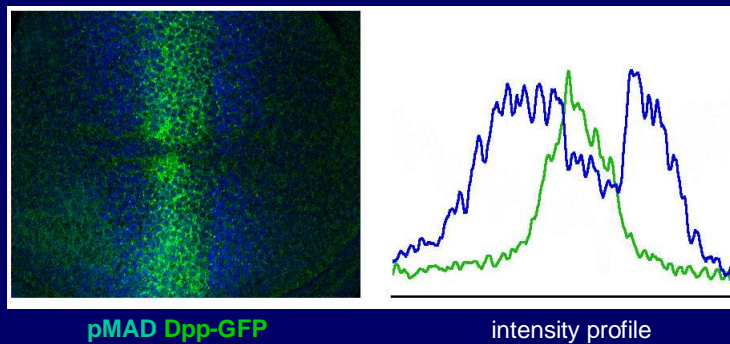
'Recent' developments

Modeling & Open Questions

- Mechanism of spreading?
- Size accomodation
- • Ligand vs. activity gradients

1. Spreading
2. Size accom.
3. Activ. grad.

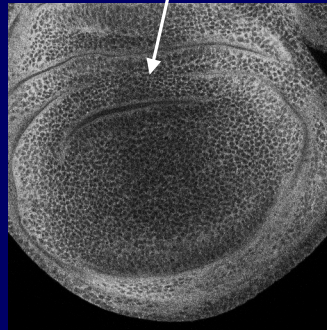
Direct comparison of ligand and activity gradients



1. Spreading
2. Size accom.
3. Activ. grad.

Tkv receptor levels are low in the Dpp producing region

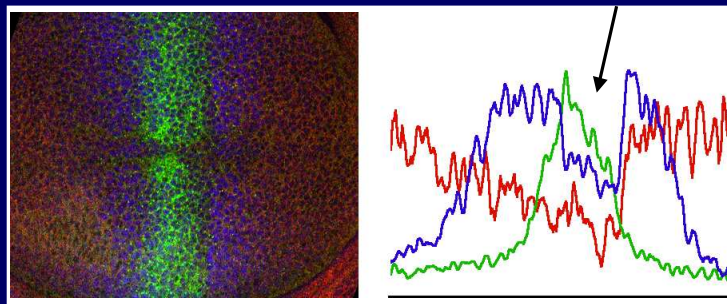
Hh signaling reduces Tkv levels



1. Spreading
2. Size accom.
3. Activ. grad.

Discrepancies between pMad and Dpp profile partly explained by Tkv levels

Hh signaling reduces Tkv levels

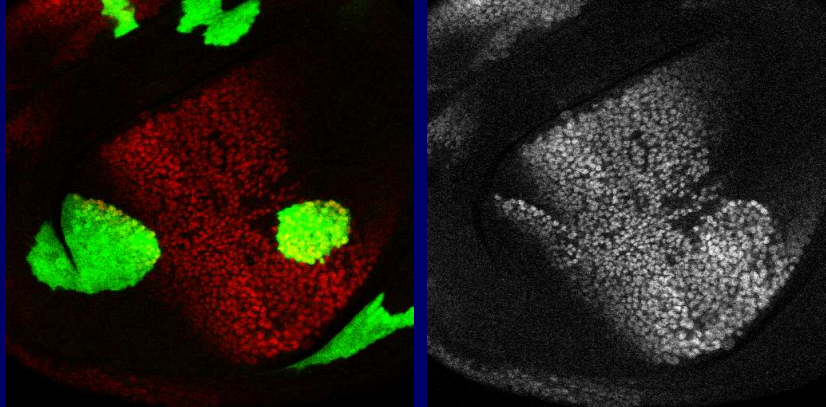


pMAD Dpp-GFP Tkv

intensity profile

1. Spreading
2. Size accom.
3. Activ. grad.

High Tkv levels both sensitize cells to Dpp and reduce Dpp diffusivity

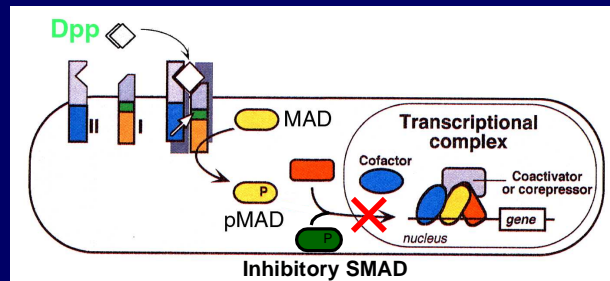


Dpp receptor expressing clones

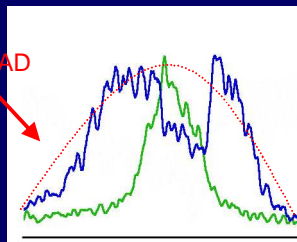
spalt

1. Spreading
2. Size accom.
3. Activ. grad.

Inhibitory SMAD also plays a role



inhibitory SMAD



1. Spreading
2. Size accom.
3. Activ. grad.

Multiple factors may affect translation of Dpp levels into Dpp signaling

- Receptor (Tkv) levels
- Inhibitory SMAD levels
- Receptor cooperativity ??
- Internalization & degradation rates ??

1. Spreading
2. Size accom.
3. Activ. grad.

Conclusions & Open Questions

Dpp gradient formation

- High flux
- Tissue growth too slow & cytonemes unlikely
- Mechanism still unclear

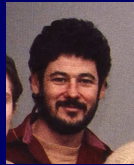
Size accommodation

- Occurs at 'high level' (ligand, receptor, etc.)
- May involve translation of Dpp levels into pMAD levels
- Mechanism still unclear

Acknowledgements



Maura Strigini



Stephen Cohen

Backups

Brinker is not responsible for size accommodation

