Anterior/Posterior Axis Formation

Developmental Biology 7.72

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The Anterior/Posterior Axis
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


- 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

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**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

(a) Dome stage. Cells intercalate radially, contributing to epithelium. (b) Shield stage. Cells at the margin intercalate and migrate toward the shield. 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 cyc26 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. cyc26 was stained by in situ hybridization (purple), while alf (red) was used to mark the blastoderm margin (A, A', open arrowhead) and developing notochord (C, F, asterisk). cyc26 is expressed in the presumptive anterior neural ectoderm (A, A') filled arrowheads and at the blastoderm margin (A, A', open arrowhead) throughout gastrulation (B-D). Subsequently, expression in the anterior neural ectoderm decreases rapidly (F, F', F'' filled arrowheads), while expression continues in the tail bud.
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)
FGF Signaling

Lloyd (2006)

Fig. 3. Fgf signaling alters the expression of early AP-specific genes. Dorsal views of whole embryos at late gastrula stages. One- to two-cell embryos were injected with in RNA. (A-D) or RNA encoding the down-regulated Fgf receptor, XFD (E-L). Embryos were stained with anti-6 (A-D), anti-1 (E-H) or anti-2 (I-L). The anterior expression of cyp76 and cat2 was suppressed by Fgf (A), and expanded in a posterior direction by XFD (B). Expression of the posterior gene head/10 was expanded by Fgf (F) and suppressed by XFD (H). 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 0.5 mM RA for 30 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 arx2 (G,H). The posterior genes hoxb1b and meis3 were ectopically induced in the anterior region by RA (B,D), while the anterior gene arx2 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), arx2 (E,F) and arx2 (Hoxb1b together) (I). hoxb1b, meis3 and the posterior domain of arx2 were suppressed by cyp26 injection (B,D,E), whereas, hoxb1b and the anterior domain of arx2 expression were not suppressed by cyp26 injection (C,F). The suppression of arx2 by RA was partially rescued by cyp26 (L, arrowhead).
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,C,E) and XFD injections (C,E,M), respectively. Epistasis with Fgf was examined by XFD injection followed by LiCl treatment (D,L,M) and by fgf3+XFD coinjection (H,L,M). Embryos were fixed at late gastrula and stained with cyp26 (A,C,Ex1; arrowhead,F,J) and hand2 (K-O). Arrowheads in C and M indicate the limit of neural expansion of cyp26 expression (C) and remaining hand2 expression (M). In some cases, one half of the embryos is affected when one of two cells is injected (K, arrowhead).

Fig. 8. Epistatic analysis of the function of Wnt and Rha in patterning the neural ectoderm. Dorsal views of whole embryos at late gastrula stage (A-E,L), and lateral views of 57% epiboly stage embryos (G,J). cyp26-injected embryos were treated with LiCl at the 50% epiboly stage (B,E), and dipl2-targeted embryos were treated with RA at 40% epiboly stage (C,F). Embryos were fixed at late gastrula and stained with cyp26 (A,C) and hand2 (D,F,G). Regulation of the expression of hand2 was examined in dipl2-targeted embryos (H). At 57% epiboly stage, cyp26 expression is not suppressed at the blastoderm margin by dipl2 injection, though the hand2-negative area in the dorsal-most margin is slightly extended (H). At late gastrula stage, cyp26 expression remains restricted to the blastoderm margin in dipl2-targeted embryos (F, arrowhead).
Morpholino Antisense Oligonucleotides

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

Fig. 6. 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 orth (A,B), foxβ (C,D), mesod (E,F) and irf (G,H). In addition, esp26-GFP fusion construct was injected with either morpholino, and fluorescence was examined (I,J). (A,C,E,G): Control morpholinos (B,D,F,H) mCYP1-injected embryos.
Fig. 10. Summary of experiments. The experimental results are summarized as a cartoon. Notch and hoxb1b 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 color) neural 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 the 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 increased by Fgf/Notch/Wnt, RA accumulation is at least in part due to the activity of Hoxb1b, and activates posterior genes such as hoxb1b and meh3. (B) At the late blastula stage, Fgf/Notch/Wnt are expressed in the mesoderm at the blastoderm margin. Fgf/Wnt signals from the animal pole, 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 preserved. After the beginning of gastrulation, a convergence-extension movement leads 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

A
a
b
c
d

B
Ectoderm
Prospective Neural

C
Ectoderm
Pre-neural
Forebrain (sensitized)

Stern et al. (2006)