# Anterior/Posterior Axis Formation

Developmental Biology 7.72

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## The Anterior/Posterior Axis

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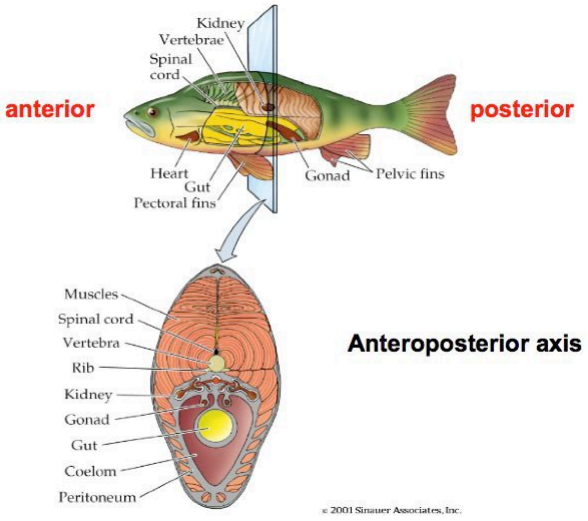
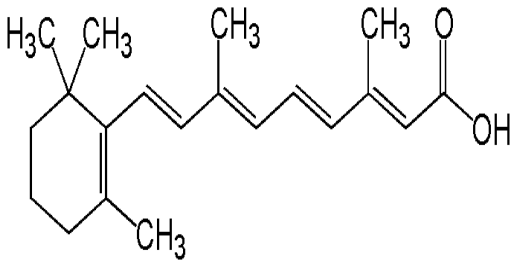
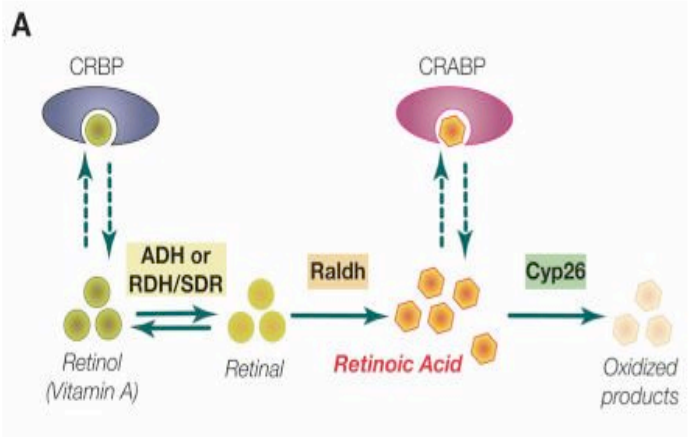


# Retinoic Acid

- Regulates gene expression in numerous cell types
- RA plays a crucial role in embryogenesis
- Dysregulation of RA levels leads to severe malformation

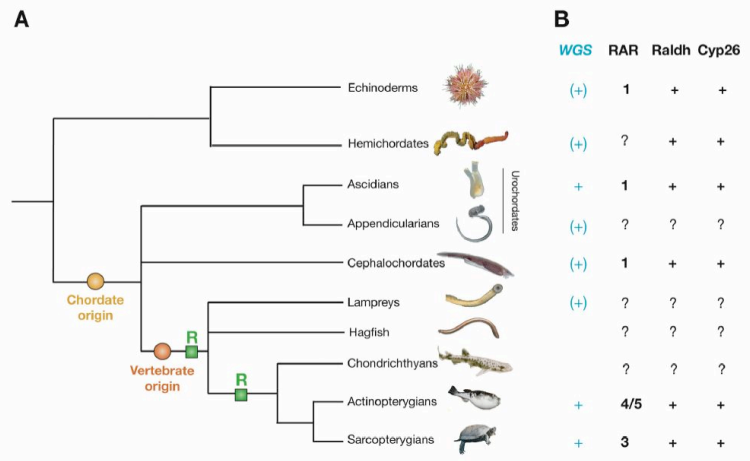



# Regulation of Retinoic Acid Levels

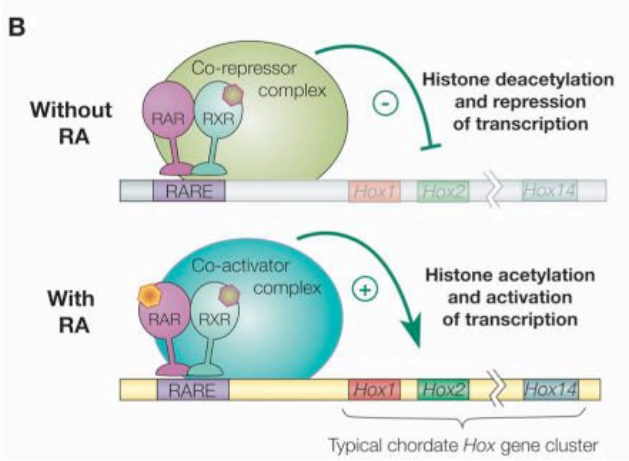



Marletaz et al. (2006)

# Deuterostome Phylogeny and Conservation of RA Signaling Components

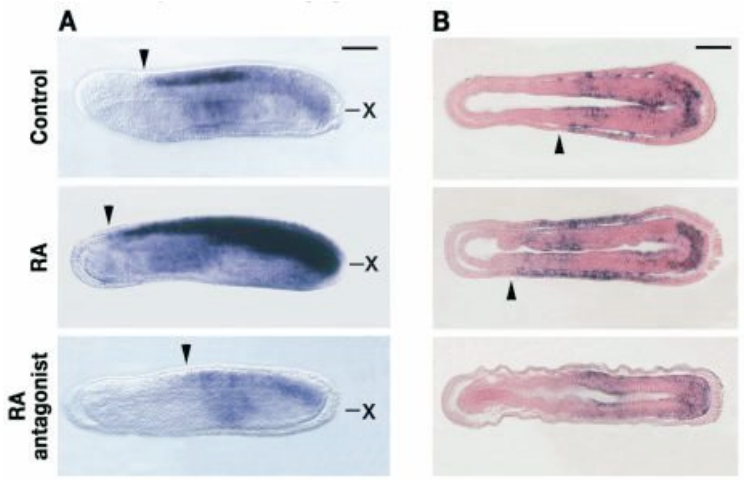



# Retinoic Acid Signaling Pathway




Marletaz et al. (2006)

# Retinoic Acid Induces Posteriorization




Marletaz et al. (2006)

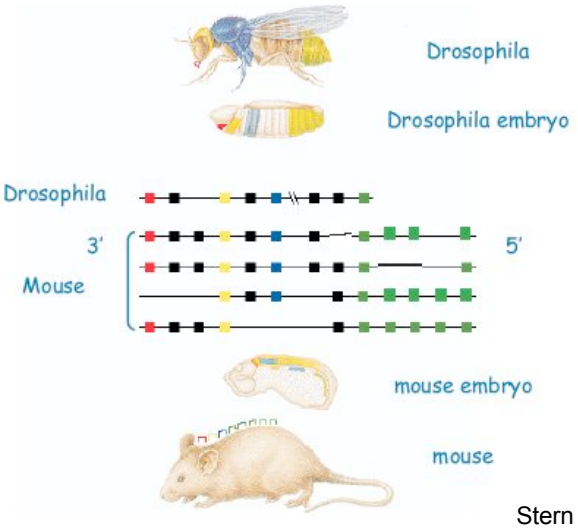
# Maden et al (1989)

- CRABP found in ecto-,mesodermal cells (RA?)
- [RA] high in neural crest region
- Likely secreted from dorsal blastopore lip
- Nieuwkoop center indirectly involved in RA regulation
- Homoebox genes

# Cracking the Hox Code

- Hox genes encode TFs that regulate embryonic development
- Its responsiveness corresponds with its location within the gene cluster (4 + 13)
- 3' end genes= low RA
- 5' end genes= high RA
- Expression pattern of Hox genes correspond linearly with gene order along DNA***
- time of onset + code = pos. address

# Chromosomal Organization and Schematic Expression of *Hox* Clusters




Stern et al. (2006)

# Distinct Roles for Fgf, Wnt and Retinoic Acid in Posteriorizing the Neural Ectoderm

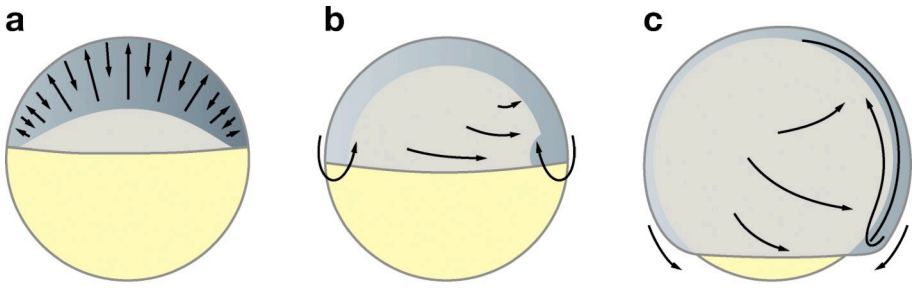
T. Kudoh, S. Wilson, and I. Dawid

*Development* 129:4335-4346 (2002)

## Definitions

- **Epiboly** = Ectodermal cell movement during gastrulation around the yolk
- **Blastoderm Margin** = Interface between the blastoderm and the yolk
- **Negative Feedback Loop** = The ability of a signaling molecule to downregulate its own expression by activating its own inhibitor

# Gastrulation in Zebrafish




Schier AF, Talbot WS. 2005.
Annu. Rev. Genet. 39:561–613

**Figure 2** Gastrulation movements. (*a*) Dome stage. Cells intercalate radially, contributing to epiboly. (*b*) Shield stage. Cells at the margin internalize and migrate toward the animal pole. Cells converge dorsally, with lateral mesodermal cells starting convergence at later stages than cells closer to the shield. (*c*) 90% epiboly stage. Epiboly, internalization, convergence and extension continue. Modified from Reference.

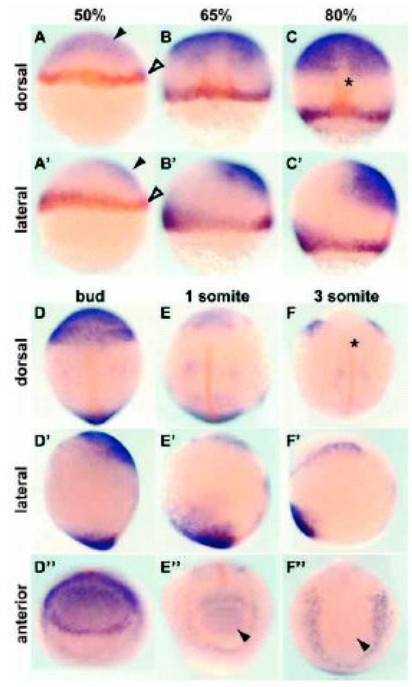



**Fig. 1.** Expression of *cyp26* at gastrula to early somitogenesis stages. Views of whole embryos (orientation indicated to the left of each row) at the stages indicated on top of each column. *cyp26* was stained by in situ hybridization (purple), while *ntl* (red) was used to mark the blastoderm margin (A, A′, open arrowhead) and developing notochord (C,F, asterisk). *cyp26* is expressed in the presumptive anterior neural ectoderm (A,A′ filled arrowhead) and at the blastoderm margin (A,A′ open arrowhead) throughout gastrulation (B-D). Subsequently, expression in the anterior neural ectoderm decreases rapidly (E″, F″ filled arrowhead), while expression continues in the tail bud.

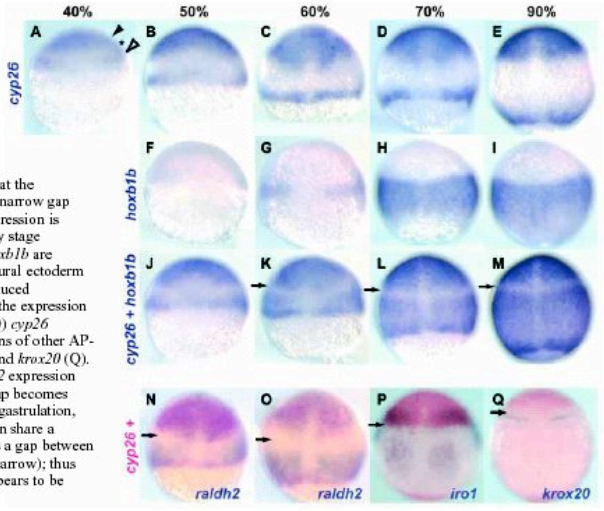
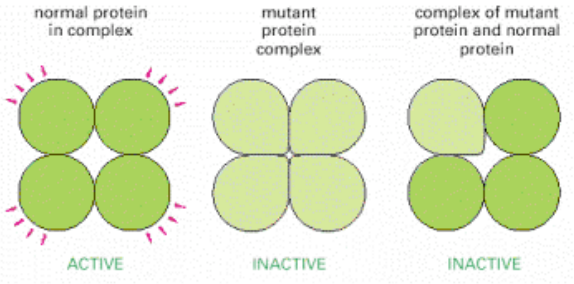



**Fig. 2.** *cyp26* and *hoxb1b* expression domains define an early AP boundary within the prospective neuroectoderm. Dorsal views of whole embryos at the stages indicated on top of. Genes analyzed are indicated at the left of the column or in the figure, with text color matching the in situ stain. *cyp26* expression starts at the 30 to 40% epiboly stage (A) in presumptive anterior neural ectoderm (filled arrowhead) and at the blastoderm margin (open arrowhead), leaving a narrow gap (asterisk). As gastrulation proceeds, *hoxb1b* expression is initiated within the gap at about the 60% epiboly stage (C,G,K). From this time onwards, *cyp26* and *hoxb1b* are expressed in a complementary manner in the neural ectoderm throughout gastrulation; a narrow domain of reduced expression is observed at the interface between the expression domains of these two genes (K-M, arrow). (N-Q) *cyp26* expression (red) in relation to expression domains of other AP-specific genes (purple), *raldh2* (N,O), *iro1* (P) and *krox20* (Q). There is a narrow gap between *cyp26* and *raldh2* expression domains at early gastrula (N, arrow), and this gap becomes wider by late gastrula (O, arrow). At the end of gastrulation, *cyp26* and the anterior domain of *iro1* expression share a common posterior boundary (P, arrow). There is a gap between *cyp26* and rhombomere3 staining of *krox20* (Q, arrow); thus the posterior boundary of *cyp26* at this stage appears to be positioned around rhombomere 1.

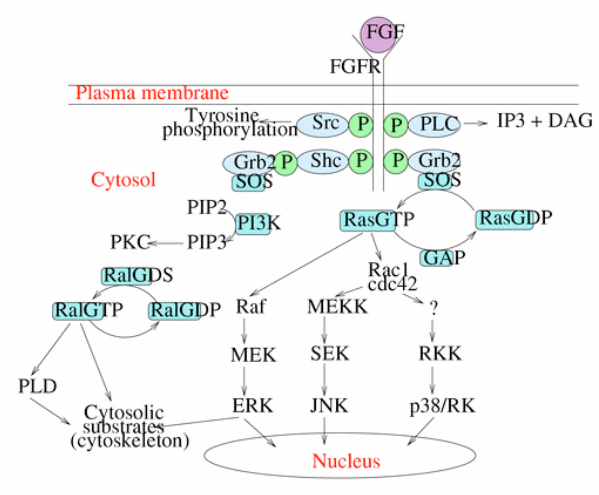
# Dominant-Negative Mutants

- A dominant mutation that blocks the activity of a wild type allele at the same gene (i.e., the mutant blocks wt activity)




Alberts et al., 4th edition

# FGF Signaling




Lloyd (2006)

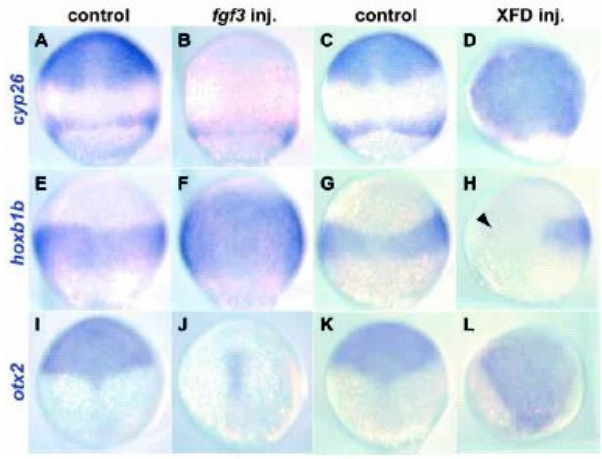



**Fig. 3.** Fgf signaling alters the expression of early AP-specific genes. Dorsal views of whole embryos at late gastrula stage. One- to two-cell embryos were injected with mRNAs for *fgf3* (B,F,J) or RNA encoding the dominant negative Fgf receptor, XFD (D,H,L). Embryos were stained either with *cyp26* (A-D), *hoxb1b* (E-H) or *otx2* (I-L). The anterior expression of *cyp26* and *otx2* was suppressed by Fgf3 (B,J) and expanded in a posterior direction by XFD (D,L). Expression of the posterior gene *hoxb1b* was expanded by Fgf3 (F) and suppressed by XFD (H, arrowhead). The entire embryo is affected by injection at the one-cell stage (B), while in some cases only half the embryo is affected when one of two cells is injected (H).

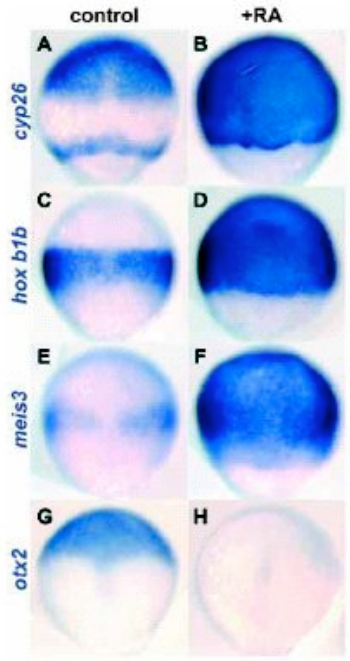



**Fig. 4.** RA alters the expression of early AP-specific genes. Dorsal views of whole embryos at late gastrula stage. Embryos at the 40% epiboly stage were treated with $10^{-6}$ M RA for 80 minutes. The embryos were fixed at the 80 to 90% epiboly stage, and stained with *cyp26* (A,B), *hoxb1b* (C,D), *meis3* (E,F) or *otx2* (G,H). The posterior genes *hoxb1b* and *meis3* were ectopically induced in the anterior region by RA (B,D), while the anterior gene *otx2* was suppressed (H). Although *cyp26* is expressed in the anterior region, its expression was activated by RA(B).

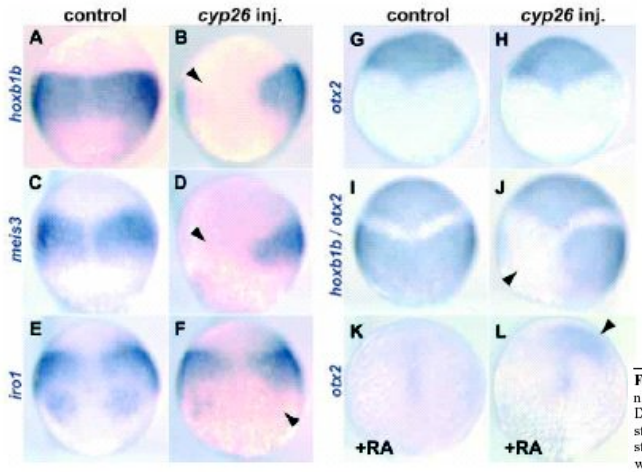



**Fig. 5.** Cyp26 can suppress posterior genes but does not induce anterior genes in the posterior region. Dorsal views of whole embryos at late gastrula stage. *cyp26* mRNA was injected at the two-cell stage into one blastomere. Some of the embryos were treated with RA (K,L). Embryos were stained for *hoxb1b* (A,B), *meis3* (C,D), *iro1* (E,F), *otx2* (G,H,K,L) and *otx2/hoxb1b* together (I,J). *hoxb1b*, *meis3* and the posterior domain of *iro1* were suppressed by *cyp26* injection (B,D,F,J, arrowhead), but *otx2* and the anterior domain of *iro1* expression were neither suppressed nor expanded (F,H,J). The suppression of *otx2* by RA was partially rescued by *cyp26* (L, arrowhead).

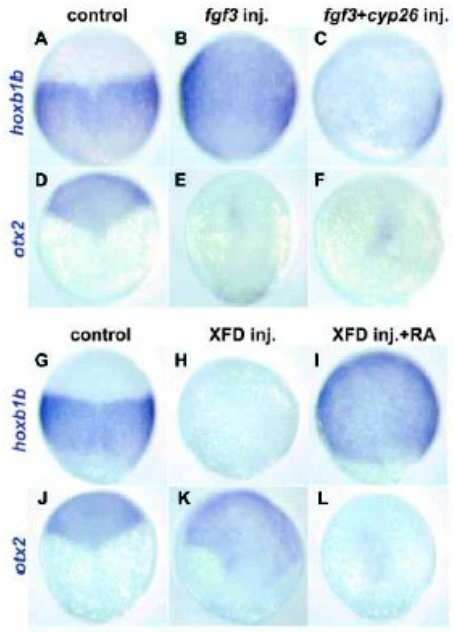
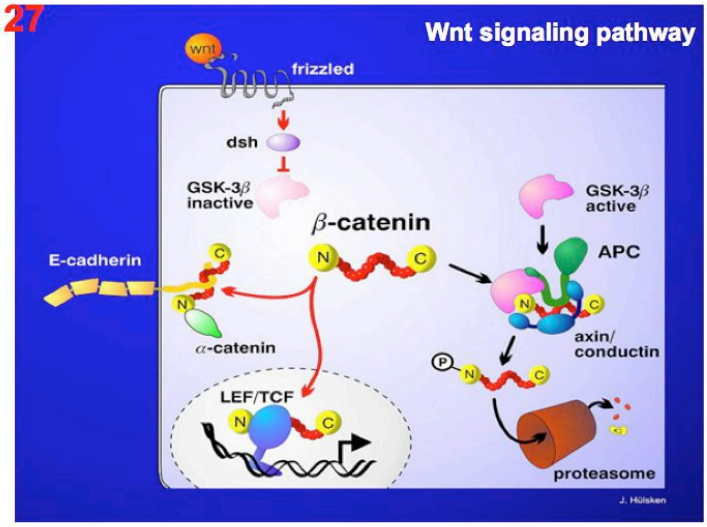



**Fig. 6.** Epistatic analysis of the function of Fgf and RA in patterning the neural ectoderm. Dorsal views of whole embryos at late gastrula stage. RNA for *fgf3*, alone or together with *cyp26* RNA, was injected at the one-cell stage. The embryos were stained for *hoxb1b* (A-C) or *otx2* (D-F). XFD mRNA was injected at the one-to-two cell stage, and half of the embryos were treated with RA. Embryos were stained for *hoxb1b* (G-I) or *otx2* (J-L).




LiCl = Activates Wnt signaling by inhibiting GSK3, thus stabilizing β-catenin

Dkk1 overexpression = Inhibits Wnt signaling by antagonistically binding to Wnt co-receptors (Lee et al., 2004)

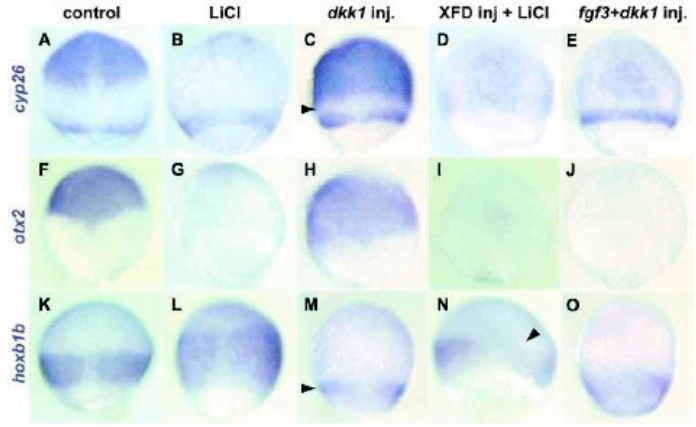



**Fig. 7.** Epistatic analysis of the function of Wnt and Fgf in patterning the neural ectoderm. Dorsal views of whole embryos at late gastrula stage. Gain and loss of function of Wnt activity was achieved by LiCl treatment (B,G,L) and *dkk1* injection (C,H,M), respectively. Epistasis with Fgf was examined by *XFD* injection followed by LiCl treatment (D,I,N) and by *fgf3 +dkk1* co-injection (E,J,O). Embryos were fixed at late gastrula and stained with *cyp26* (A-E), *otx2* (F-J) and *hoxb1b* (K-O). Arrowheads in C and M indicate the limit of caudal expansion of *cyp26* expression (C) and remaining *hoxb1b* expression (M). In some cases, only half the embryo is affected when one of two cells is injected (N, arrowhead).

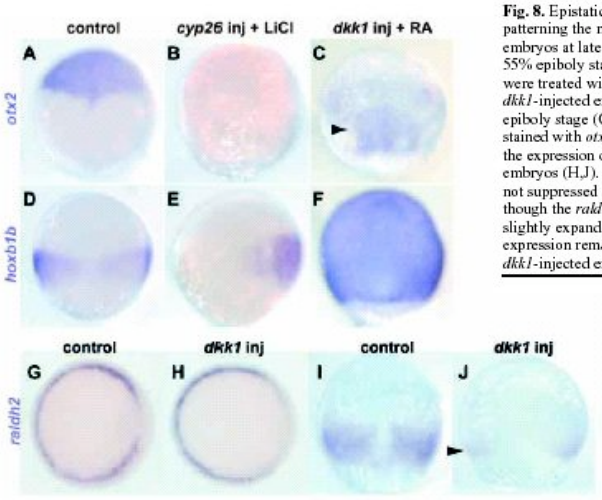
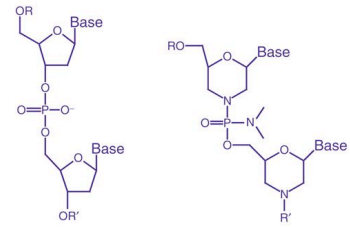



**Fig. 8.** Epistatic analysis of the function of Wnt and RA in patterning the neural ectoderm. Dorsal views of whole embryos at late gastrula stage (A-F,I,J), and animal views of 55% epiboly stage embryos (G,H). *cyp26*-injected embryos were treated with LiCl at the 50% epiboly stage (B,E), and *dkk1*-injected embryos were treated with RA at 40% epiboly stage (C,F). Embryos were fixed at late gastrula and stained with *otx2* (A-C) and *hoxb1b* (D-F). Regulation of the expression of *raldh2* was examined in *dkk1*-injected embryos (H,J). At 55% epiboly stage, *raldh2* expression is not suppressed at the blastoderm margin by *dkk1* injection though the *rakdh2* negative area in the dorsal-most margin is slightly expanded (H). At late gastrula stage, *raldh2* expression remains restricted to the blastoderm margin in *dkk1*-injected embryos (J, arrowhead).

# Morpholino Antisense Oligonucleotides

- DNA analogs that that use Watson-Crick basepairing to sequester a specific mRNA, thus preventing its translation
- Knock-down approach




Phosphodiester DNA

Morpholino

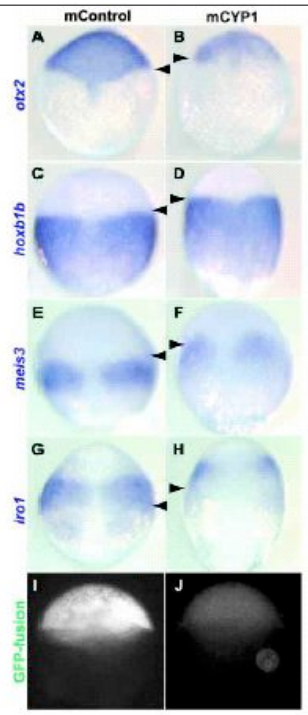



**Fig. 9.** Abrogation of Cyp26 activity causes moderate posteriorization. Dorsal views of whole embryos at late gastrula stage (A-H) and lateral views of late blastula stage embryos (I,J). A control morpholino or mCYP1, which is complementary to *cyp26* mRNA sequence, was injected at 5 ng per embryo. Embryos were stained with *otx2* (A,B), *hoxb1b* (C,D), *meis3* (E,F) and *iro1* (G,H). In addition, cyp26-GFP fusion construct was injected with either morpholino, and fluorescence was examined. (I,J). (A,C,E,G,I) Control morpholino; (B,D,F,H,J) mCYP1-injected embryos.

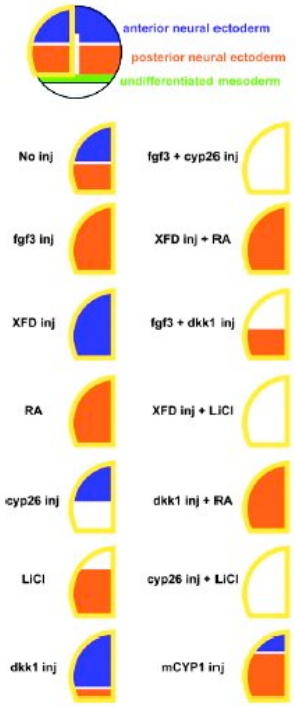



**Fig. 10.** Summary of experiments. The experimental results are summarized as a cartoon. *otx2* and *hob1b* are used as representative markers of anterior and posterior neural ectoderm, respectively, as these genes were examined in all of experiments. The top picture is a dorsal view of a late gastrula embryo divided into anterior (purple) and posterior (red) neural ectodermal regions and undifferentiated mesendoderm at the blastoderm margin (green). One half of the dorsal ectoderm is surrounded by yellow line and experimental results are summarized in this area as marker genes for mesoderm and axial area were not analyzed in any detail in this study.

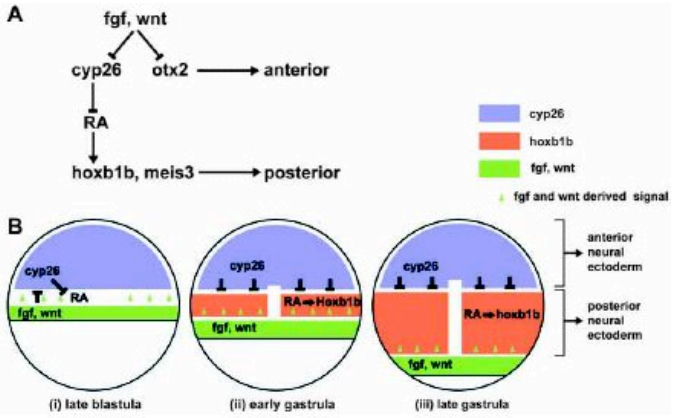
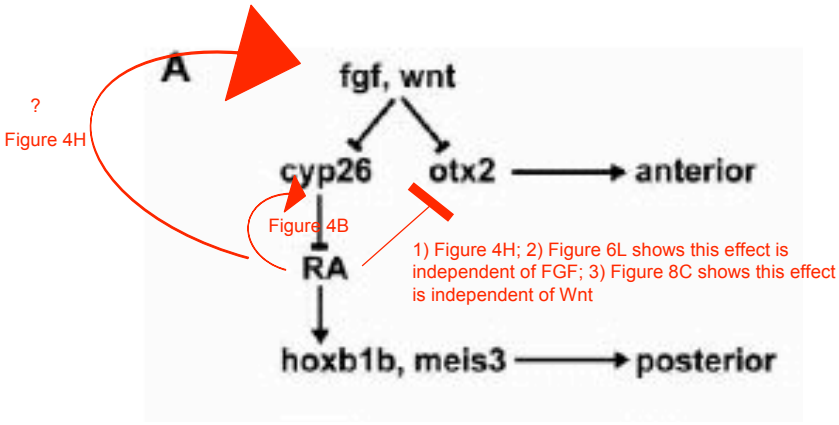
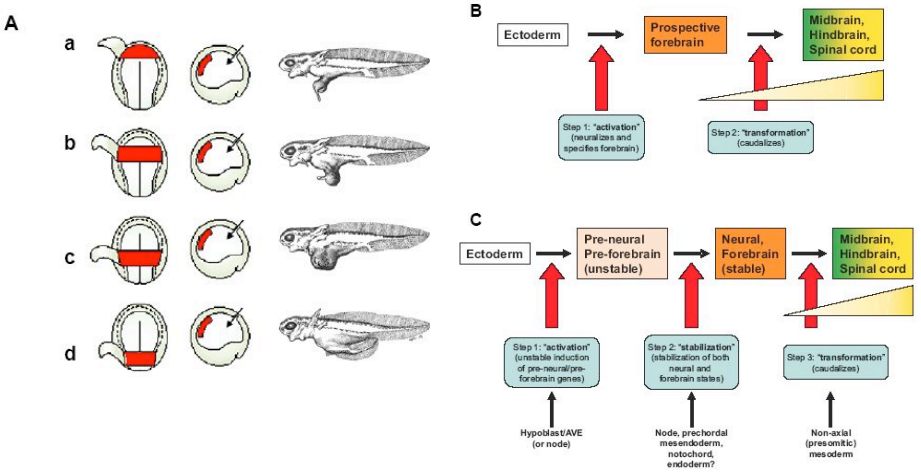



**Fig. 11.** A model for interactions between Fgf, Wnt and RA signaling in the neural ectoderm during gastrulation. (A) Sequence of posteriorization signals. Fgf and/or Wnt signals initiate the first step of posteriorization by suppressing expression of anterior genes, represented here by *cyp26* and *otx2*. This process is not mediated by RA. In the posterior domain, where *cyp26* expression is suppressed by Fgfs/Wnts, RA accumulates at least in part due to the activity of Raldh2, and activates posterior genes such as *hoxb1b* and *meis3*. (B) At the late blastula stage, Fgfs/Wnts are expressed in the mesoderm at the blastoderm margin. Fgf/Wnt signals from the margin block the expression of *cyp26* in the adjacent ectoderm, which will give rise to the posterior neural plate. *cyp26* is expressed in the anterior domain, at a distance from the source of the Fgf/Wnt signals; as a consequence, RA is degraded and the expression of posterior genes is prevented. After the beginning of gastrulation, convergence-extension movements lead to a widening of the *cyp26*-negative area, allowing RA to accumulate to a level where it can activate posterior genes such as *hoxb1b*. Subsequent cell movements expand the domain that will give rise to the posterior neural ectoderm.




In red: additional relationships suggested by this study.
The Figure numbers refer to the particular data that suggest each relationship. Relationships which not have been explicitly demonstrated, but nonetheless are consistent with the authors' data, are indicated with a "?".

# 3 Models to Explain A/P Patterning




Stern et al. (2006)