

Chapter 4: Patterning the vertebrate body plan II: the mesoderm and early nervous system

Overview: In this chapter, the antero-posterior patterning of the mesoderm and the central nervous system are discussed. Think of your own body and the relationship between your vertebral column, which derives from paraxial mesoderm, and your spinal cord, which is enclosed within it. Clearly, their development must be intimately related. The coordination of their development culminates in the phylotypic stage. At the phylotypic stage, all vertebrates exhibit a remarkably similar organization, despite diverse strategies for earlier development, and a diversity of final forms once their development has been completed. This implies that common mechanisms are at play in all vertebrates at the phylotypic stage. Indeed, the expression patterns of the Hox genes along the antero-posterior axis are virtually a molecular description of this point in vertebrate development, and have long been a focus of research into the common mechanisms involved in patterning the vertebrate body plan. We will see that the Hox gene products specify the identity of structures along the antero-posterior axis: neck versus thorax, hind brain versus spinal cord, and so on. We will also see that the Hox gene expression patterns are themselves controlled by an interplay of cell-cell signaling events and changes in gene expression, which establishes a segmental periodicity along the axis, reminiscent of the strategy by which the insect body plan is organized.

Study tip: Despite the similarities between all vertebrates, differences do exist. As you read the chapter, note carefully whether reference is being made to chicks or frogs, to help you avoid confusion. It may be useful to read the chapter twice, focusing on *Xenopus* during the first reading, then add to your knowledge during a second reading by focusing on chicks.

Keywords: Briefly compare and contrast the following pairs of terms. Check your answer by using the glossary and the text.

axial mesoderm / pre-somitic mesoderm
dermomyotome / sclerotome
myotome / dermatome
homeodomain / homeobox
homolog / paralog
homeosis / apoptosis

Factual recall questions:

- The central nervous system is derived from _____, the axial skeleton is derived from _____, and the muscles of the trunk are derived from _____?
(a) ectoderm, mesoderm, endoderm
(b) mesoderm, endoderm, endoderm
(c) ectoderm, mesoderm, mesoderm
(d) all are derived from mesoderm
(e) all are derived from ectoderm
- What would be the effect on the timing of somite formation, if a piece of the pre-somitic mesoderm of a chick embryo is rotated by 180° and reinserted in its original position?
(a) there would be no effect because the embryo would regulate and development would proceed normally
(b) antero-posterior development would be blocked at that point, and more posterior structures would fail to form
(c) the rotated mesoderm would degenerate and a gap would form in the antero-posterior pattern
(d) the rotated mesoderm will reverse the gradient of somite formation, so that somites formation posterior to the rotated mesoderm will begin in the posterior and move forward to meet the previously formed somites
(e) the timing of somite formation will be reversed in the rotated block only, proceeding from posterior to anterior, but the rest of the somites will form in a normal fashion, proceeding from anterior to posterior
- Delta is a cell-surface bound ligand, whose receptor is Notch. Would mutations in *Delta* be predicted to have effects similar to, or different from, those in *Notch*, and why?
(a) Similar, since the two proteins cooperate to activate the same gene expression pathway
(b) Different, since mutations in two different proteins will always produce two different phenotypes
(c) Similar, since the two proteins are involved in somite formation, although they do this through independent pathways
(d) Different, since Delta and Notch are present on separate cells
(e) Similar, since all mutations that affect development cause similar defects
- The effect of grafting an extra piece of notochord into a dorsal position adjacent to pre-somitic mesoderm is

- (a) the pre-somitic mesoderm will degenerate
 - (b) somites will form but fail to differentiate
 - (c) the majority of the somite will be specified as sclerotome
 - (d) the somite will be converted almost entirely to muscle
 - (e) the somite is incorporated into the neural tube
5. Hox genes
- (a) encode transcription factors which specify position along the anterior-posterior axis in vertebrates
 - (b) make signaling molecules used during somite formation
 - (c) control the formation of vertebrae in mice, but are not found in other animals
 - (d) are expressed such that the low-numbered genes, a1, b1, and so on, are expressed in the posterior regions, whereas high-numbered genes are expressed in more anterior regions
 - (e) are expressed only in the mesoderm
6. The mutation of genes in the production of "knock-out" mice is accomplished by
- (a) chemical mutagenesis of adult mice
 - (b) radiation treatment of cultured mouse embryos
 - (c) homologous recombination in ES cells
 - (d) injection of genes into mouse blastocysts
 - (e) breeding experiments between different mouse lines
7. The grafting of the dorsal lip of the blastopore from a late *Xenopus* gastrula onto the ventral side of an early embryo will result in
- (a) the formation of two separate and independent embryos
 - (b) the formation of two complete embryos joined along the ventral axis
 - (c) the formation of two sets of anterior structures joined along the ventral axis: a two-headed embryo
 - (d) the formation of two sets of posterior structures joined along the ventral axis: a two-tailed embryo
 - (e) no effect: only dorsal lips from early embryos have organizer activity
8. The avian structure equivalent in developmental activity to the *Xenopus* organizer is the
- (a) embryonic shield
 - (b) primitive streak
 - (c) notochord
 - (d) Henson's node
 - (e) head fold
9. Neural tissue can be experimentally induced in non-neural *Xenopus* ectoderm by
- (a) transplant of non-neural ventral ectoderm into a dorsal region of presumptive neurectoderm
 - (b) transplant of organizer tissue or notochord to the ventral side of a recipient embryo
 - (c) injection of *gooseoid* mRNA into the ventral side of an early frog embryo
 - (d) inhibition of BMP-4 by addition of chordin or noggin proteins to cultured blastula animal caps
 - (e) all of the above

Concept questions:

1. Examine Figure 2.2 and recall what is meant by the phylotypic stage. Identify the somites. Where is the central nervous system: is it yet enclosed by the bony skull and vertebral column? Now move to Figure 2.3 and identify the stage of development we had reached by the end of Chapter 3. If fertilization had occurred at dawn, what time would be at the end of Chapter 3? What will be the time when the phylotypic stage is reached? What events must occur during this long night, about which we have yet to learn?
2. Figure 4.2 illustrates an experiment which explores the effect of manipulating the pre-somitic mesoderm on the timing of somite formation along the antero-posterior axis. In contrast, how would you describe the experiment shown in Figure 4.5? Putting Figures 4.2 and 4.5 together, hypothesize on the result of rotating pre-somitic mesoderm, with regard to positional identity along the antero-posterior axis. Examine Figure 4.5 carefully: what structures might you attempt to target in your rotation experiment to best demonstrate your point, and why would you choose these particular structures?
3. Describe the origin and movements of the cells which will become the somitic mesoderm. What other mesodermal structure is forming from cells that leave Henson's node during retraction of the primitive streak?
4. Cells which make up somite number 22 in the chick will form thoracic vertebrae, with ribs. Describe an experiment which would allow you to demonstrate this point, including the experimental methods (Hint: refer to Figure 4.6 in your

description of the method).

5. Complete the following sentence: The dermatome forms in the (dorsal/ventral, medial/lateral) portion of the somite in response to signals from (epidermis, notochord, neural tube, lateral plate mesoderm) in the form of (Wnt, BMP-4, Shh); these signals lead to the expression of the transcription factor (Pax 1, Pax 3, myoD) and subsequent formation of (dermis, axial muscle, limb muscle, vertebrae and ribs). Use this sentence as a guide to fill in the following table:

	location	signaled by	signaling molecule	transcription factor	structures formed
dermatome					
medial myotome					
lateral myotome					
sclerotome					

6. Speculate on the consequences of the following two experiments: In the first experiment, a newly formed somite is rotated 180°, so that its presumptive dorsal side is now ventral and vice versa. In the second experiment, the neural tube and notochord are rotated so that the notochord is dorsal and the neural tube lies ventral to it.

7. The "combinatorial model" for Hox gene activity would say that each cell's identity is defined by the combination of Hox genes being expressed in that cell. Thus, we might describe a cell which is fated to form a cervical vertebra as a "a1-a4-a5-b1-b4-b5-d3-d4" cell (see Figure 4.12). Using this notation, "name" typical thoracic, lumbar, sacral, and caudal cells. What patterns emerge in these names, as you move from anterior to posterior?

8. Describe the organization of the Hox genes in clusters and paralogous groups on the chromosomes, and the correlation of that organization to their expression along the antero-posterior body axis.

9. Describe three lines of evidence that Hox genes specify cell identity: expression patterns in different species, mouse knock-outs, and overexpression experiments.

10. Contrast the effects you might expect of a knock-out of *Hoxc6* versus the ectopic expression of *Hoxc6* in cervical somites. Use the term "homeotic transformation" in your answer. For which of these experiments might the functional redundancy of Hox genes in separate clusters influence your predicted results, and what would you do to address this?

11. Integrate your knowledge of somite formation, colinearity of Hox cluster organization and Hox gene expression, and Hox gene control of somite identity along the antero-posterior axis, with the facts presented in Section 4.5 regarding retinoic acid. Propose a coherent model for patterning of somites along the axis. Propose an experiment that would test your model.

12. Examine the embryo depicted in Figure 4.17. Has this embryo yet established an anterior-posterior axis? How would you describe the stage of development this embryo has reached? What experimental protocol was used to derive the information shown in the figure?

13. Recall what you have learned about the role of the *Xenopus* organizer in patterning the dorsal ventral axis through the production of chordin and the inhibition of BMP activity. Relate that story to the role of the organizer in patterning the anterior-posterior axis, with special reference to cerberus and the inhibition of WNTs as well as BMPs. What happens when only WNTs are blocked? Hypothesize on a gradient of activities that would generate the antero-posterior pattern shown in Figure 4.17.

14. Contrast the "two-signal" model (the "activation-transformation" model) for neural patterning with the "graded signal" model. Which is considered most likely? What signals appear to be involved? (Keep in mind as you think this through that the WNTs are actually a family of separate proteins, which may have different activities from each other.)