“The molecular nature of the zebrafish tail organizer”

Agathon, Thisse & Thisse, *Nature* 2003

Presented by Lara Rogers

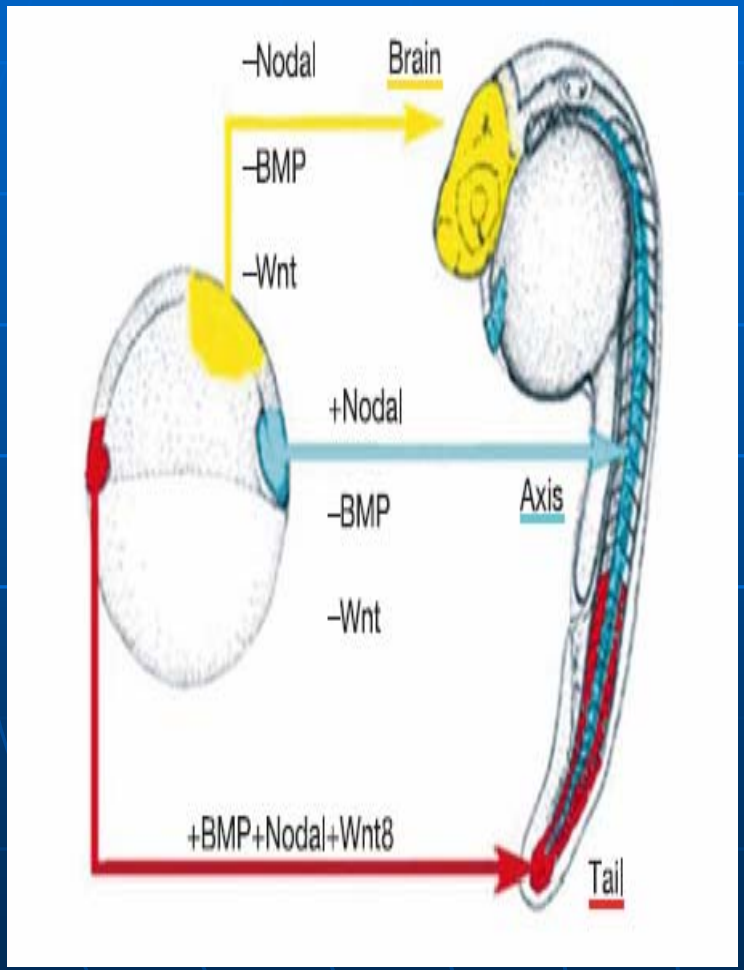
02/10/05

Main Questions

- Does development of the zebrafish tail depend on an organizer region distinct from the dorsal (Spemann) organizer?

- Which signaling pathways are necessary/sufficient for tail development?

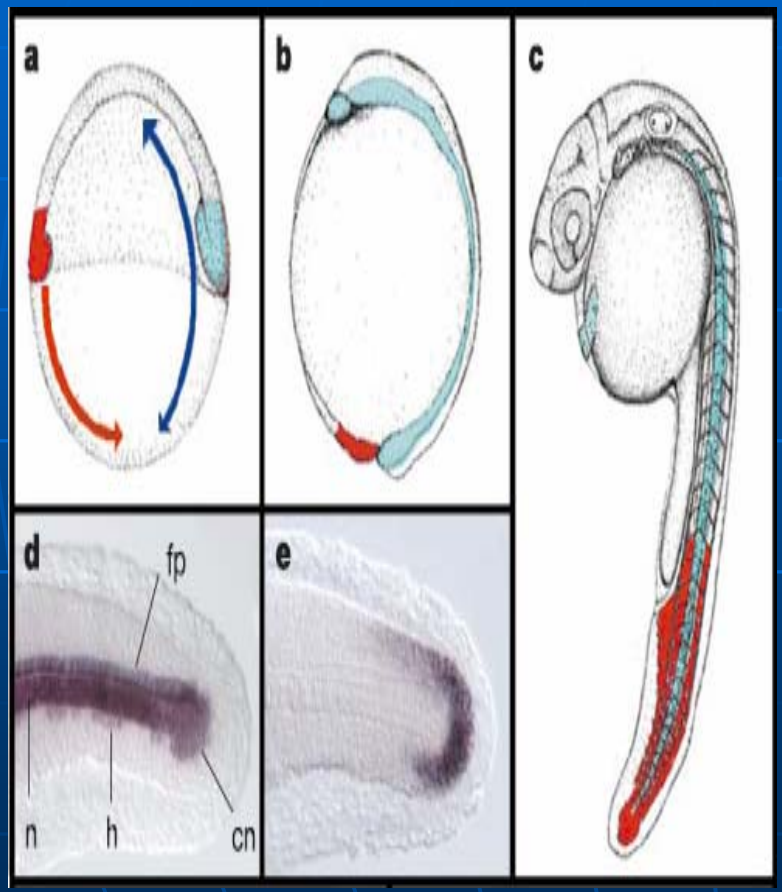
Answers/ Take-home message



- Tail organizer derives from ventral margin of zebrafish embryo
- Tail induction results from triple stimulation of BMP, Nodal and Wnt8 signaling pathways

Source: Agathon, A., C. Thisse and B. Thisse. "The molecular nature of the zebrafish tail organizer" *Nature* 424 (2003): 448-452. Courtesy of the authors. Used with permission.

Normal tail development: axial and non-axial structures



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a. Beginning of gastrulation; Red=ventral marginal cells; Blue=dorsal marginal cells

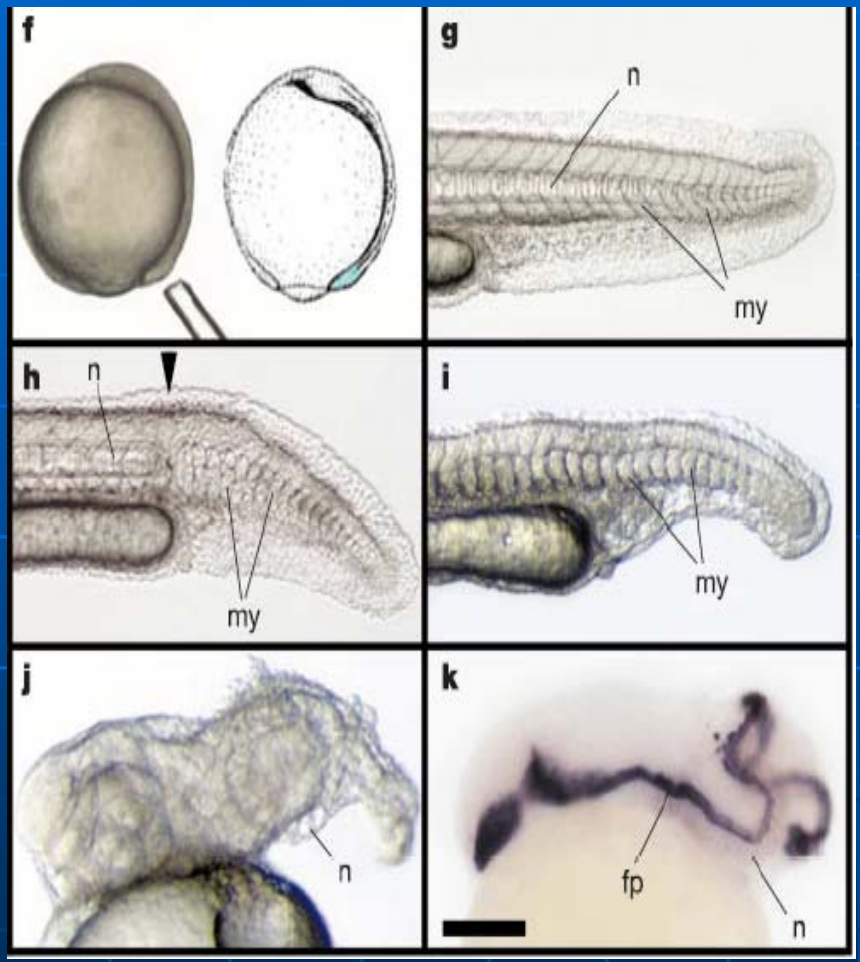
b. End of gastrulation

c. 24 h after fertilization

d. axial structures labeled with shh

e. non-axial structures labeled with evel

Axial and non-axial tail structures can develop independently



F. Surgical extirpation of dorsal margin

G. Wildtype tail (n for notochord, my for myotomes)

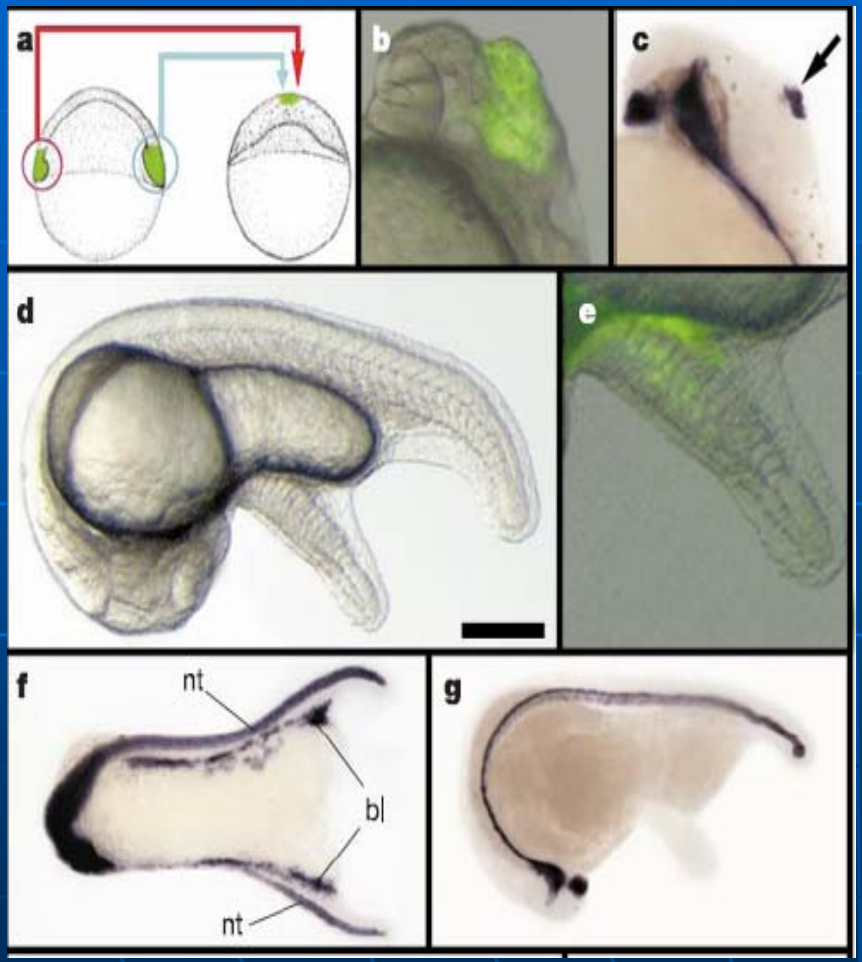
H. Surgically extirpated dorsal margin→no axial structures

I. Tail of ventralized embryo (BMP overexpressed)→ lacks axial structures

J. Embryo injected with frzb RNA (Wnt8 inhibitor overexpressed) →no non-axial tail structures

K. Embryo axial territory labeled with shh→no non-axial structures

Transplant experiments: dorsal margin vs. ventral margin



A. Transplanted dorsal or ventral margin cells into animal pole.

B,C. Dorsal transplant → ectopic axial structures (labeled with shh)

D-G. Ventral transplant → no axial structures, 2nd tail induced

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Taking stock so far: organizer from ventral marginal cells

Transplant studies show that ventral marginal cells induce 2nd tail formation in animal pole, while dorsal marginal cells induce axial structures but not 2nd tail

Ventral marginal cells appear to be tail organizer

Molecular nature of tail organizer

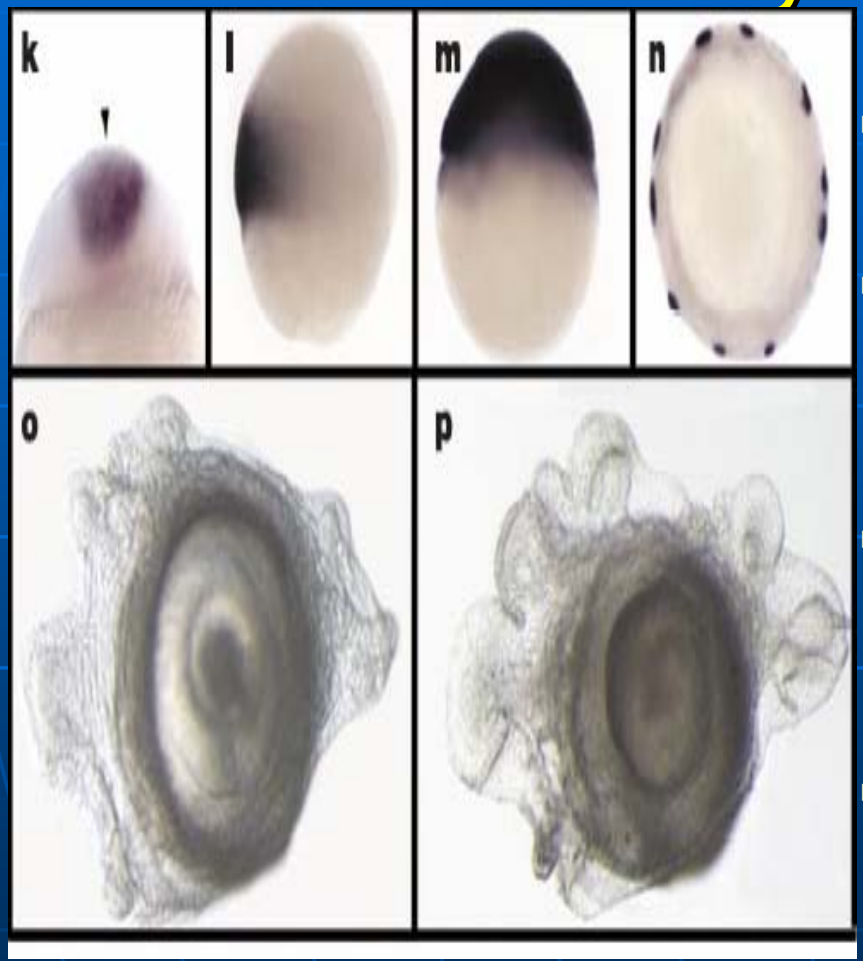
- Nodal, BMP4, Wnt8—overlap in expression at ventral margin of zebrafish blastula
- Loss-of-fxn of any one of these pathways prevents tail formation*

- Nodal, BMP, Wnt8 good candidates for tail-organizing activity

Testing the tail organizing activity of Nodal, BMP, Wnt8

- Misexpression experiments: injected Nodal, BMP, Wnt8 sense RNA alone and in combination (bmp4/znr1; bmp4/wnt8; bmp4/znr1/wnt8) into animal pole blastomere
- Combinations w/ BMP resulted in secondary tail formation at animal pole
- BMP is necessary but not sufficient for tail development

Induction of ventral marginal cell fate as measured by evel expression



- Evel stains ventral marginal cells
- K. localized animal pole injection of bmp/znr1 RNAs induces ectopic ventral margin at early blastula
- M. Misexpression of bmp, znr1 leads to massive expression of evel (compare M with wildtype L)
- Embryos that survive misexpression: after 24 h (n), multi-tailed w/ muscles but no axial structures after 36 h (o,p)

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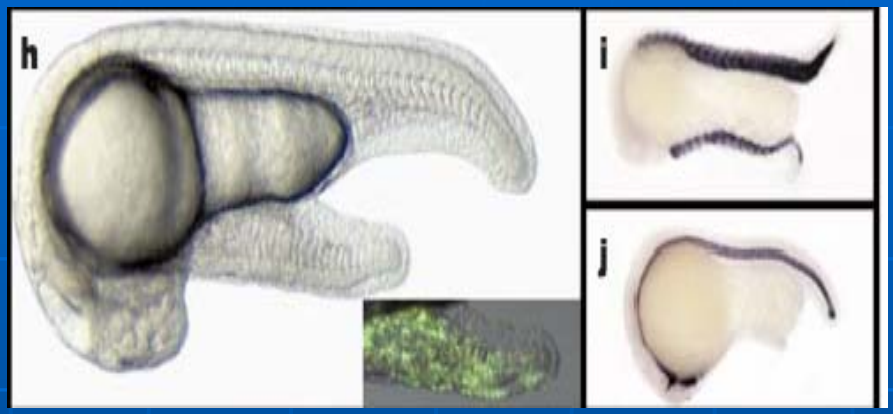
Induced secondary tail contains donor and recruited cells



H. Induction of ectopic tail in animal pole of 128-256 cell stage embryo by injection of a single cell with bmp4/znr1/gfp RNAs

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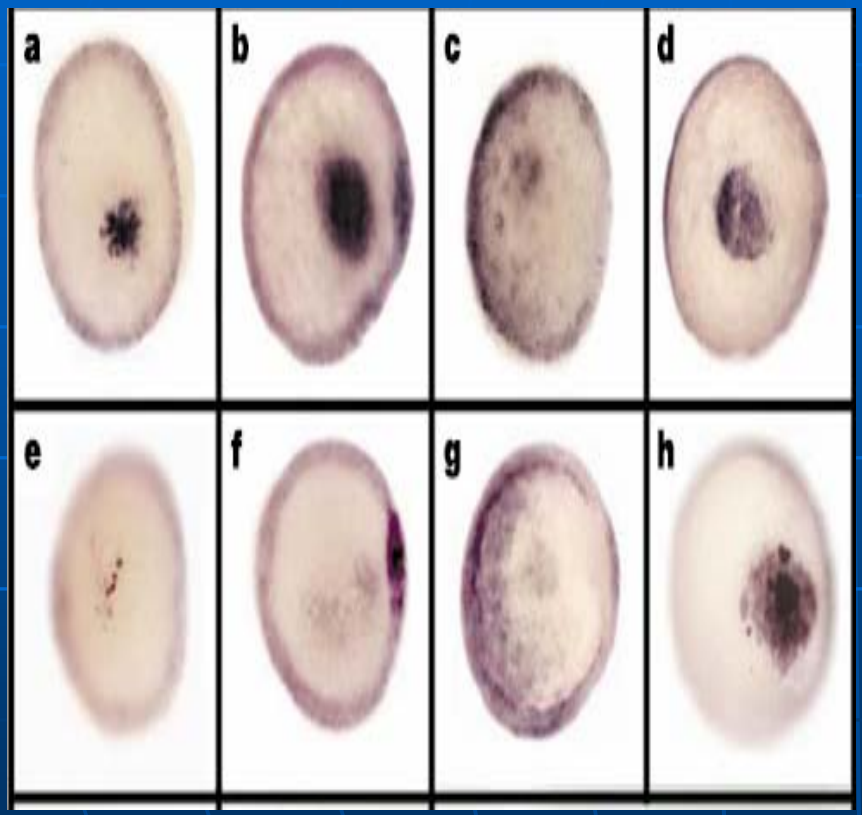
- gfp labelling shows injected cells; non-labelled are recruited animal pole cells
- I. Induced tail contains muscle (labeled with myoD)
- J. Induced tail contains no axial structures (shh)

Cross-regulation of BMP, Nodal, Wnt8



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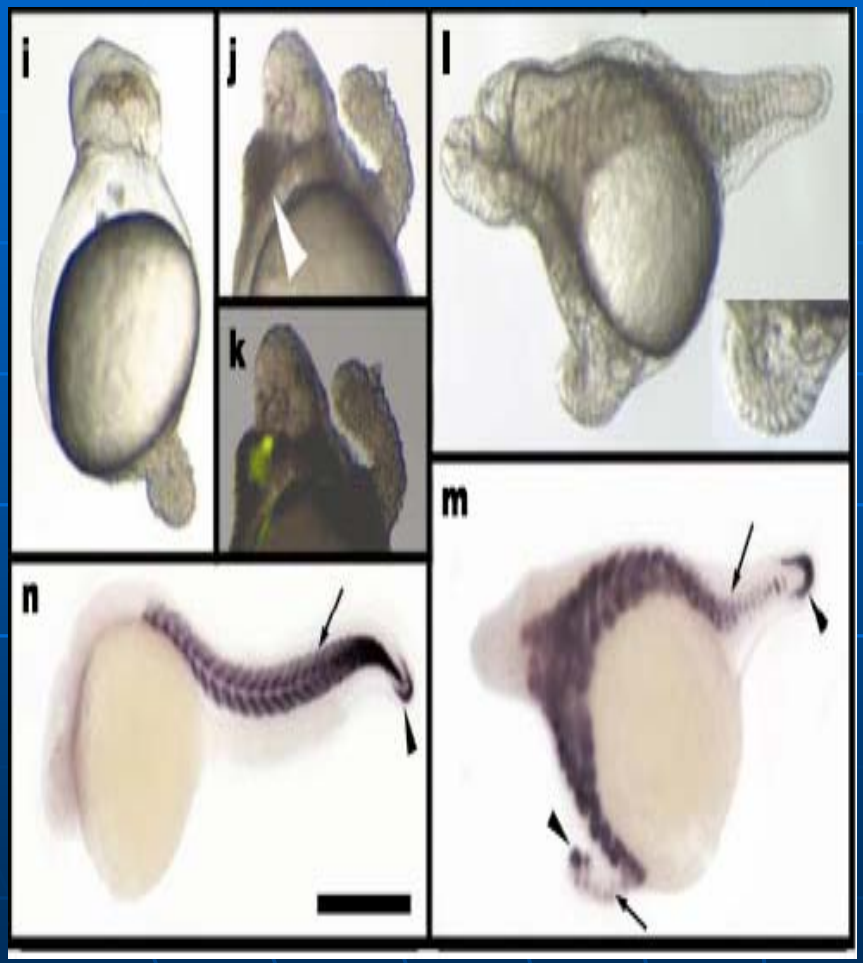
- BMP alone does not influence Nodal or Wnt8 expression
- Injection of znr2 (Nodal) in animal pole results in induction of A) znr2; B) znr1; C)bmp2 and D) wnt8
- Injection of wnt8 in animal pole: induction of E) znr1; F) znr2; G) bmp2 and H) wnt8
- BMP with either Nodal or Wnt8 leads to tail formation→ BMP necessary but not sufficient for tail dev.

Nodal signaling is necessary for ectopic tail induction; ectopic tail induction independent of dorsal organizer



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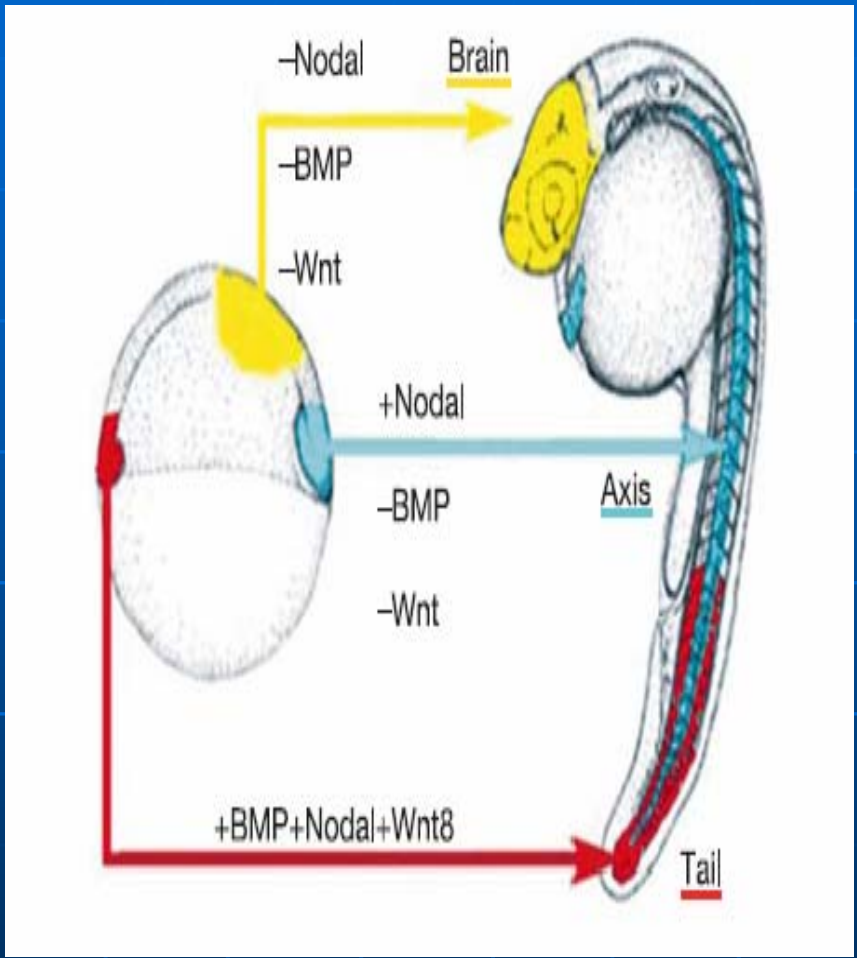
- I. Complete inhibition of Nodal signaling by injection of antivin (competitive inhibitor of Nodal)
- J. Antivin cells injected into animal pole blastomere at 128 day stage with bmp/wnt8/gfp RNAs
- K. GFP-labeled injected cells located close to head; ectopic tail is NOT induced
- L-n. Embryo devoid of dorsal organizer through BMP overexpression is injected into an animal pole blastomere at 128 cell stage w/ bmp4/znr1 RNAs;
- m—stained with eve1 (arrowhead) and myoD(arrow); note 2ndary tail in comparison with wildtype n

Recap of the evidence

- Ventral, not dorsal, marginal cell transplants to the naïve animal pole can induce ectopic tail formation→suggests ventral tail organizer
- Ectopic tail formation can be induced by the injection of an embryo lacking a dorsal organizer into an animal pole blastomere→ tail organizer independent of dorsal organizer
- BMP signaling is necessary but not sufficient for tail induction
- Misexpression of BMP + Nodal, BMP +Wnt8, and BMP + Nodal + Wnt8 in animal pole of blastomere can induce ectopic tail formation
- Nodal and Wnt8 cross-regulate; loss of function of Nodal or Wnt8 pathway prevents tail development

Tail Development Conclusions



- There is a tail organizer, and tail induction results from triple stimulation of BMP, Nodal, and Wnt8 signaling pathways

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Questions