# Finding a morphogen gradient using forward genetics:

## Dorsal-ventral patterning by BMPs in Drosophila embryos

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11/15/2006

# Fruit flies…

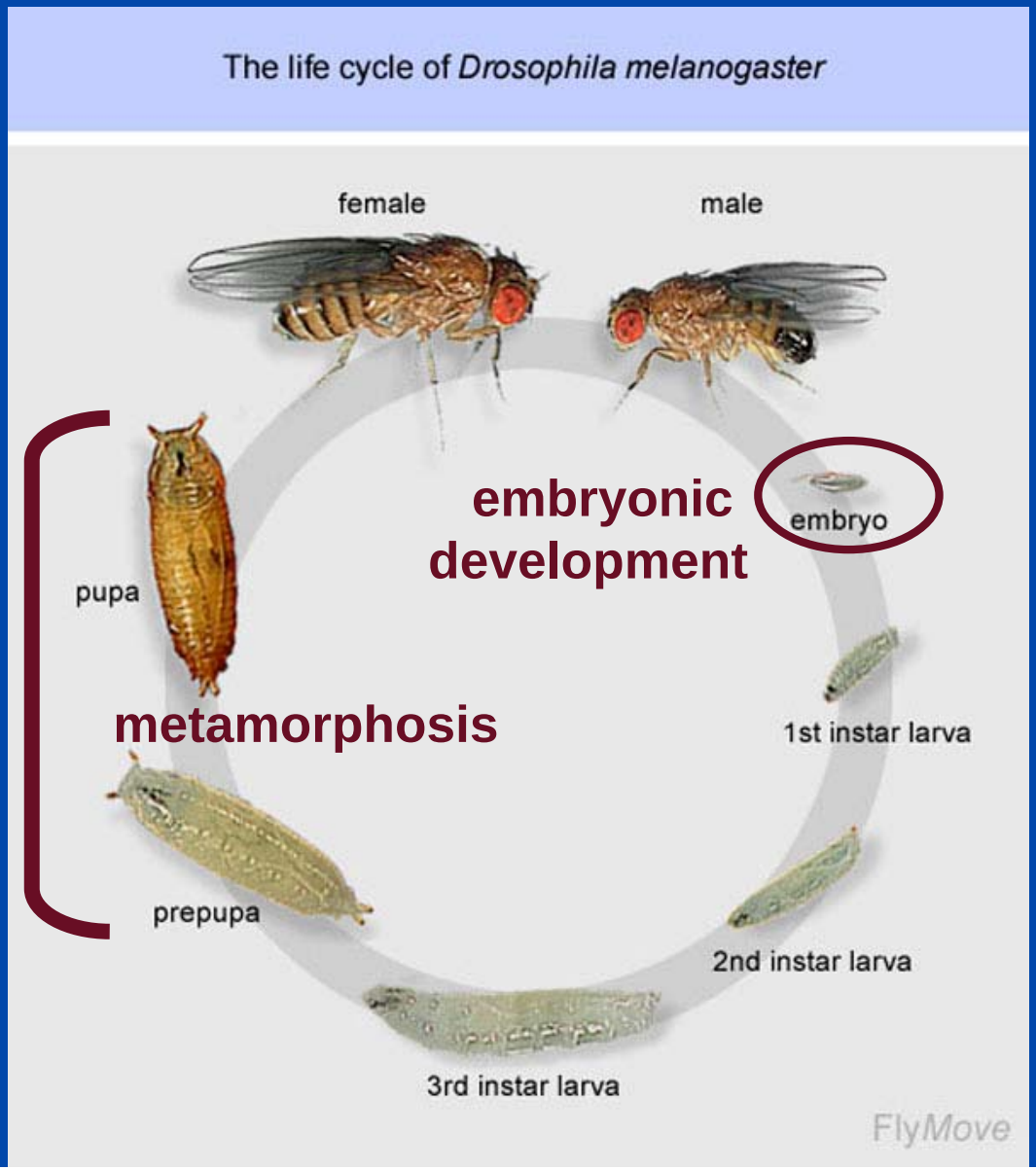
***Drosophila melanogaster***

www.istoica.com/.../ 20060618174337_genetics.jpg

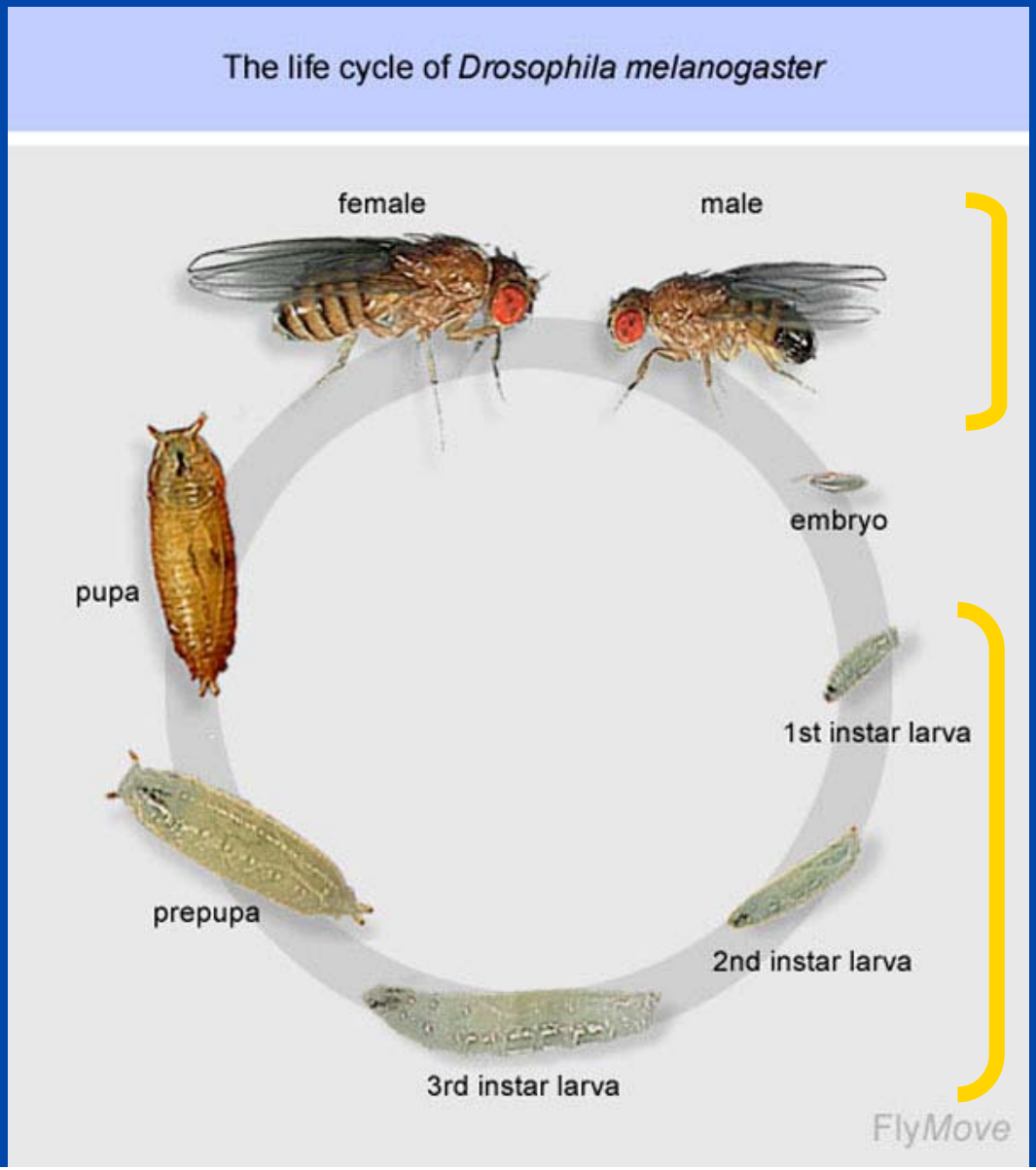
**Artist's conception**

www.hubbo.com/images/ Fly-and-banana.jpg

# Drosophila life cycle: two distinct developmental phases



# Drosophila development:
# two different bodies for two different purposes




Dispersal and reproduction

feeding

- Why Drosophila genetics?
- What is a morphogen gradient?
- Inference of a morphogen gradient from genetics
- Identification of signaling pathway from genetics
- Visualization of a gradient from signal transducers
- Shaping the gradient
- Computational predictions of molecular mechanisms

- Why Drosophila genetics?

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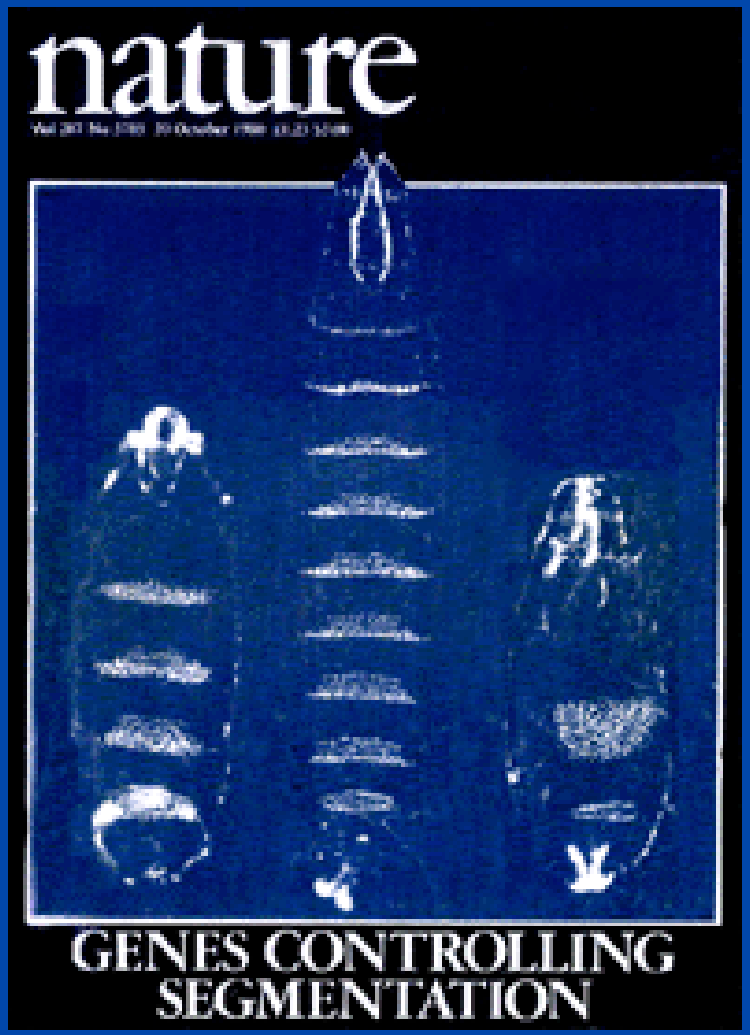
# Why I chose to study Drosophila development:




1980

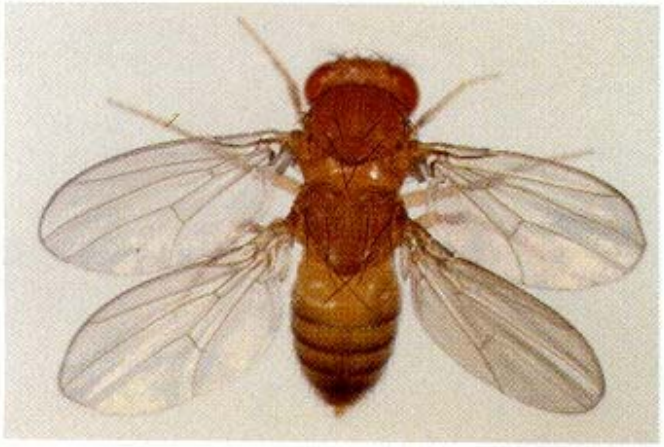
**the most powerful system to dissect the logic of development**

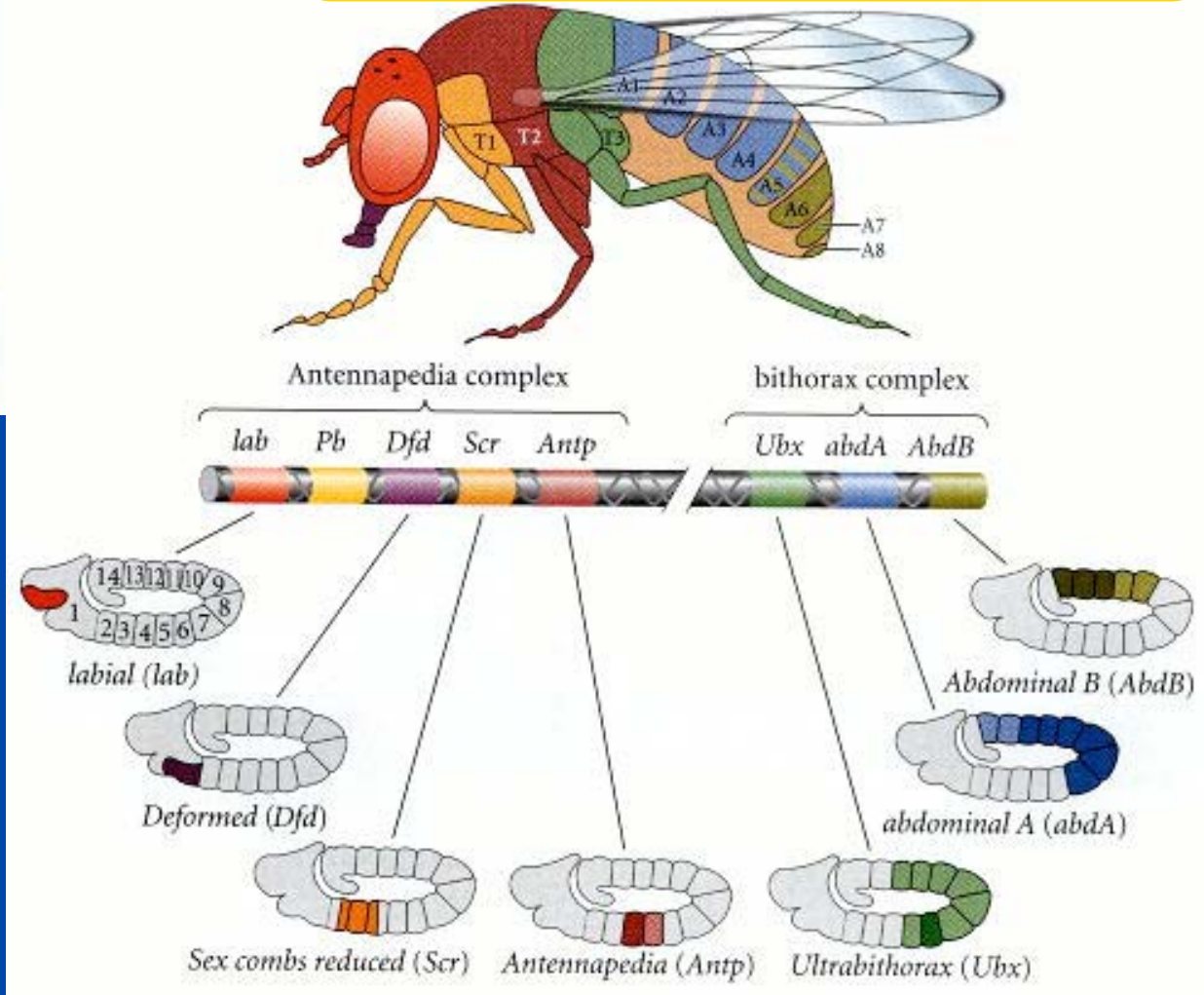
**Nusslein-Volhard and Wieschaus**

Nobel prize 1995

# Ed Lewis:
# Homeotic mutations

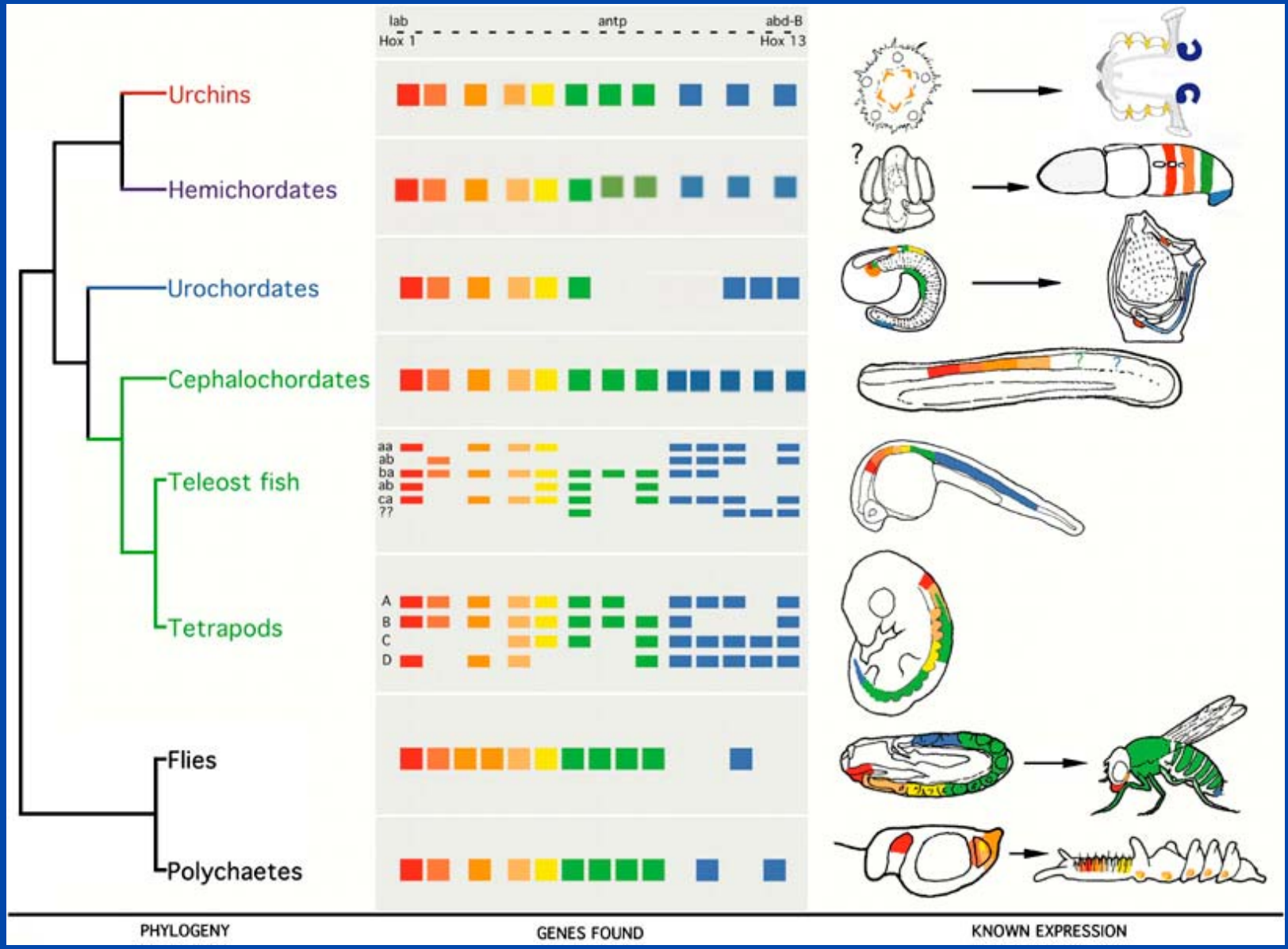
"...and when that happened
a revolution occurred in the field of biology"
-Scott Gilbert






From: Scott F. Gilbert, 2000. Developmental Biology 6th Edition

# Homeobox genes establish body plans for all multicellular organisms




From: Swalla, 2006, Heredity 97: 235-243

# Why people choose Drosophila today:

- Multicellular organisms use the same tools
  - e.g. the gang of five for organogenesis:
    - TGFβ ligands: BMPs*
    - Receptor tyrosine kinase ligands: EGFs and FGFs
    - Hedgehogs*
    - Wnts*
    - Notch ligands*

- It is easier to understand a simple system
  - e.g. 7 TGFβ ligands in flies
    57 in mice
    34 in zebrafish

## Practical reasons to study flies:

- They have a 10 day generation time
- They are inexpensive to rear
- Genetic tools are sophisticated and easy to use

- Because flies are fast and inexpensive you can
  - Develop a developmental hypothesis
  - test it
  - Define a molecular mechanism
  - Test it

    all in one graduate student's thesis project

## Practical reasons to study flies:

- They have a 10 day generation time
- They are inexpensive to rear
- Genetic tools are sophisticated and easy to use

- Regulators don't care about flies
- People share reagents, even before publication

- Because flies are fast and inexpensive you can
  - Develop a developmental hypothesis
  - test it
  - Define a molecular mechanism
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    all in one graduate student's thesis project

- Why Drosophila genetics?
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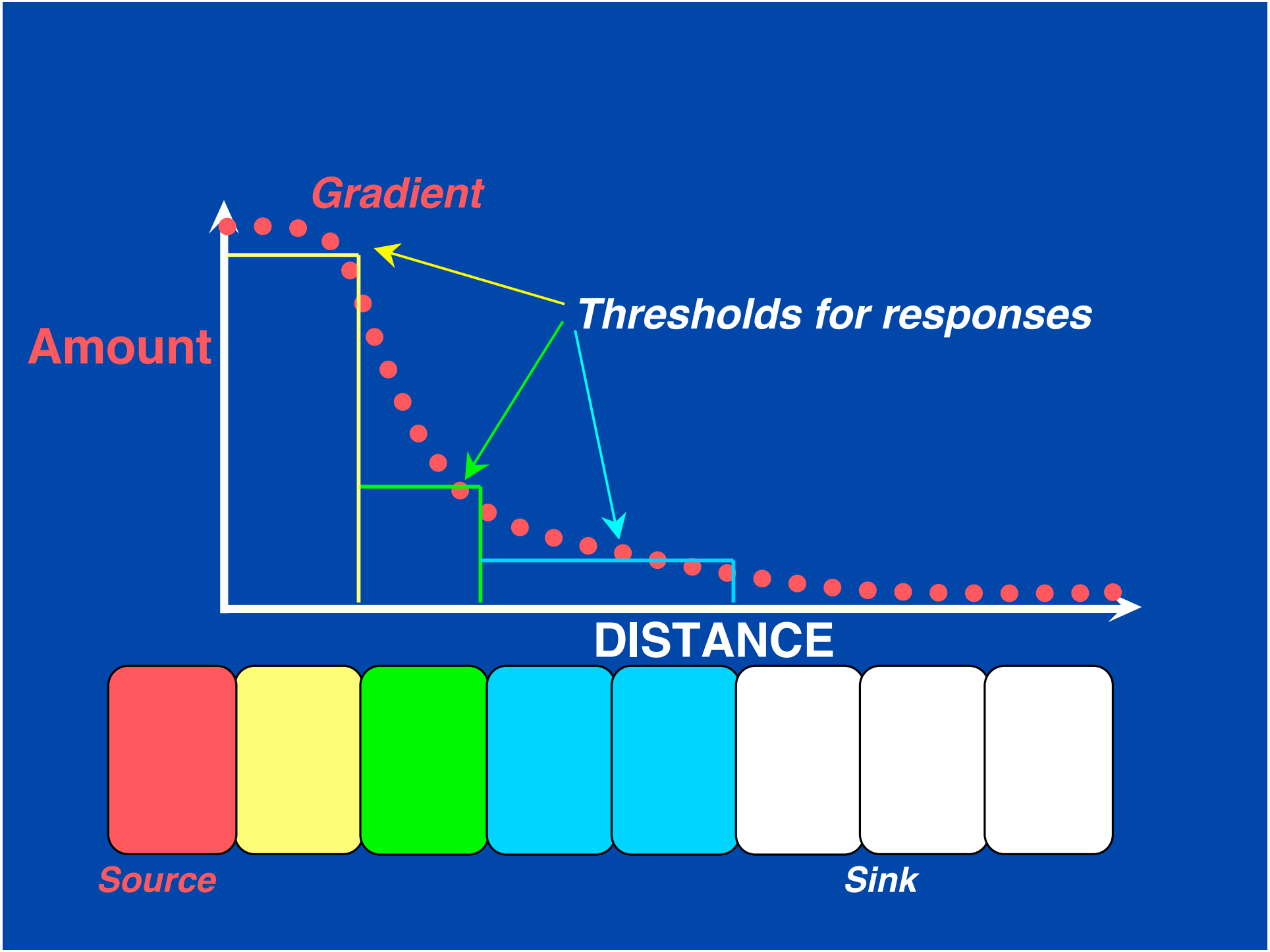
•Different cell fates can be determined by different concentrations of a morphogen, a diffusible molecule present at variable concentrations
(A. Turing, 1952)

2002 Curr. Biol.

**A cell decides its fate based on its position within the tissue. The pattern of cell fates is established through a coordinate system of positional information.**
**(L. Wolpert, 1969)**


Gradient
Amount
Thresholds for responses
DISTANCE
Source
Sink

- Why Drosophila genetics?
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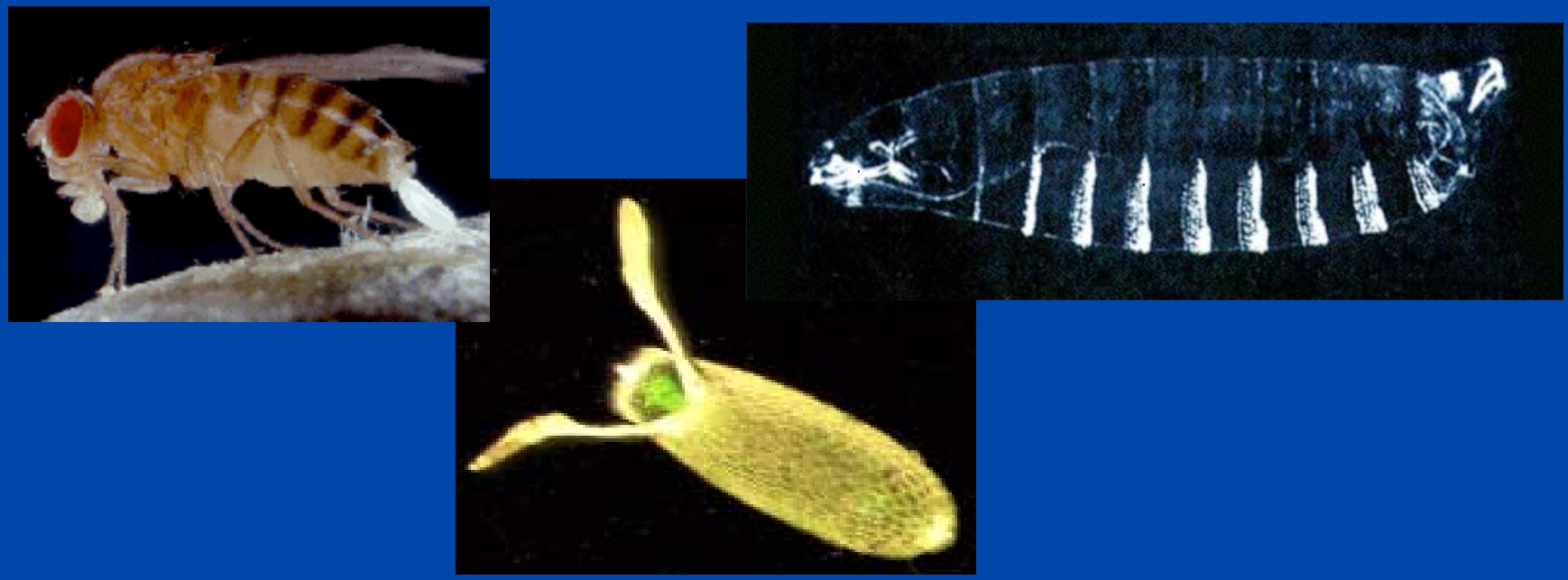
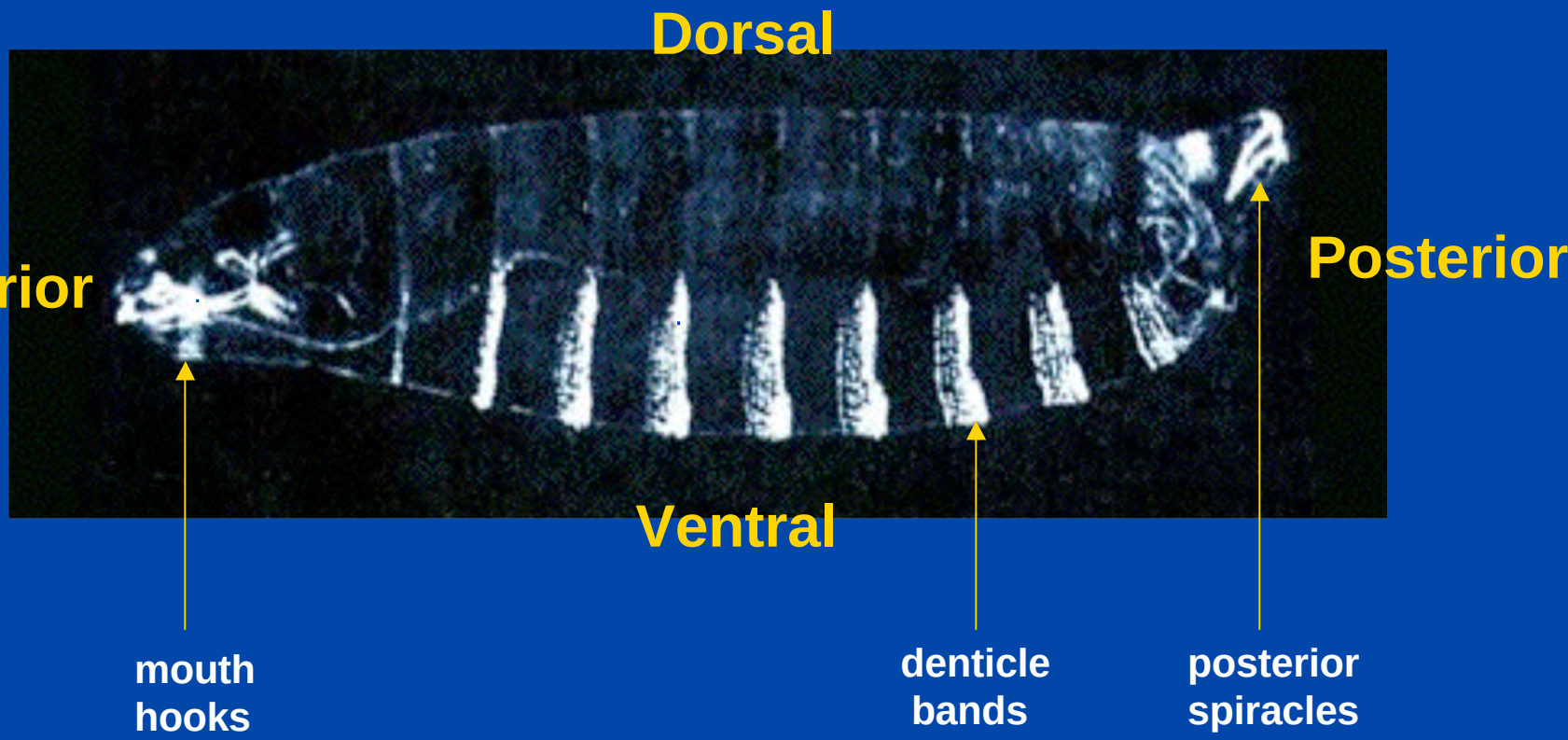
# The *Drosophila* body plan is prepatterned in the egg

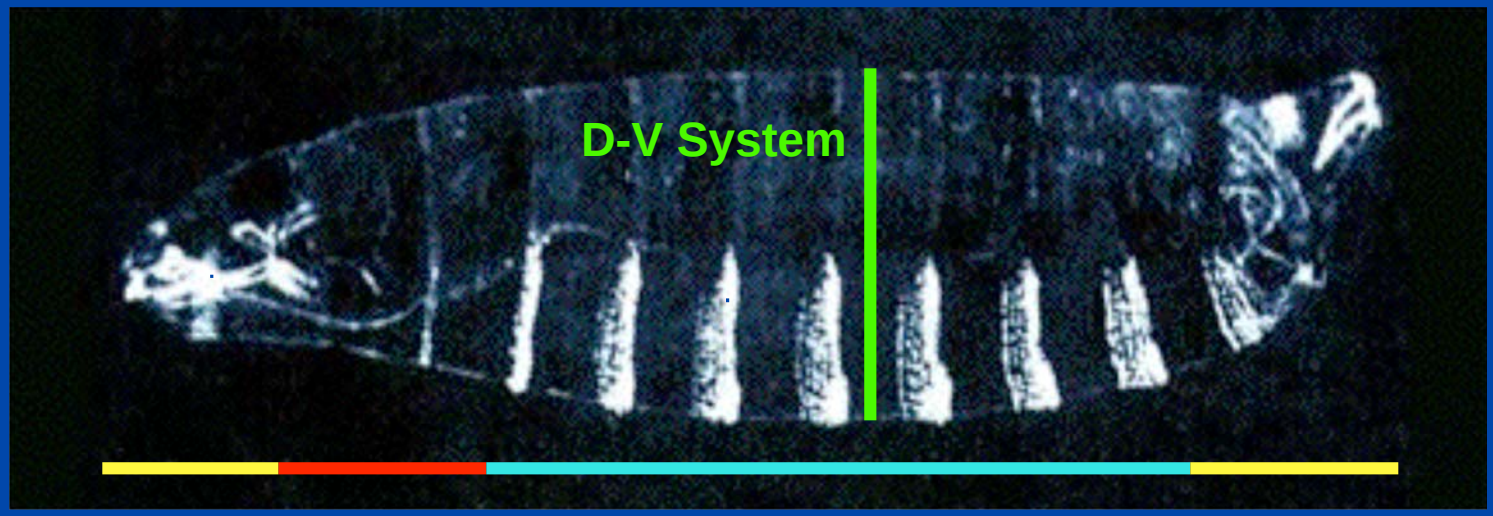


**By RNA and protein from the mother**

# The *Drosophila* body plan is visible in the exoskeleton (cuticle)



# A set of maternal genes establishes each aspect of the body plan



# Maternal genes were identified by embryo phenotypes

**Anterior**
**posterior**
**termini and**
**dorsal-ventral**

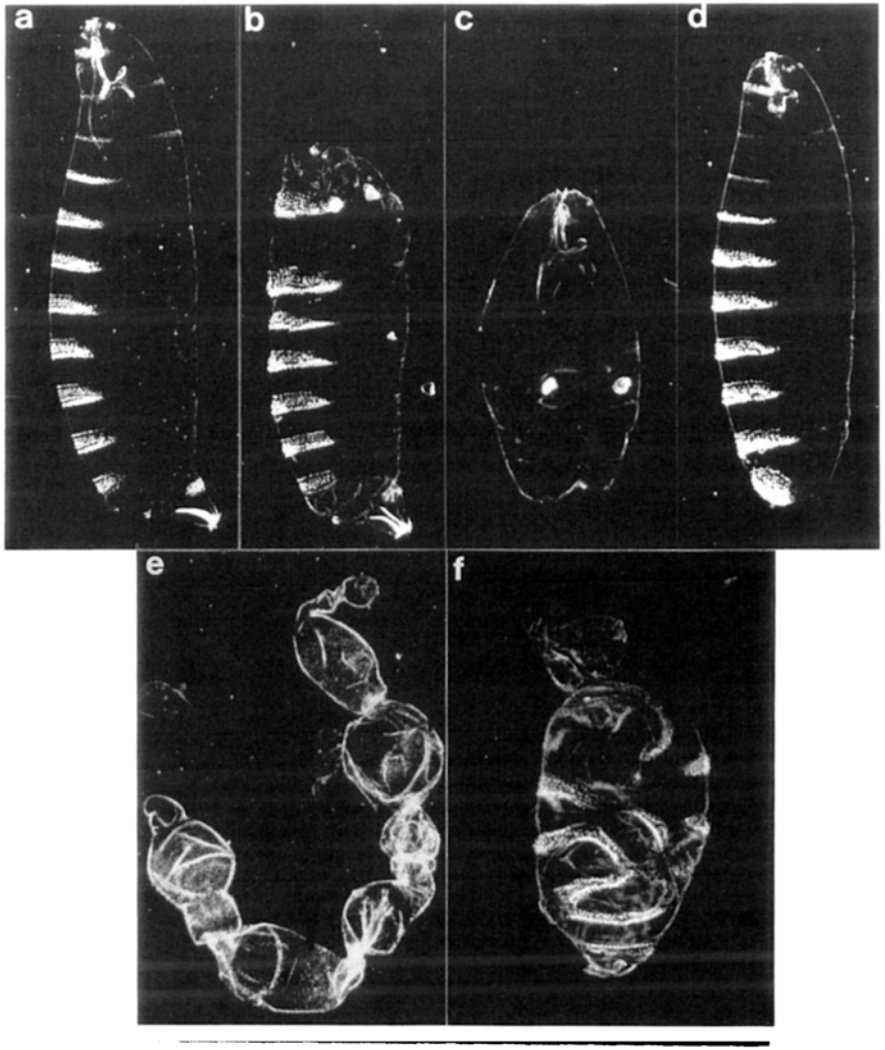


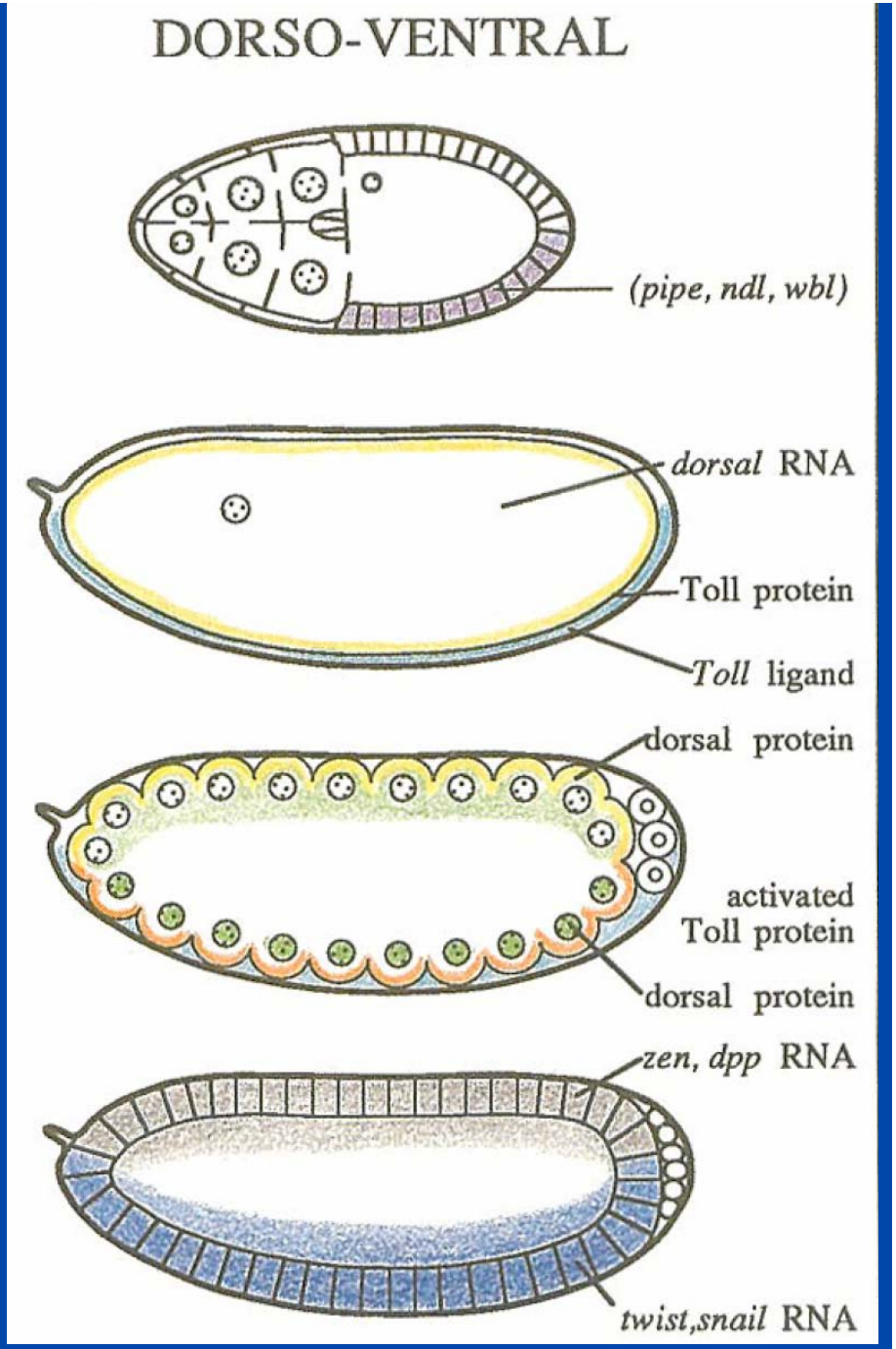

Figure 3. The Cuticular Patterns of Wild-Type and Mutant Embryos
(a) wild-type, (b) anterior (*bicoid*), (c) posterior (*oskar*), (d) terminal (*torso-like*), (e) dorsalized (*dorsal*), (f) ventralized (*cactus*).

reviewed by St. Johnston & Nüsslein-Volhard, 1992, Cell 68. 201

# Dorsal-ventral axis is patterned by sequential morphogen gradients

**Maternal genes:**
**NF-κB gradient subdivides mesoderm/ectoderm**

**Zygotic genes:**
**BMP gradient subdivides ectodermal territories**




St. Johnston & Nüsslein-Volhard, 1992, Cell 68. 201

# Why a *gradient* of Dpp in embryos?

- Uniform RNA levels, protein undetectable

➡ An inference from the genetic analysis of ectoderm patterning

- Dpp was known to be a BMP, and hypothesized as a morphogen for appendage development.

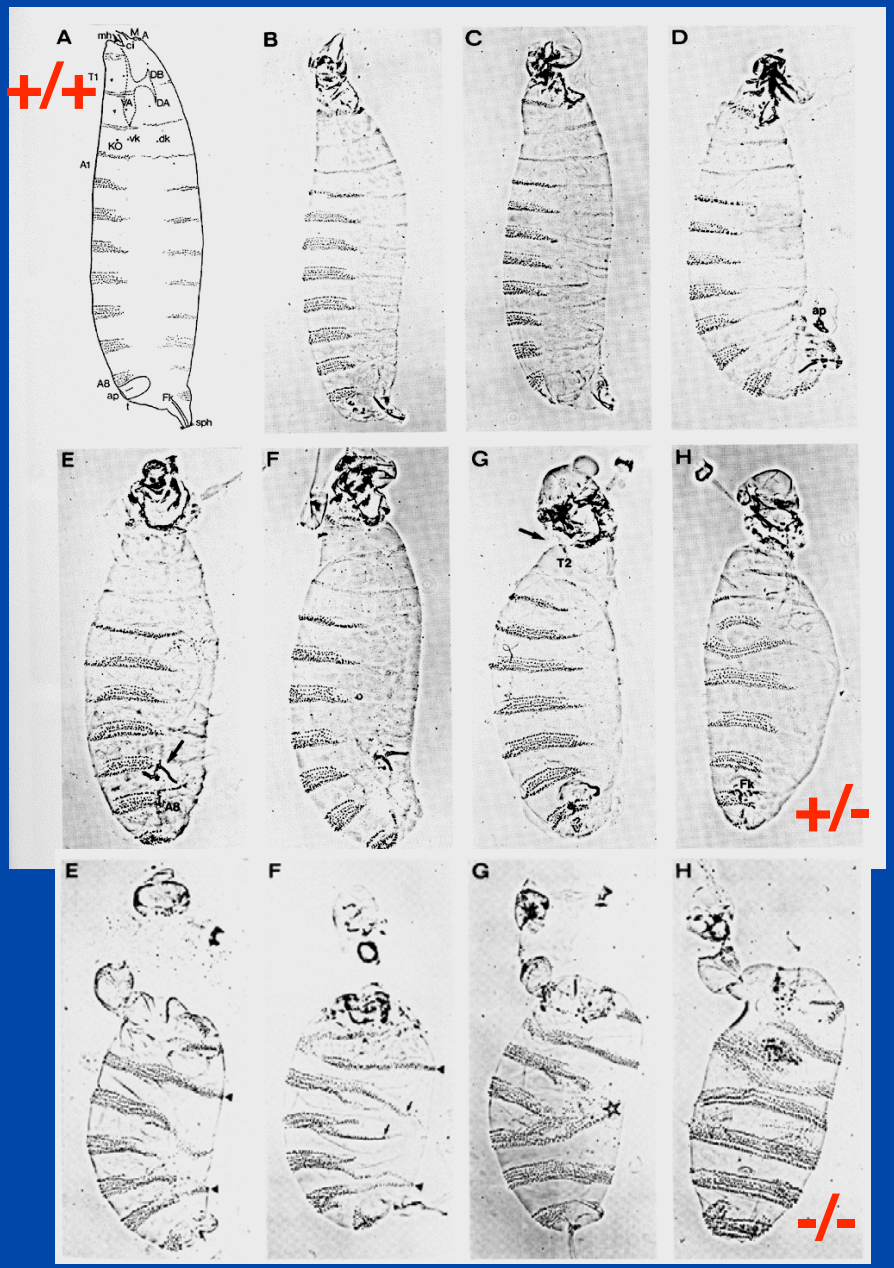
# Why a *gradient* of Dpp in embryos?

- **Uniform RNA levels, protein undetectable**

➡ **An inference from the genetic analysis of ectoderm patterning**

- **Different Dpp mutations cause loss of more or fewer dorsal ectoderm structures**
  - Wharton et al, 1993, Development

- **Dpp was known to be a BMP, and hypothesized as a morphogen for appendage development.**



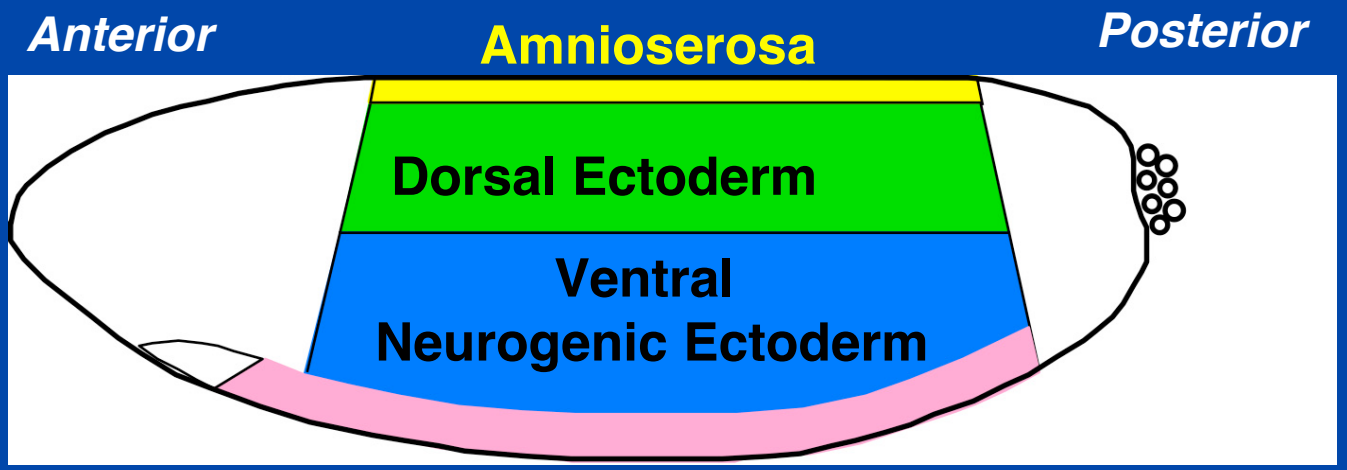

# Different mutations in *dpp* eliminate different amounts of dorsal fates

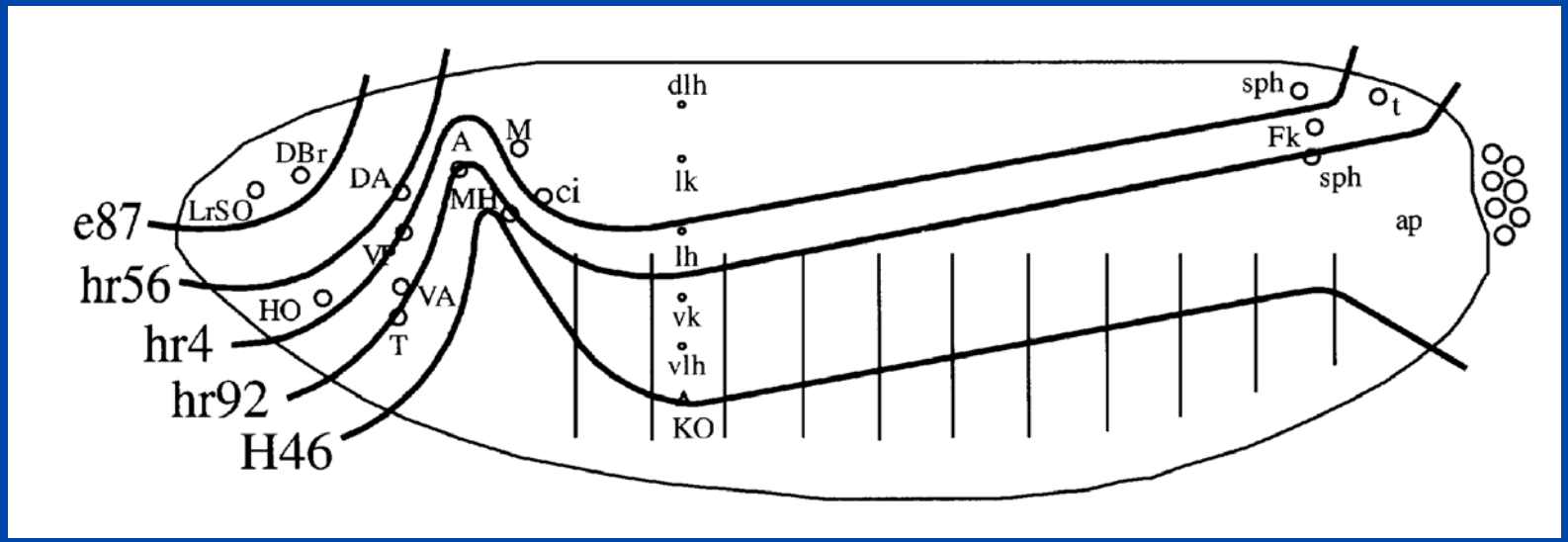
## Dpp is *necessary* to make dorsal ectoderm and amnioserosa

Irish & Gelbart, 1987, Genes Dev. 1, 868
Wharton et al. 1993, Development 117, 807

# The less DPP, the fewer dorsal structures

Lateral View




Wharton et al. 1993, Development 117, 807

# Why a *gradient* of Dpp in embryos?

- **Uniform RNA levels, protein undetectable**

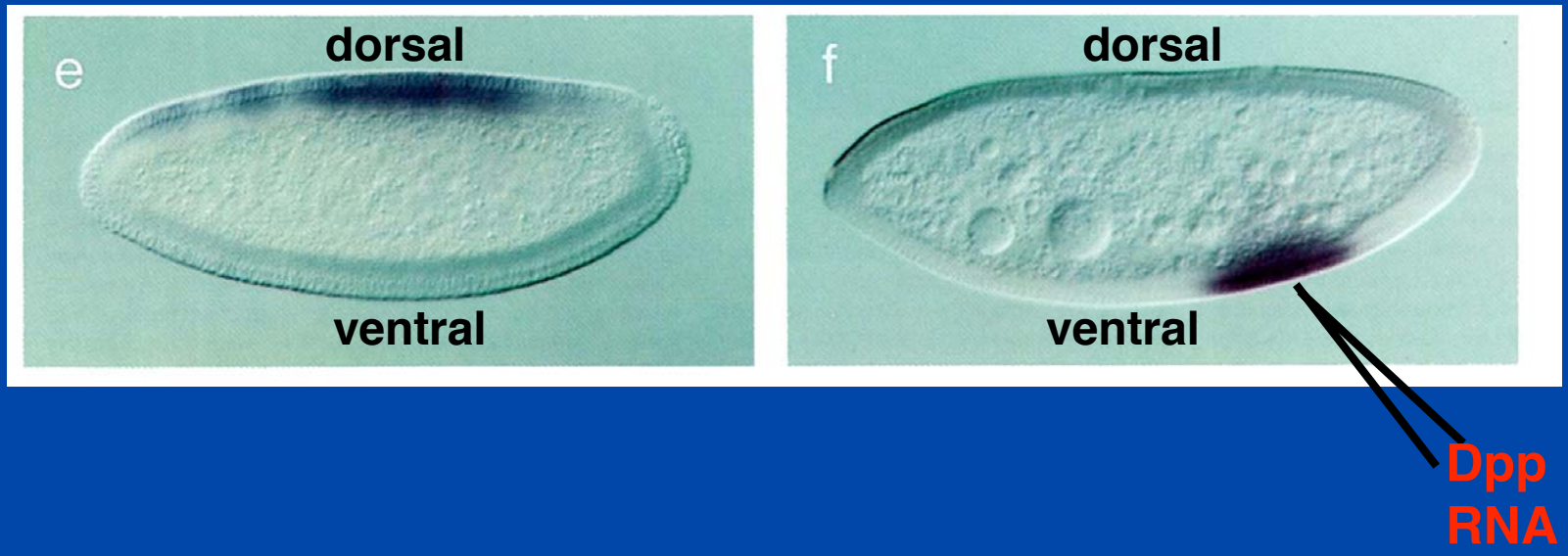
- **Different Dpp mutations cause loss of more or fewer dorsal ectoderm structures**
  - Wharton et al, 1993, Development

- **Injections of Dpp RNA induced dorsal fates**
  - Ferguson and Anderson, 1992, Cell

# Dpp RNA is *sufficient* to induce dorsal fates

***zen*** **is expressed in amnioserosa primordium**

***zen*** **expression is induced in ventral cells by Dpp RNA**




Ferguson & Anderson, 1992, Cell 71, 451

- Why Drosophila genetics?
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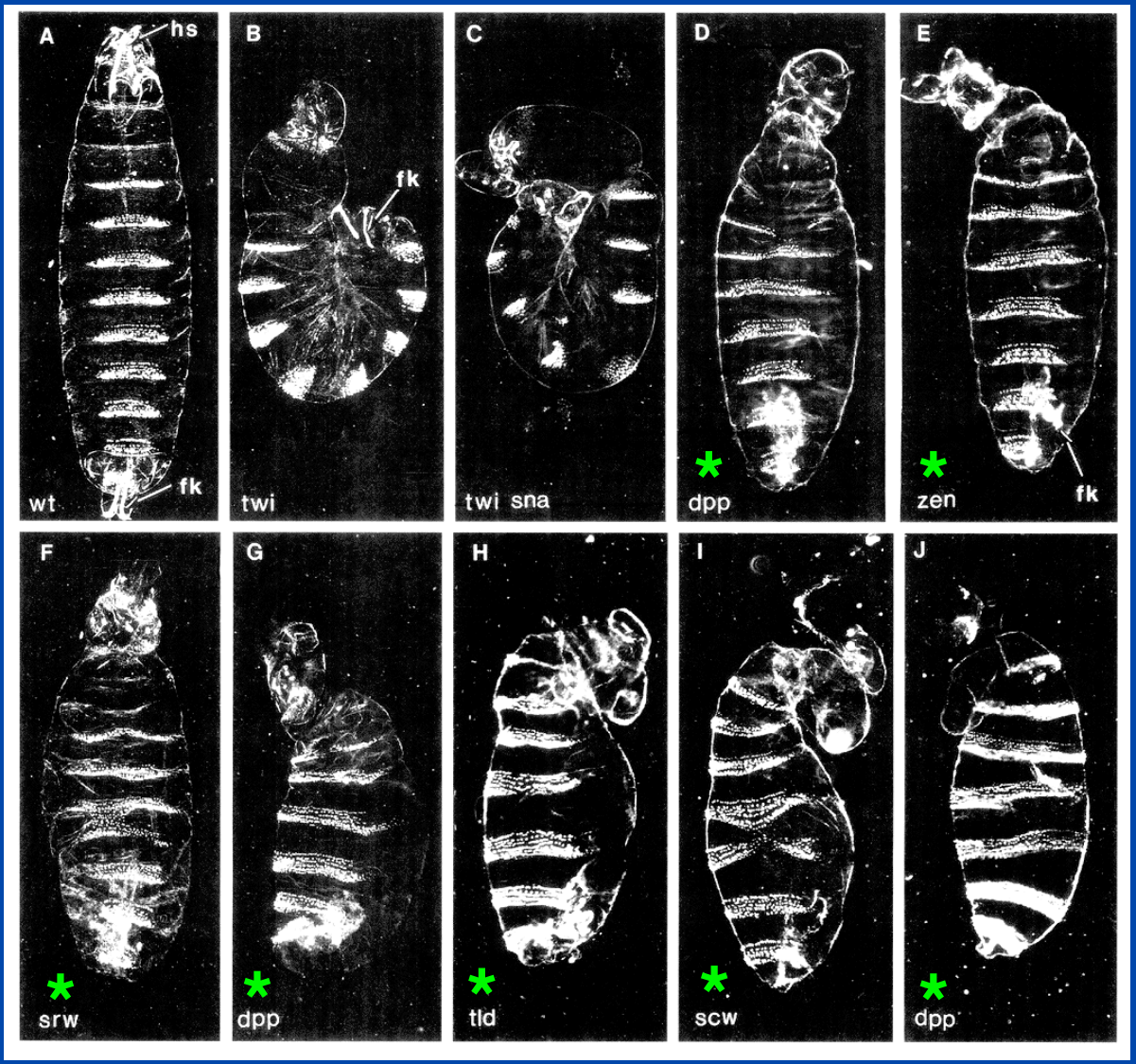
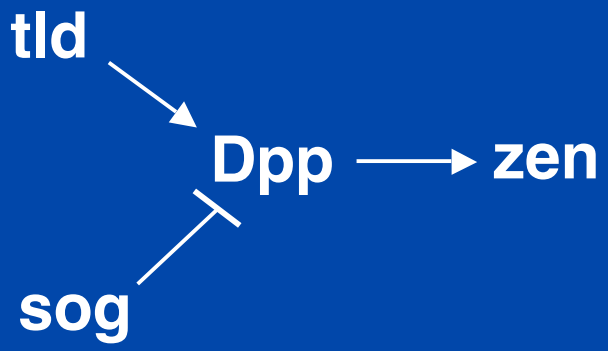
# Mutational screens identified classes of genes with shared D-V patterning defects

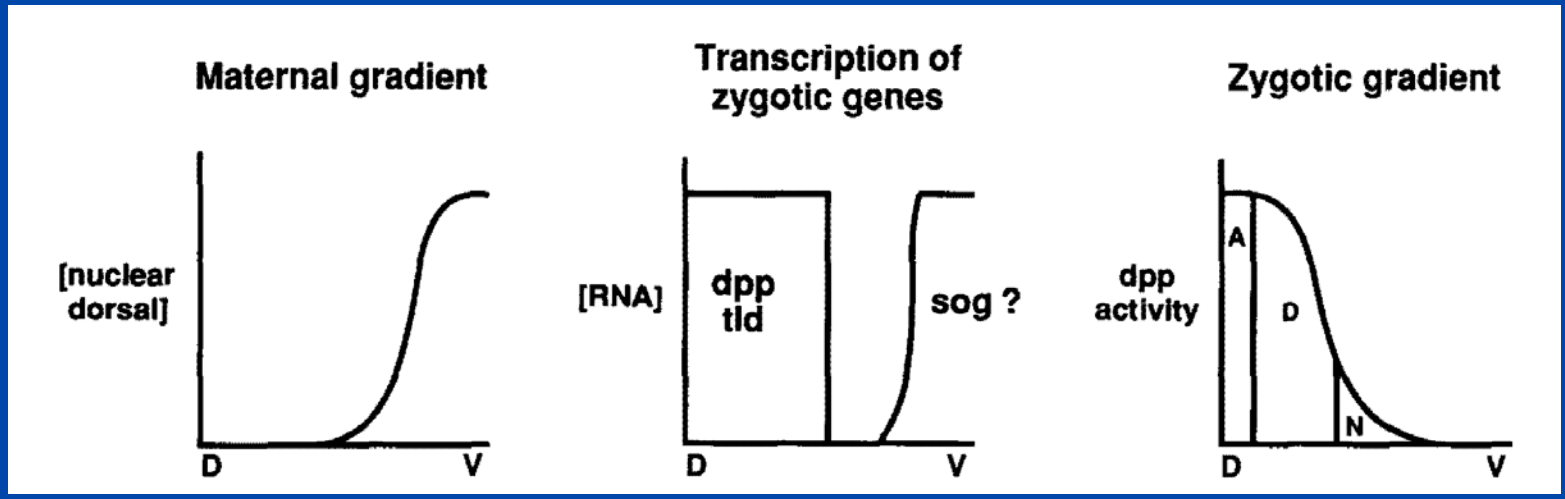



***tld and scw*** **are almost as severe as *dpp***

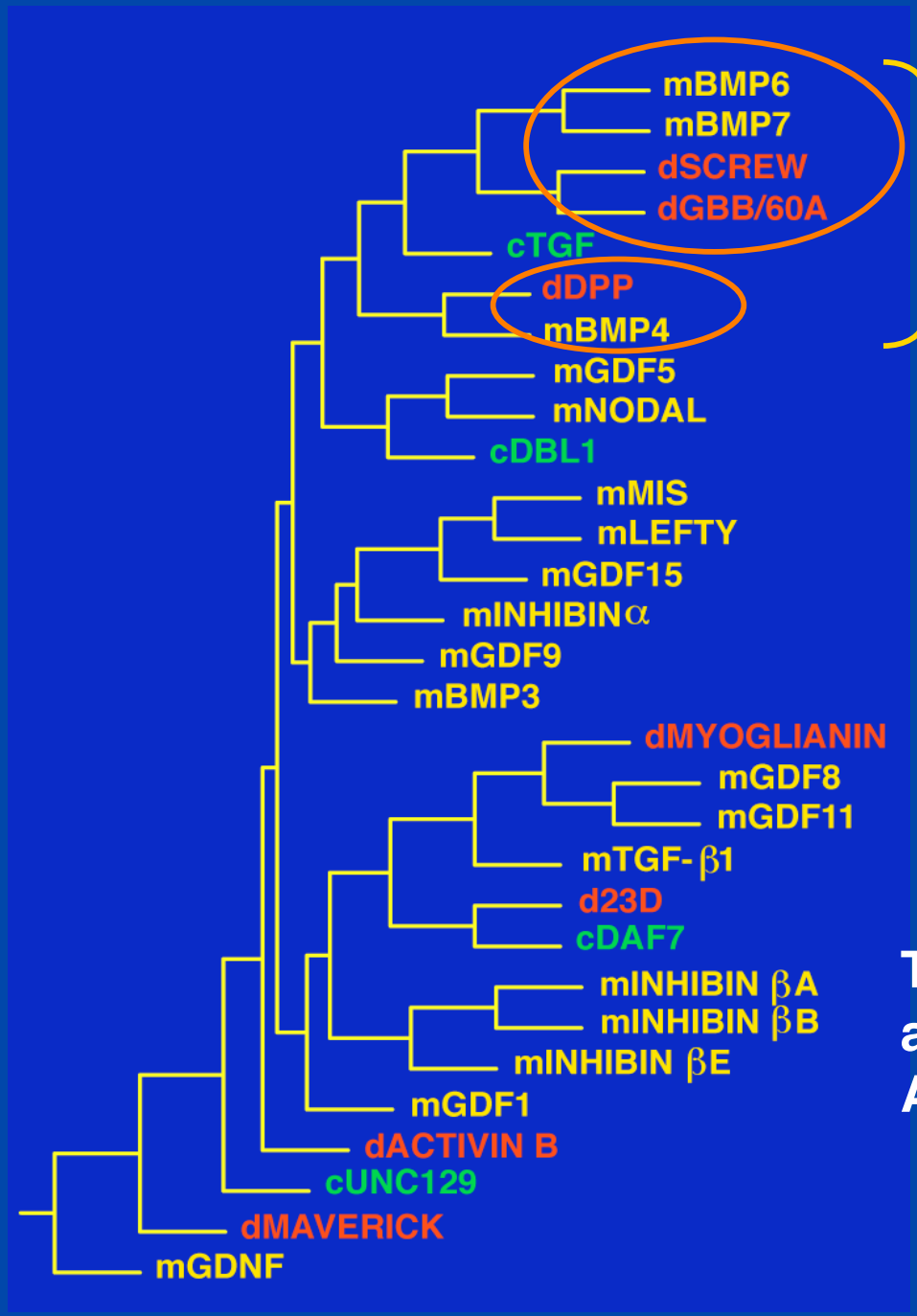
Arora et al, 1992,
Development 114, 1003

# Dpp activity is modulated by *tld* and *sog*




Ferguson & Anderson, 1992, Development 114, 583

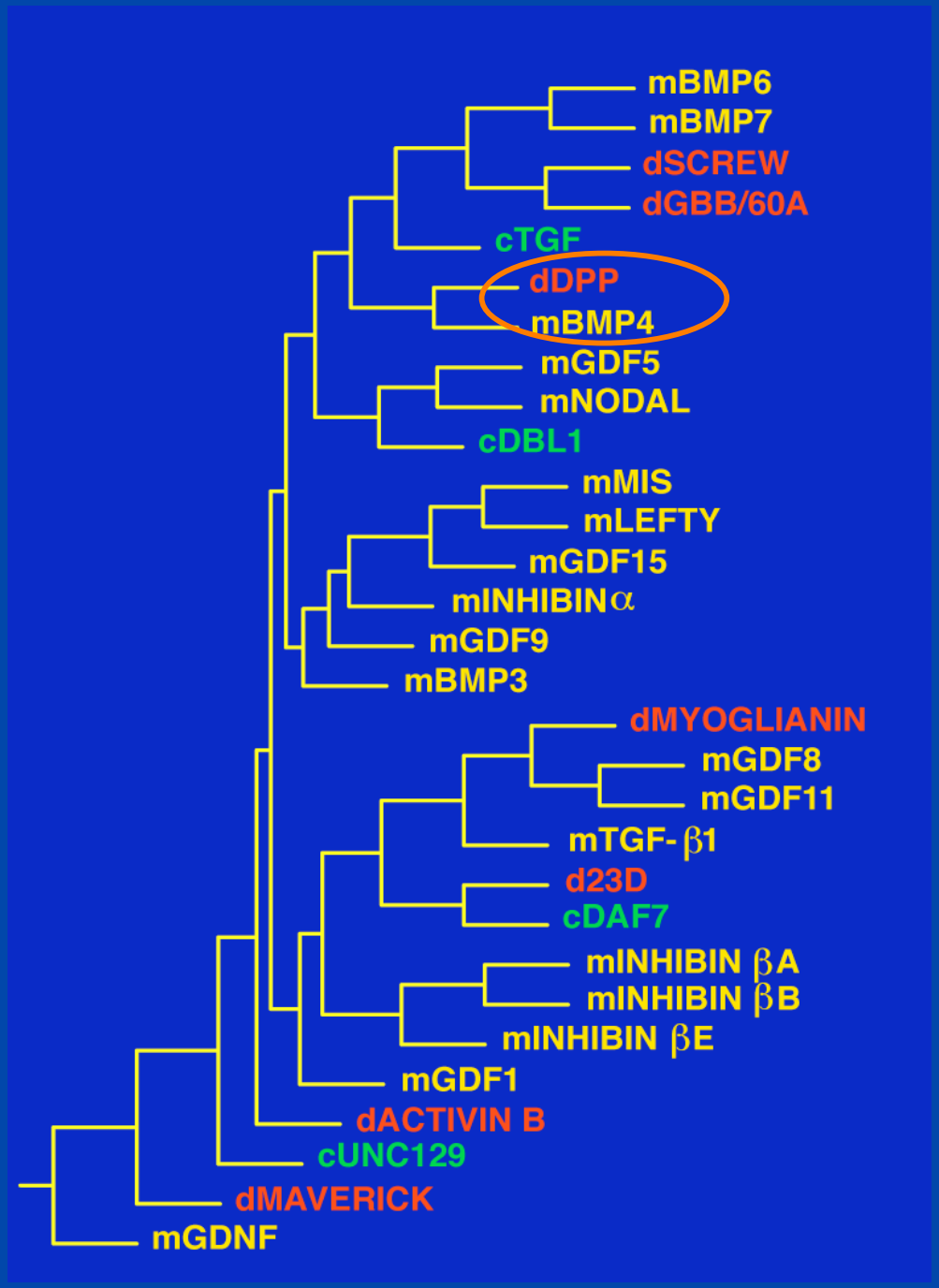



## Dpp and Screw are BMPs

(bone morphogenetic proteins)

**TGFβ proteins organized according to sequence similarity: A phylogenetic tree**

Mouse 57
Fly 7
Worm 4



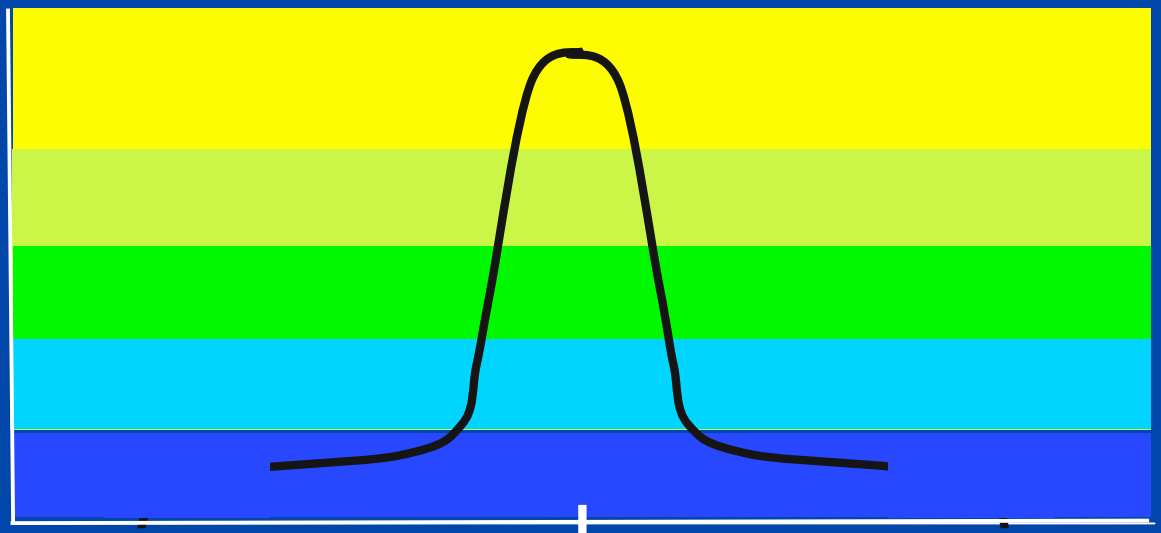

**Experimental tests say that the most similar BMPs are from different species:**

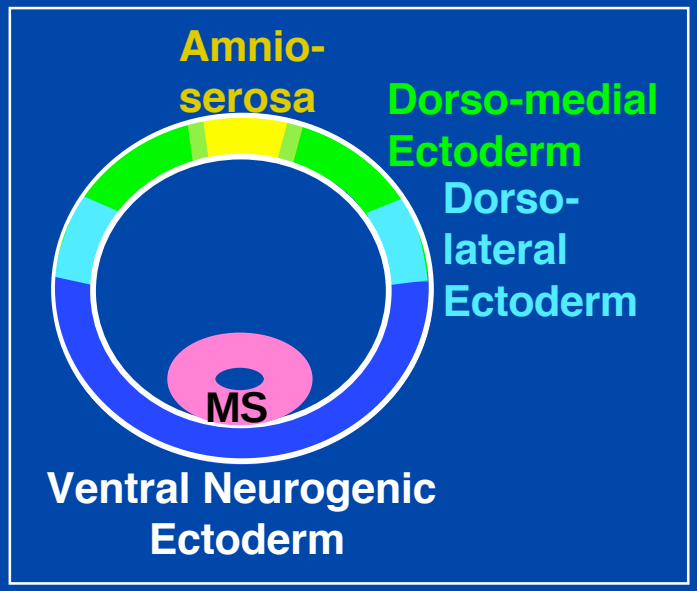
**Fly DPP can induce bone formation in rats.**

**Mouse BMP2 can replace fly DPP in dorsal-ventral patterning.**

**DPP, BMP2, BMP4 are orthologs**

# BMPs regulate nested patterns of gene expression

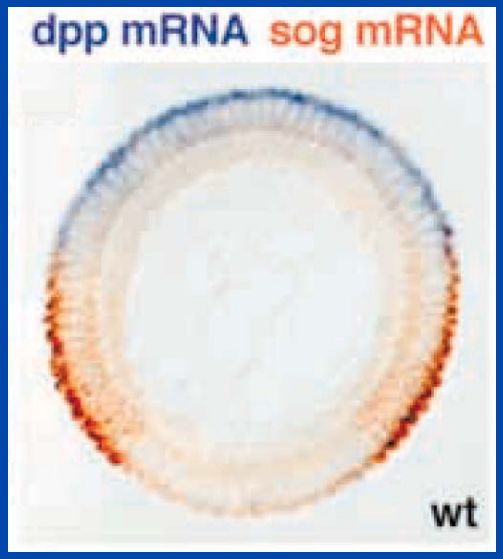



reviewed by
Raftery & Sutherland, 2003 Trends Genet. 19, 701

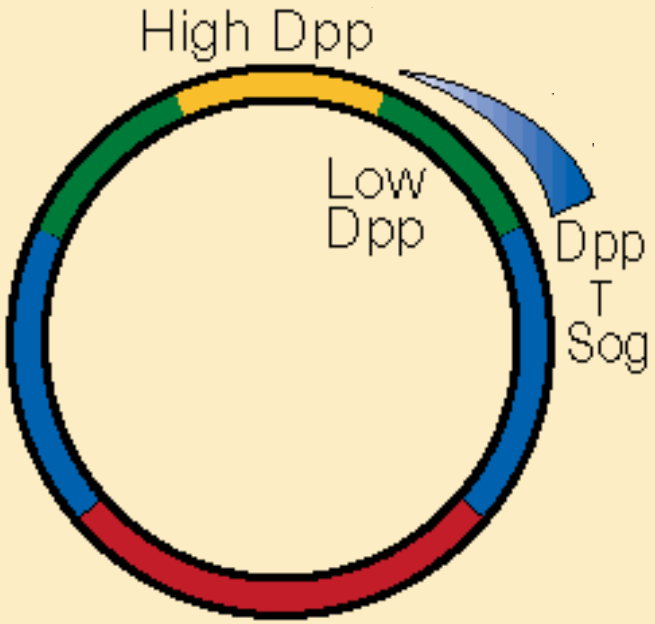
# Model: Sog removes BMPs at low end of gradient

**Screw is everywhere**



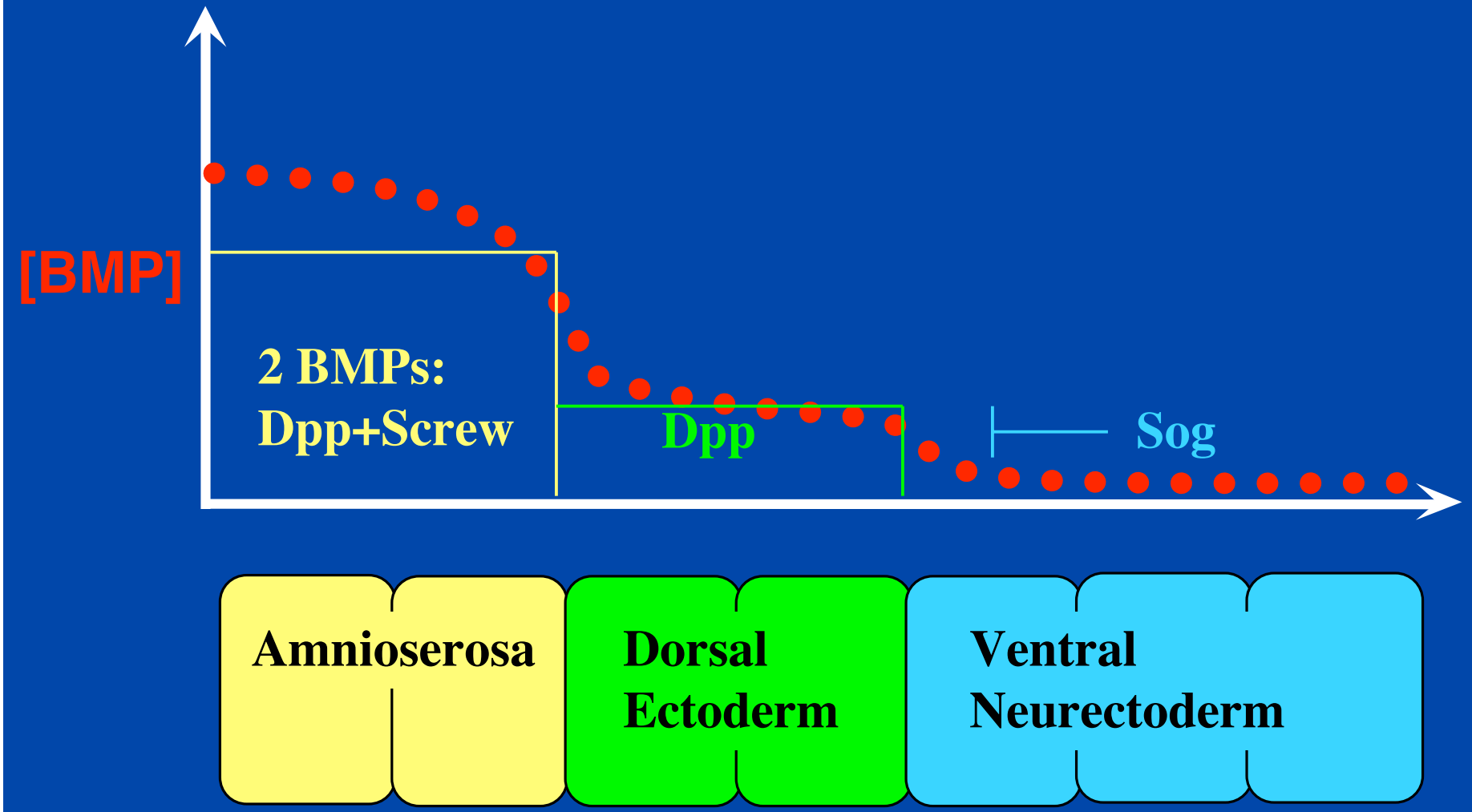

Jazwinska et al, 1999,
Development 126, 3323

**Sog binds Dpp**



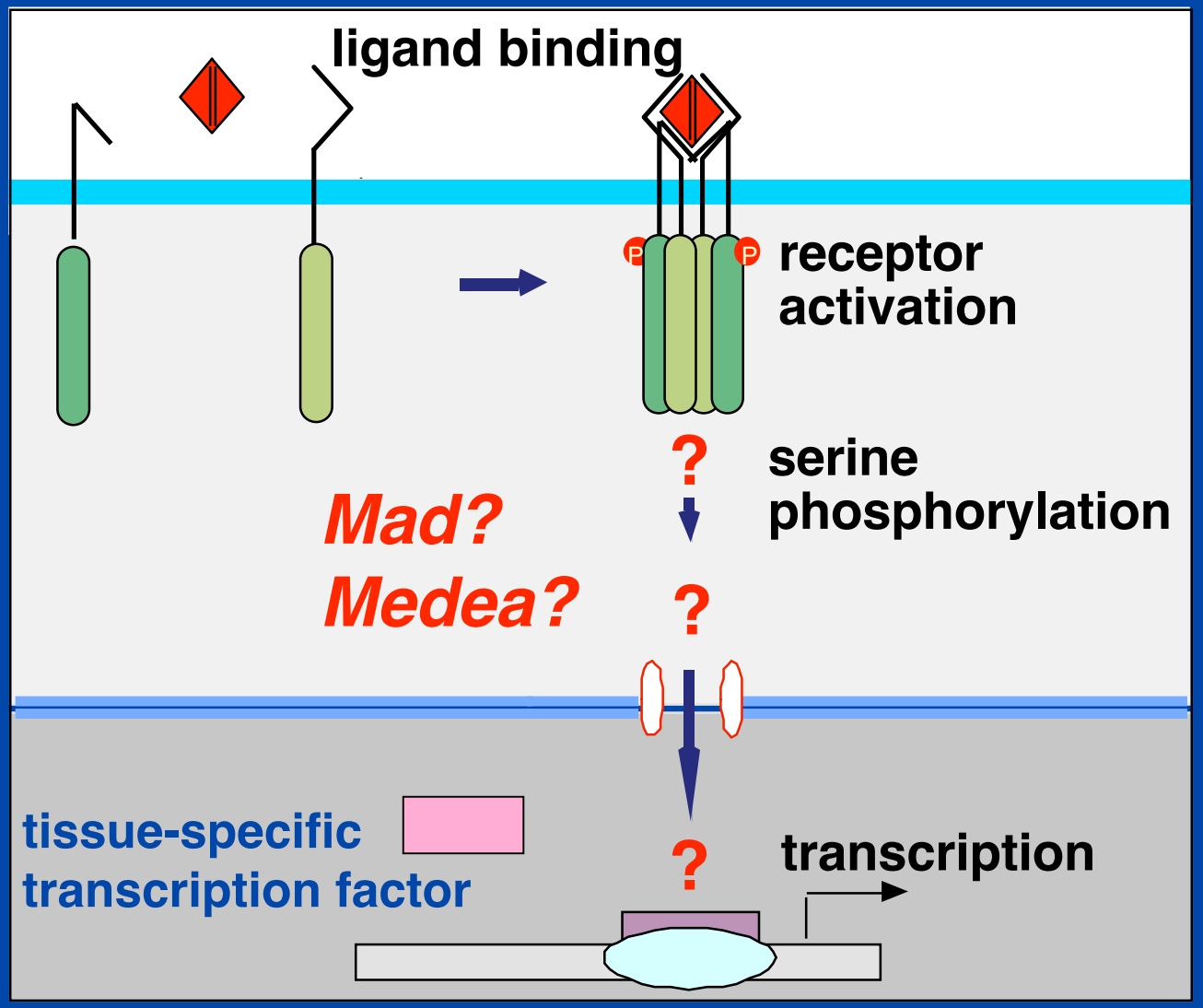

reviewed by Bier, 1999, Nature 398, 375

# Model: a BMP activity gradient at blastoderm induces dorsal ectoderm fates



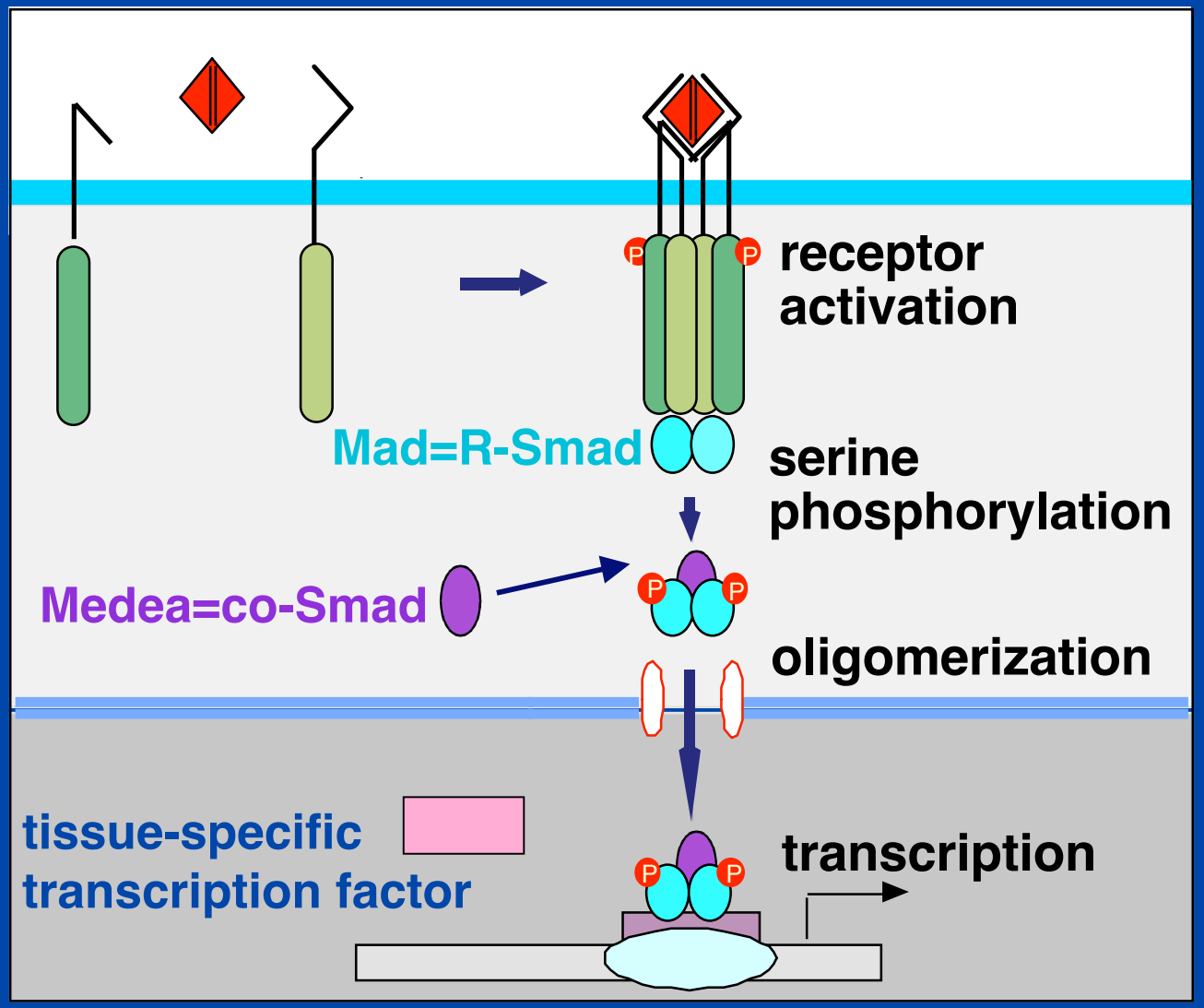
# How do cells respond to the BMP gradient?

## Mad and Medea identified in screens for genes that interact with Dpp




Raftery et al, 1995, Genetics 139, 241

# Smad proteins transduce TGFβ family signals to the nucleus




Reviewed by Raftery & Sutherland, 1999, Dev. Biol. 210, 251

- Why Drosophila genetics?
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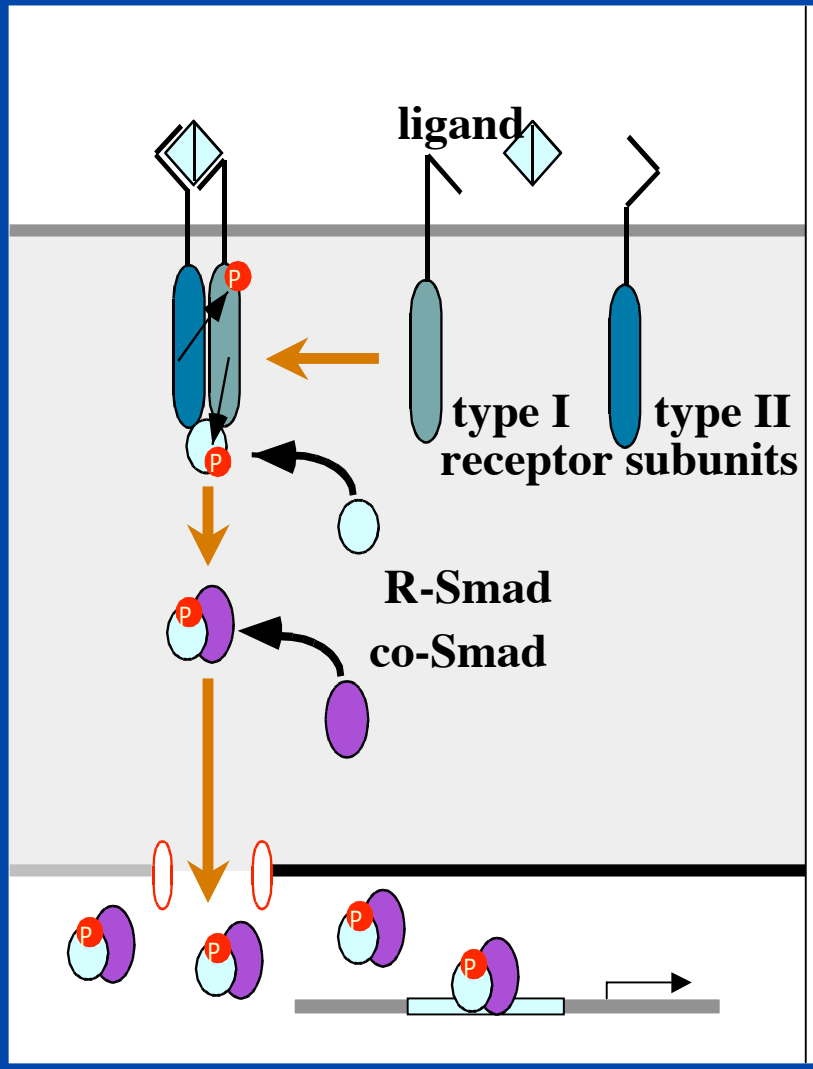
## How can we visualize a BMP activity gradient?

- Dpp RNA expression is low
- Dpp protein hasn't been visualized
- Graded distribution of diffusible antagonist, Sog

  Srinavasan et al, 2002, Dev. Cell 2, 91

⇒ Look at distribution of activated signal transducers

# Medea nuclear localization and phospho-Mad predicted to indicate level of BMP activity *in vivo*

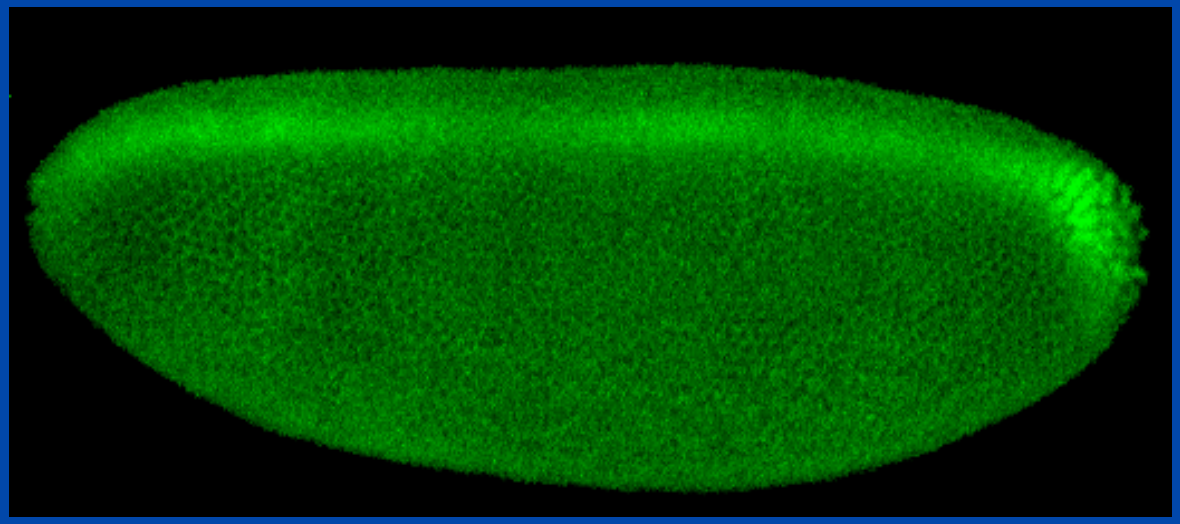



| | *BMP4* | *BMP7* |
|---|---|---|
| ligands | Dpp | Screw |
| type I receptor | Tkv | Sax |
| R-Smad | Mad | |
| co-Smad | Medea | |

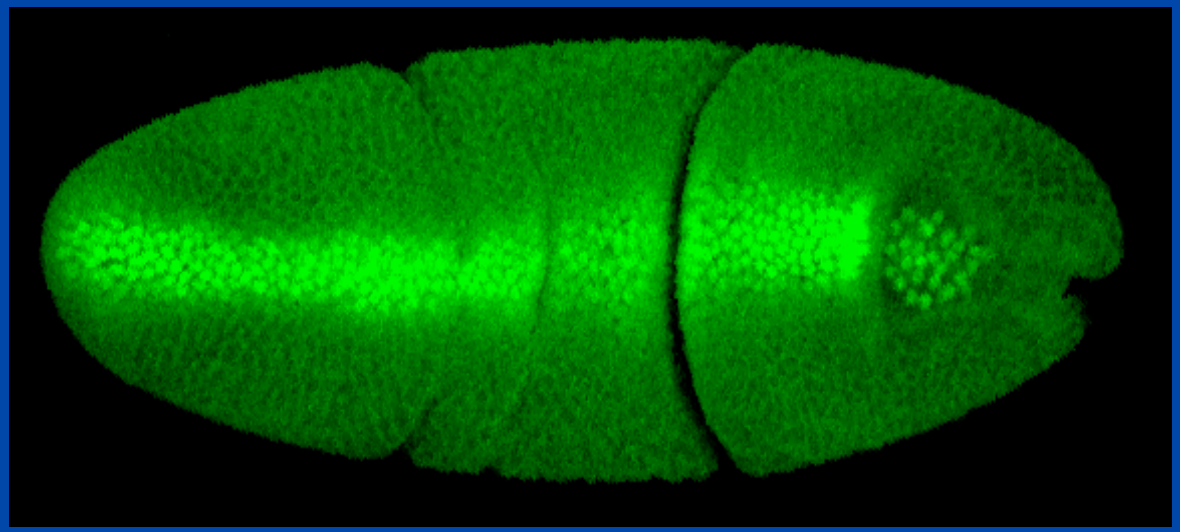
direct patterning of dorsal ectodermal fates

# A dorsal midline stripe of strong nuclear Medea

**End blastoderm**
**Side view**
**2.75hr**



**Gastrulation**
**Dorsal view**
**3.25hr**

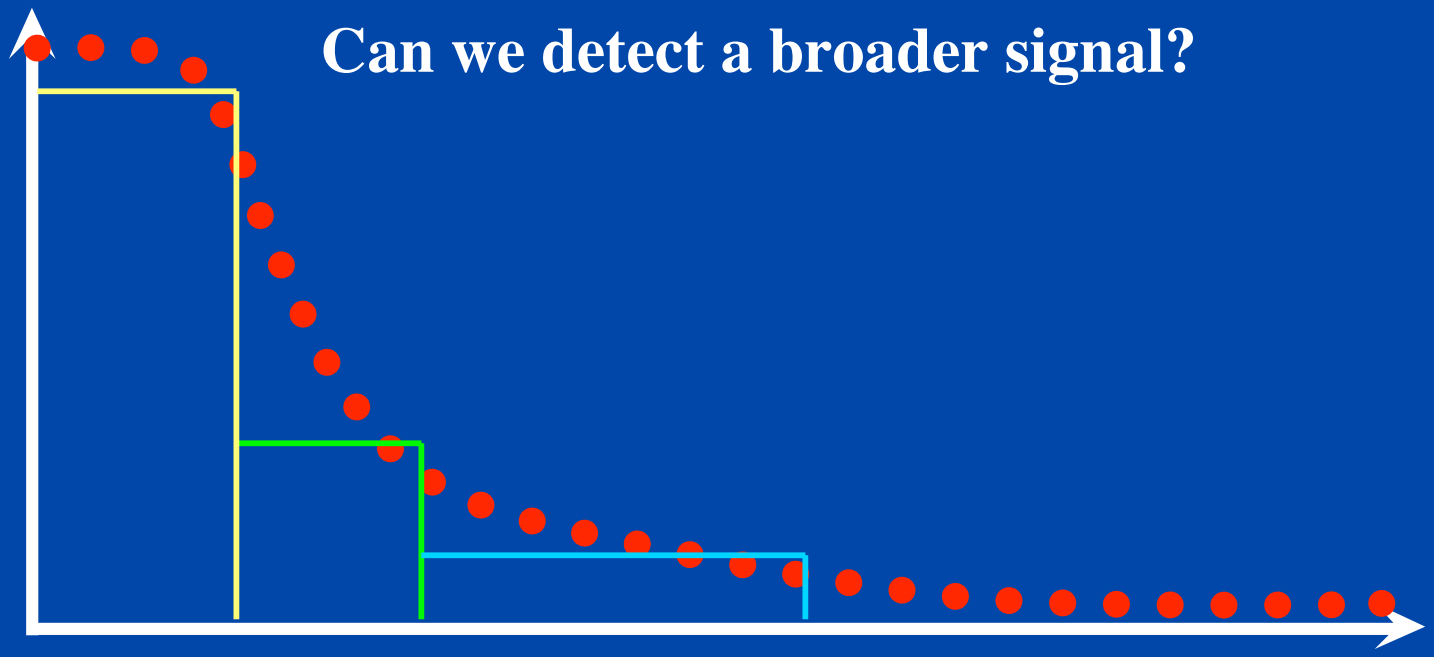


Sutherland et al, 2003, Development 130, 5705

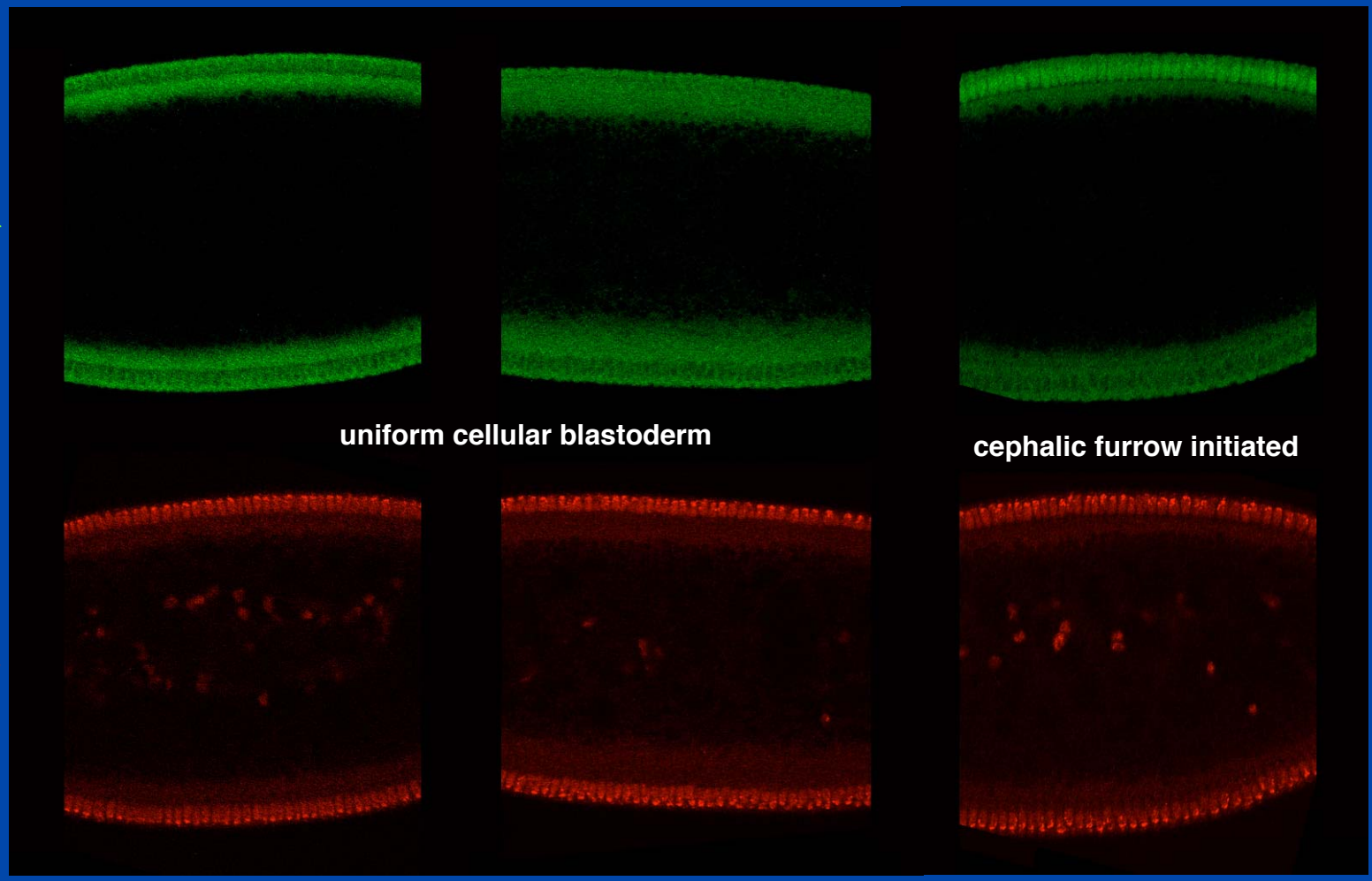
# BUT…?

**Stripe of intense response is only wide enough to account for amnioserosa induction…**

**Can we detect a broader signal?**

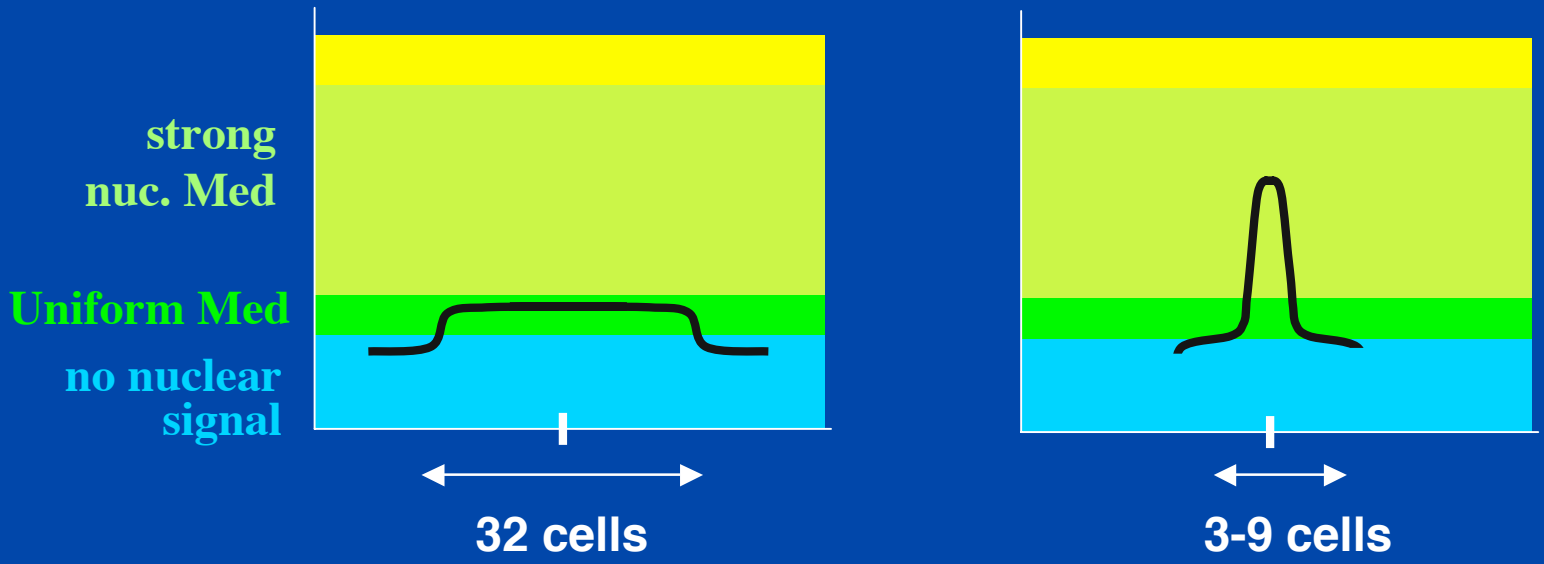


# A low level signal in some blastoderm embryos




Sutherland et al, 2003

# co-Smad response domain narrows as peak forms at the dorsal midline

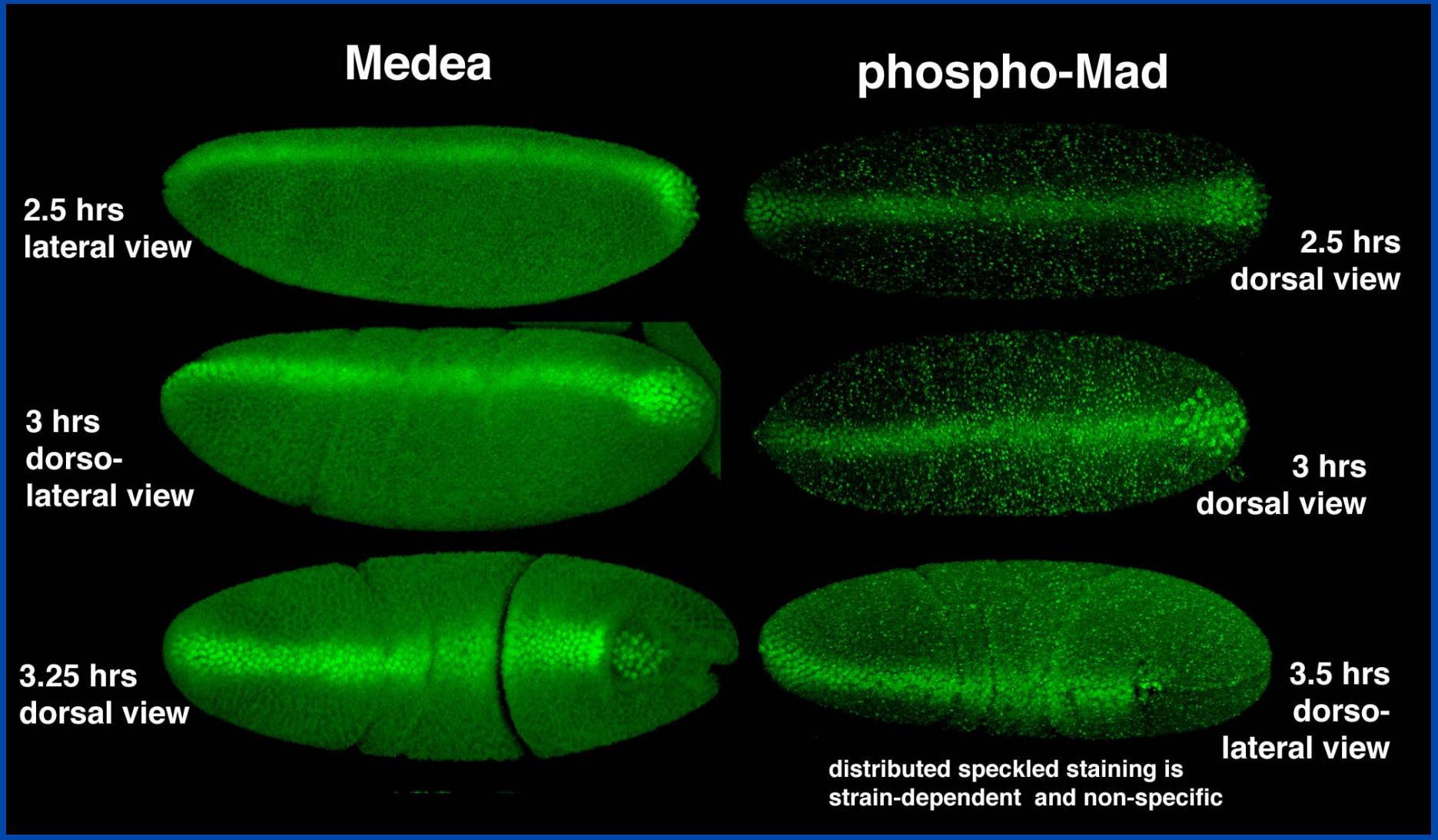



transient, or occasional

every embryo at the onset of gastrulation

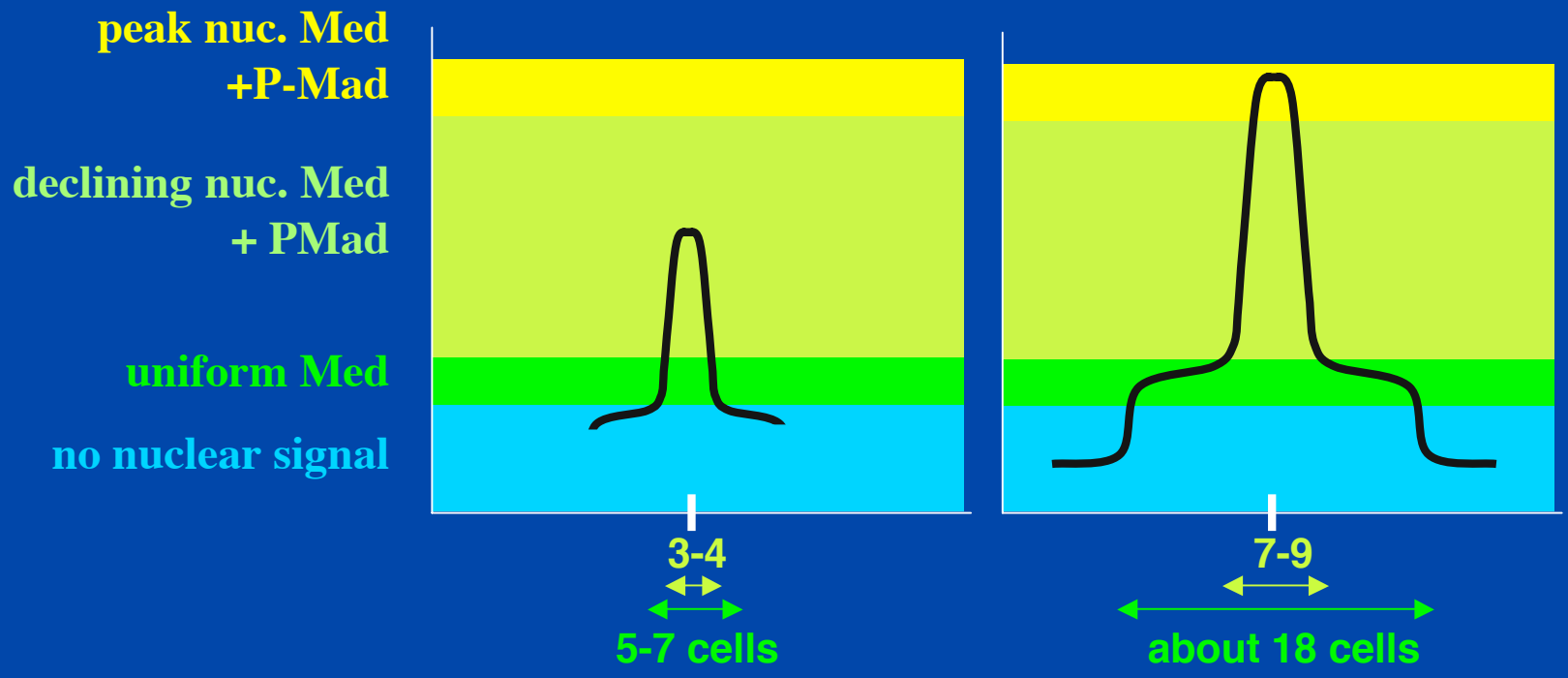
Ross et al, 2001 and Rushlow et al, 2001 have similar observations for PMad

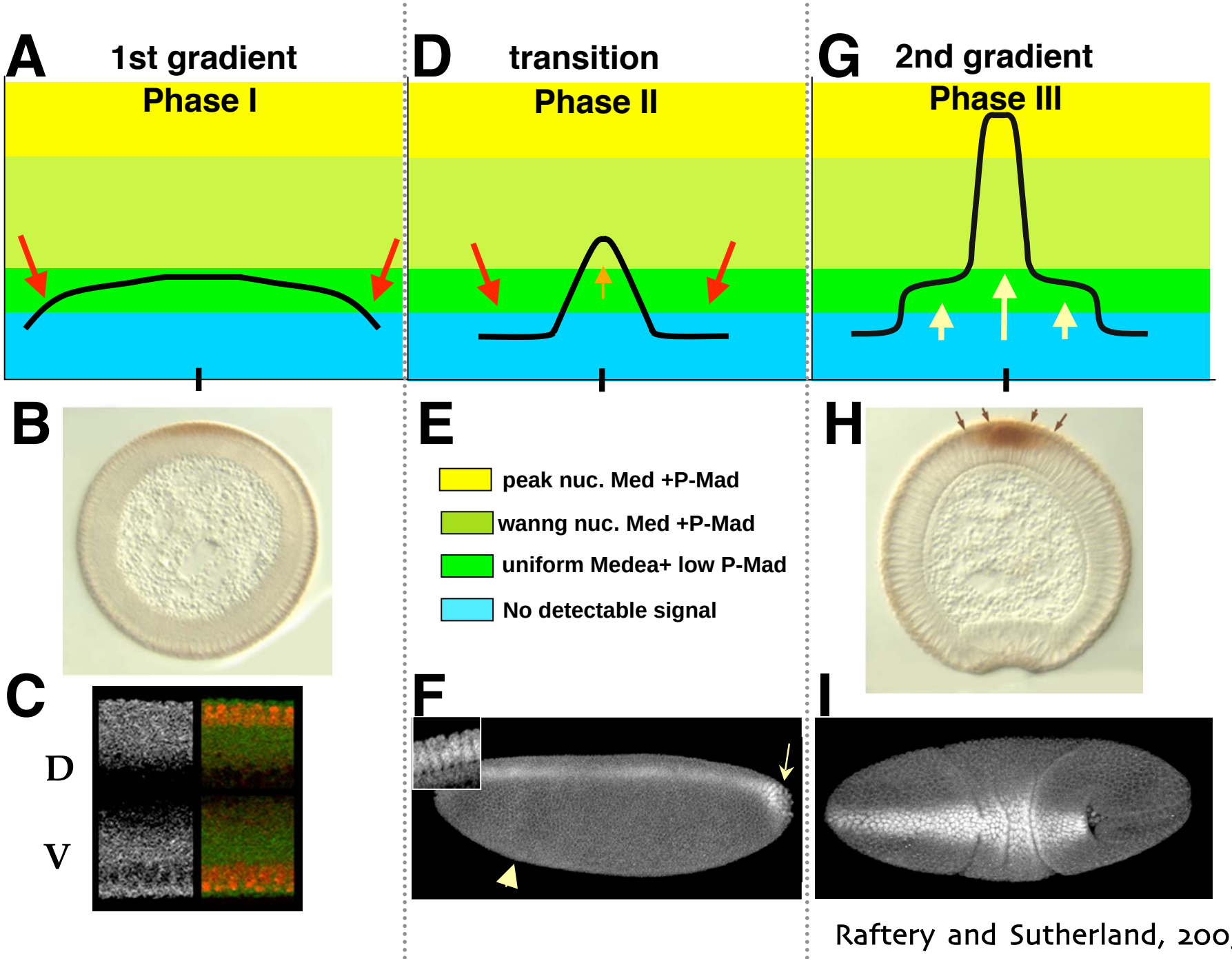
# Both co-Smad and phospho-Mad responses elevate during gastrulation




Sutherland et al, 2003

# During gastrulation, peak co-Smad response is further elevated and more cells are involved






Raftery and Sutherland, 2003

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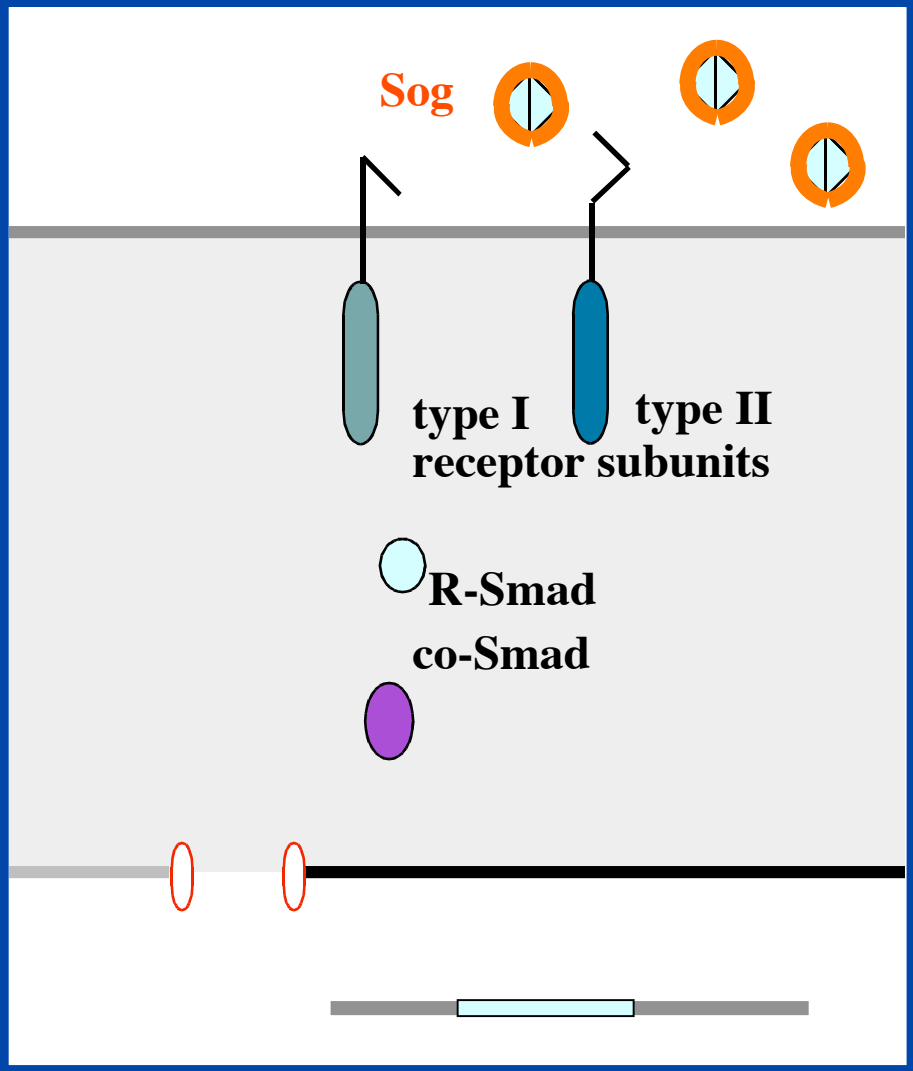
# How is the BMP gradient shaped?

| | | |
|---|---|---|
| *Mid-blastoderm* | Wide band | weak signal |
| | ↓ | ↓ |
| *Gastrulation* | Narrow stripe | strong signal |

# Sog is an extracellular BMP binding protein that can block signaling

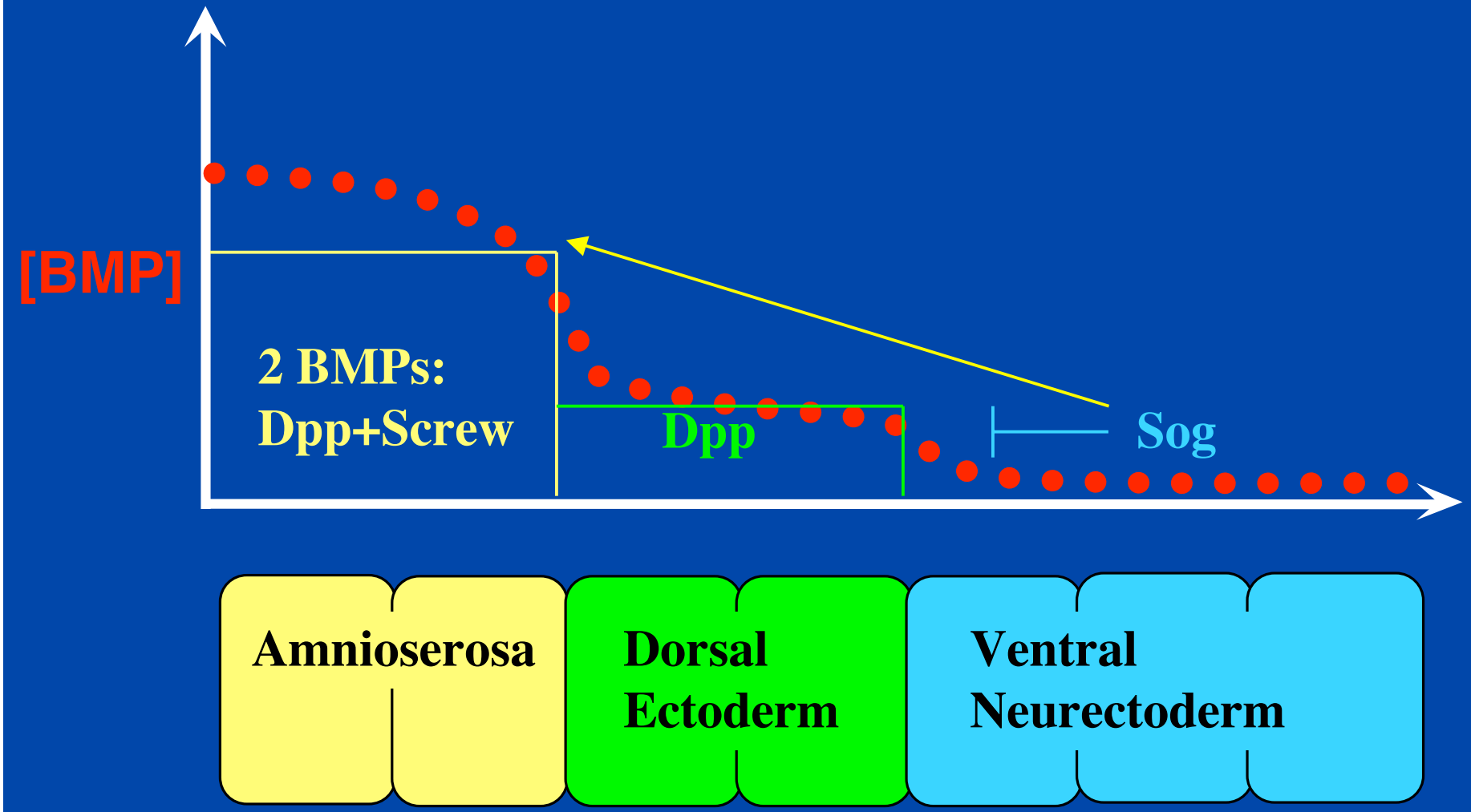




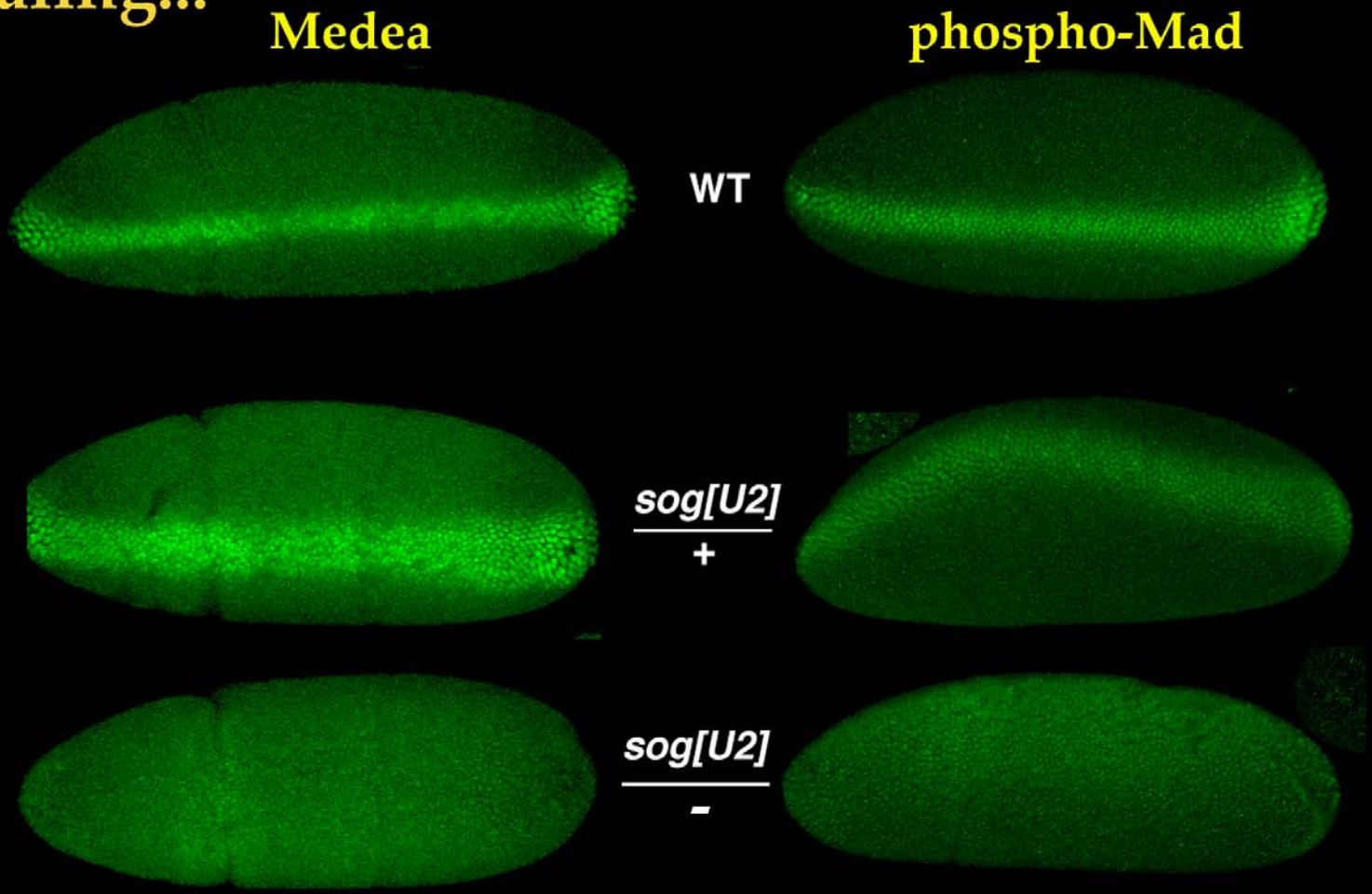
Jazwinska et al, 1999,
Development 126, 3323

but, Sog is also required to get a normal number of amnioserosa cells

# How does Sog affect the nuclear co-Smad signal?



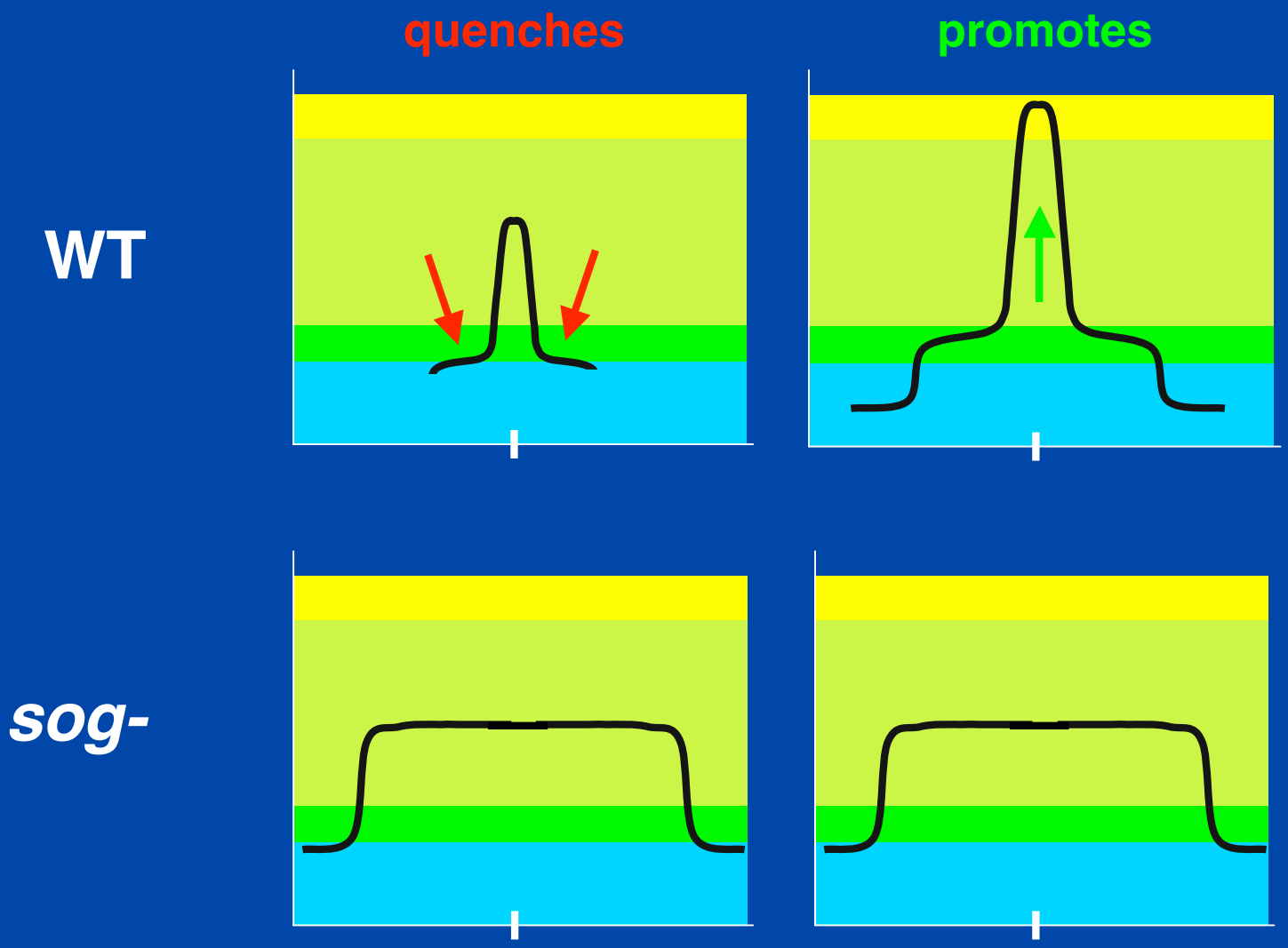
# The BMP antagonist Sog limits the domain of signaling...




## but Sog is also necessary for the stripe of peak signal.

Sutherland et al, 2003

# Sog shapes the BMP activity gradient over time



# Modulation of BMP levels: 1992 to 2002

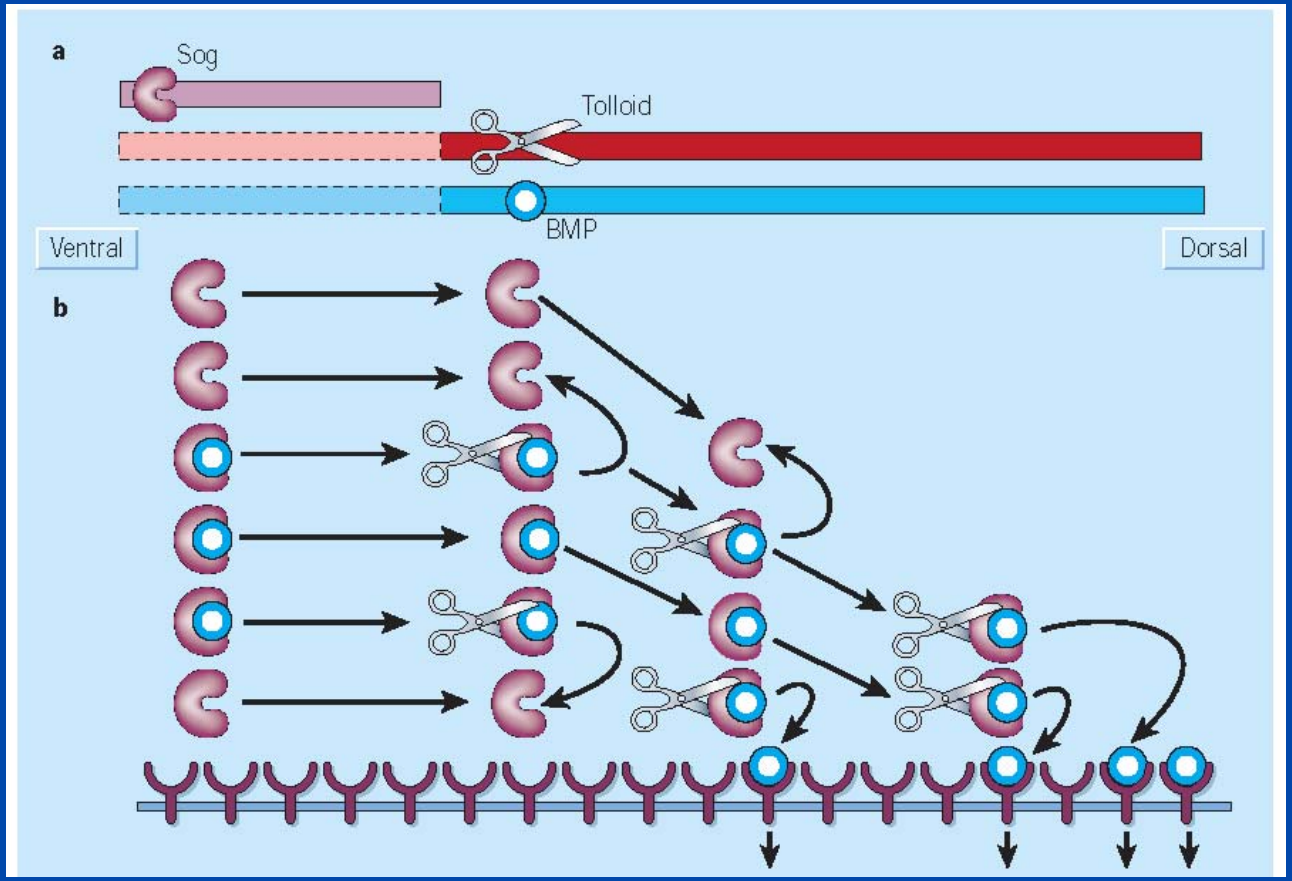
tld → Dpp → zen

sog ⊣ Dpp

Tld=
Metalloprotease

Sog=
BMP binding protein

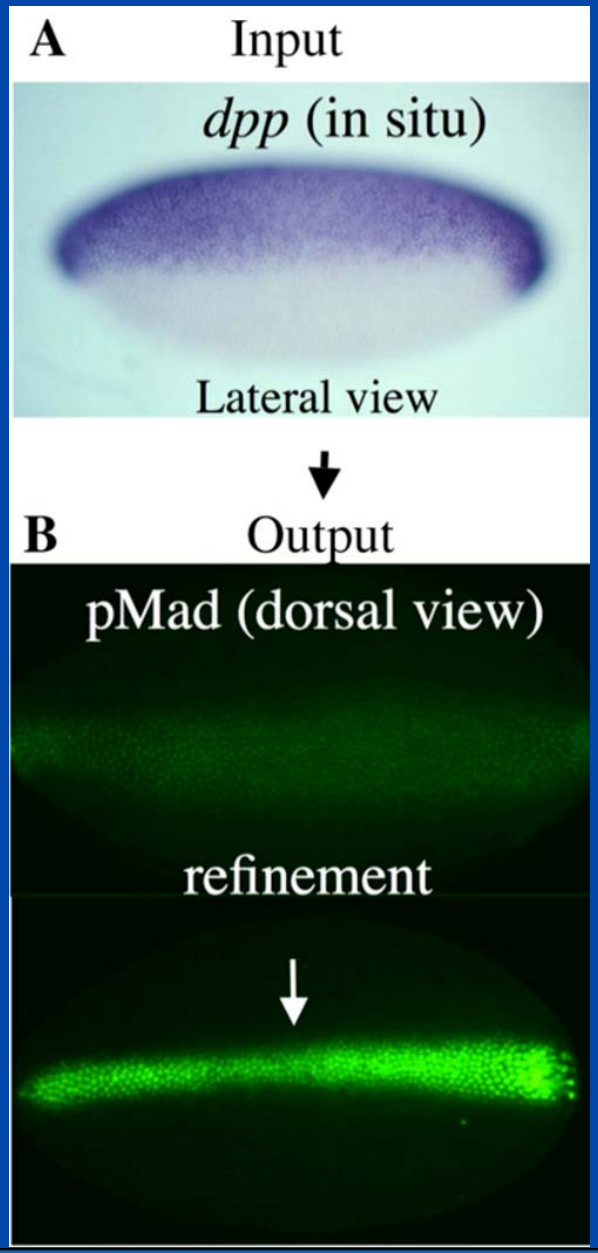
Zen=
transcription factor,
Partner to Smads

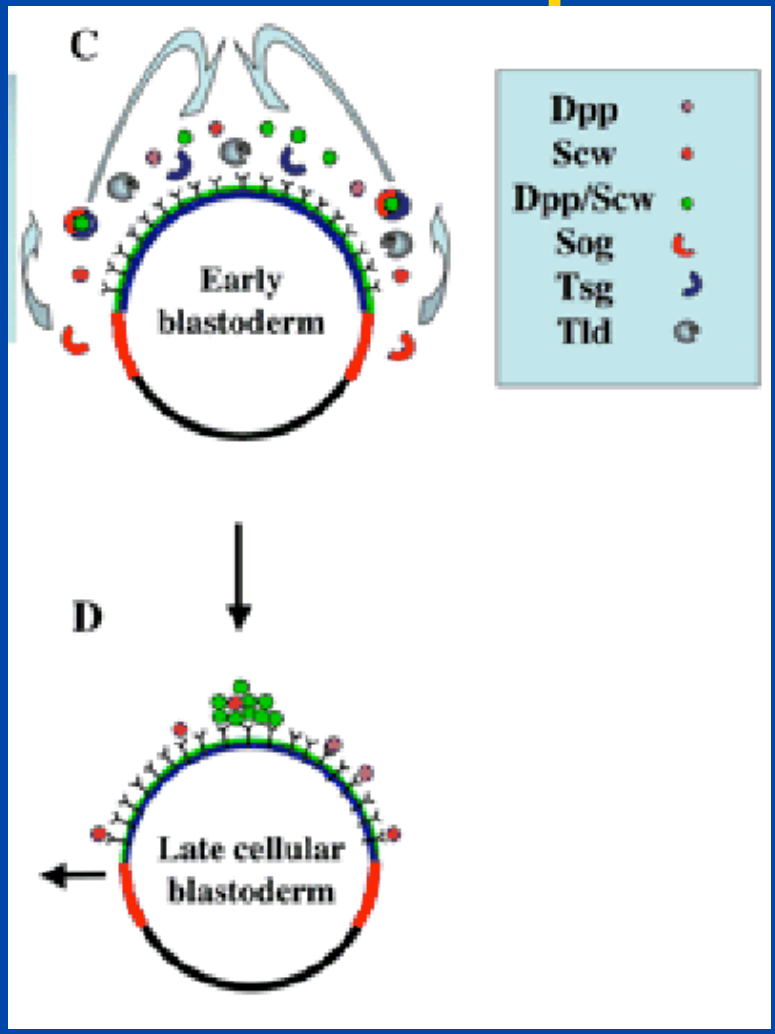



Reviewed by Meinhardt & Roth, 2002, 1999, Dev. Biol. 210, 251

# But how does this work?

# A BMP transport model:




O'Connor, M. B. et al. Development 2006;133:183-193

- Why Drosophila genetics?
- What is a morphogen gradient?
- Inference of a morphogen gradient from genetics
- Identification of signaling pathway from genetics
- Visualization of a gradient from signal transducers
- Shaping the gradient
- Computational predictions of molecular mechanisms

# Changing BMP gradient can be described mathematically

**Free ligand concentration :**

$$\frac{\partial[L]}{\partial t} = D_L \frac{\partial^2[L]}{\partial x^2} - k_{on}[L](R_0 - [LR]) + k_{off}[LR] - j_{on}[L][ST] + (j_{off} + \tau)[LST] + V_L(x)$$

**Ligand bound to receptor:**

$$\frac{\partial[LR]}{\partial t} = k_{on}[L](R_0 - [LR]) - (k_{off} + k_{deg})[LR]$$

**Sog concentration over time:**

$$\frac{\partial[S]}{\partial t} = D_S \frac{\partial^2[S]}{\partial x^2} - n_{on}[S][T] + n_{off}[ST] + V_S(x)$$

**Sog bound to Tld:**

$$\frac{\partial[ST]}{\partial t} = D_{ST} \frac{\partial^2[ST]}{\partial x^2} + n_{on}[S][T] - n_{off}[ST] - j_{on}[L][ST] + j_{off}[LST]$$

**Tld concentration over time:**

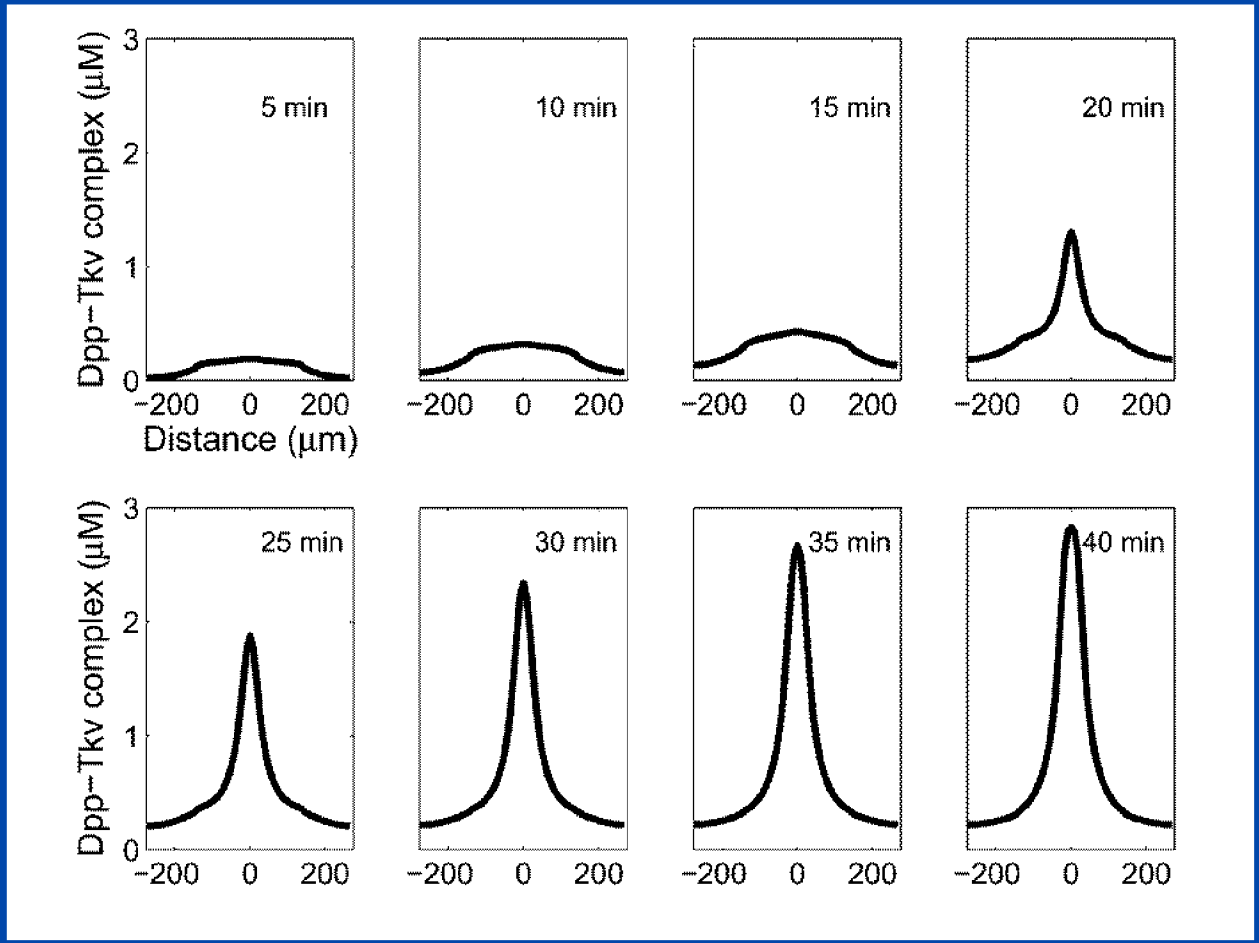
$$\frac{\partial[T]}{\partial t} = D_T \frac{\partial^2[T]}{\partial x^2} - n_{on}[S][T] + n_{off}[ST] + \tau[LST] + V_T(x)$$

**Ligand bound to Sog and Tld:**

$$\frac{\partial[LST]}{\partial t} = D_{LST} \frac{\partial^2[LST]}{\partial x^2} + j_{on}[L][ST] - (j_{off} + \tau)[LST]$$

Mizutani et al, 2005, Dev Cell 8:915-924

# Computational modeling of the changing pattern of responses…




**Can be used to predict important parameters**

Mizutani et al, 2005, Dev Cell 8:915-924

# Computational modeling to test the importance of molecular mechanisms…

- **Degradation of ligand-receptor complexes**
  Mizutani et al, 2005, Dev Cell 8:915-924

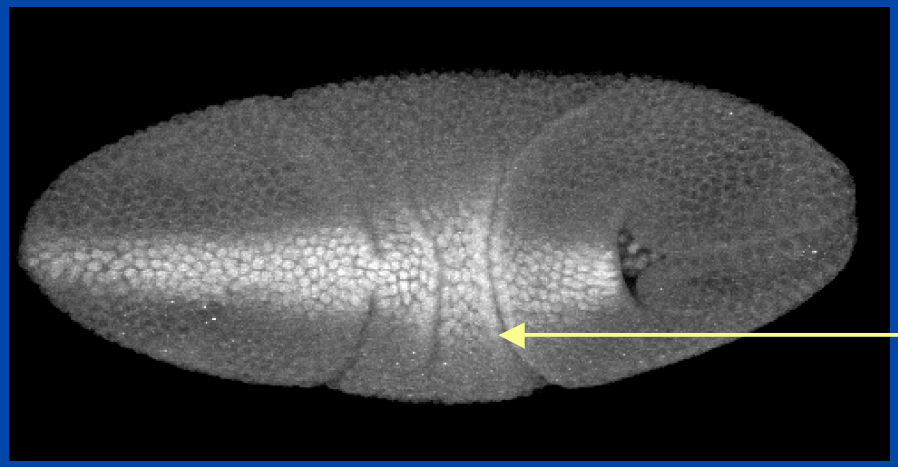
- **Positive feedback to stabilize narrow, high level gradient**
  O'Connor et al., 2006, Development 133:183-193

- **Contribution of Screw ligand: heterodimers versus two homodimers**
  Shimmi et al. 2005, Cell 120: 873-886

- Pathway genetics
- Prediction of a morphogen gradient
- Identification of signaling pathway from genetics
- Visualization of a gradient from signal transducers
- Computational predictions of molecular mechanisms

We still have questions…

# How is a threshold response organized to make a tissue boundary?



amnioserosa/
dorsal ectoderm boundary