

# 5. MESODERM FORMATION / SEGMENTATION

**Dr. Ann -Judith Silverman**

Department of Anatomy & Cell Biology

Telephone: 305-3540

E-mail: as36@columbia.edu

**READING ASSIGNMENT:** Larsen Human Embryology, 3rd Edition, pp. 79-85, 102-110.

## **SUMMARY:**

During the third and fourth weeks of embryonic development the mesoderm is established as the 2nd germ layer. The mesodermal cells are organized into 4 regions: the axial mesoderm of the prechordal plate and notochord, paraxial mesoderm, intermediate mesoderm and lateral plate mesoderm. Each of these undergoes some form of segmentation. The most evident and complete segmentation occurs in the paraxial trunk mesoderm, where each segment becomes an entirely separate somite. Much of the paraxial and lateral plate mesoderm develops into mesenchyme, an embryonic connective tissue. The derivatives of mesenchyme are connective tissue proper, cartilage, bone and blood. The cardiovascular and lymphatic systems are derived from mesoderm as well. Part of the paraxial mesoderm gives rise to all skeletal muscle cells. The intermediate mesoderm gives rise to most of the urogenital system. Part of the lateral plate mesoderm develops into the lining of the pericardial, pleural and peritoneal cavities.

## **LEARNING OBJECTIVE:**

At the conclusion of this segment of the course you should be able to:

1. Define the 4 regions of mesoderm and describe their spatial relationships within the embryo.
2. Define mesenchyme.
3. Describe for each of the 4 regions of mesoderm their further development during the embryonic period and their specific fate in the adult human organism.
4. Describe these structures: somitomeres, somites, dermatomes, myotomes and sclerotomes.
5. Discuss the steps in the process of somitogenesis.
6. Discuss the development and fate of the different segments of a somite.
7. Describe the effect of the specific combinatorial codes of Hox genes on the patterning of developing mesoderm.
8. Discuss the meaning of the clock-wave gene expression pattern and its role in somite maturation.

## **GLOSSARY:**

**Axial** - Medial/midline: site of the prechordal plate and notochord.

**Caudal** - Toward the tail end of the organism.

**Cephalic** - Relating to the head or toward the head.

**Dermatome** - The dorsolateral part of the somite that will contribute to the dermis of the skin.

**Epimere** - Segment of myotome that is dorsal to the body axis.

**Hypomere** - Segment of myotome that is ventral to the body axis.

**Mesenchyme** - A primordial embryonic connective tissue, consisting of stellate cells and a loose, fluid extracellular matrix. Can originate from the mesoderm or from neural crest.

**Mesoderm** - The middle of the 3 germ layers. It gives rise to all connective tissues (except in the head and neck regions), all body musculature, blood, cardiovascular and lymphatic systems, most of the urogenital system and the lining of pericardial, pleural and peritoneal cavities.

**Myotome** - That part of the somite that gives rise to the skeletal muscle cells.

**Notochord** - Mesodermal rod-like structure, defining the cephalic-caudal body axis.

**Paraxial** - Next to the body axis.

**Prechordal** - Located rostral to the notochord.

**Rostral** - Toward the snout end of the organism.

**Sclerotome** - A group of mesenchymal cells that emerge from the ventromedial part of a somite. These cells migrate toward the notochord where they form the vertebrae.

**Somite** - Mesodermal segments, formed in pairs in the early embryonic paraxial mesoderm, along the length of the trunk.

**Somitogenesis** - The development of a somite.

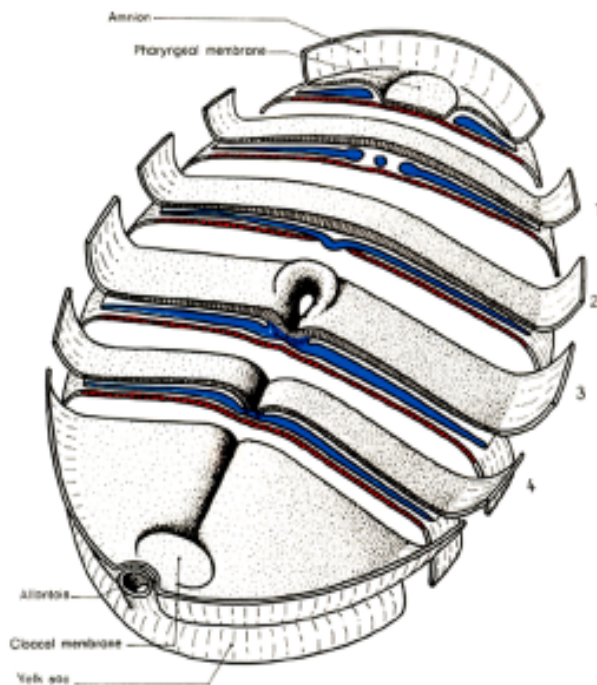
**Somitomere** - Metameric pattern in the preaxial mesoderm that precedes the development of a somite. In the head region 7 somitomeres are formed, which do not go on to form somites.

## TEXT:

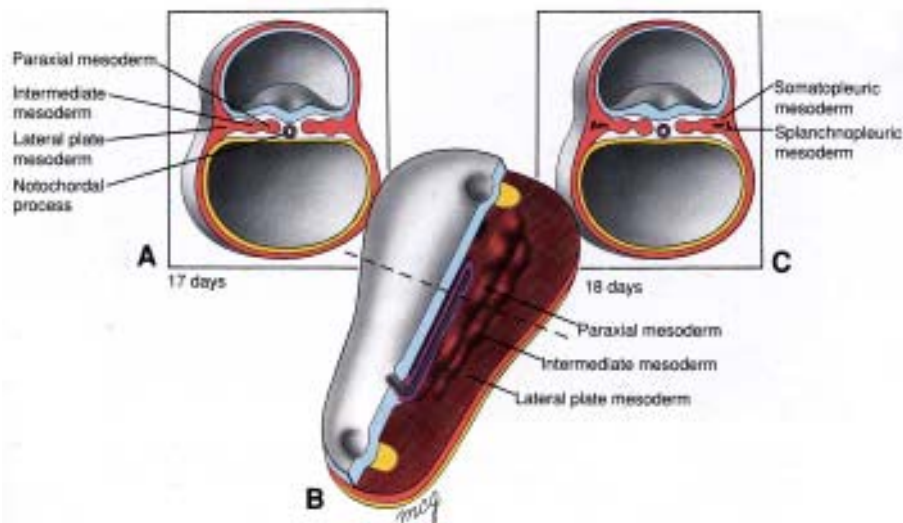
### Mesoderm Formation

Gastrulation is a series of cell movements that transforms the bilaminar germ disc (epiblast and hypoblast) into a 3 layered embryo (ectoderm, mesoderm, and endoderm, Fig. 5-1). Not only are the 3 germ layers established but cells also become committed to endodermal or mesodermal lineages during this process. The critical factors that determine the different fates of the mesodermal cell populations are: **1)** the point of entrance of the epiblast cells into the primitive streak and **2)** the direction of their subsequent migration. Depending on these 2 events, mesodermal cells can form tissues as varied as muscle, heart, kidney, or bone.

After gastrulation the mesodermal sheet on either side of the notochord is a connected layer of undifferentiated mesenchymal cells (Fig. 5-1). During the third week, this undifferentiated mesoderm will begin to condense on both sides of the notochord to form the **1)** paraxial, **2)** intermediate, and **3)** lateral plate mesoderm (Fig. 5-2). Starting on day 20 at what will be the base of the skull, the paraxial mesoderm (just lateral to the notochord;



**Fig. 5-1.** Diagrammatic view with cross sections of embryo during gastrulation.

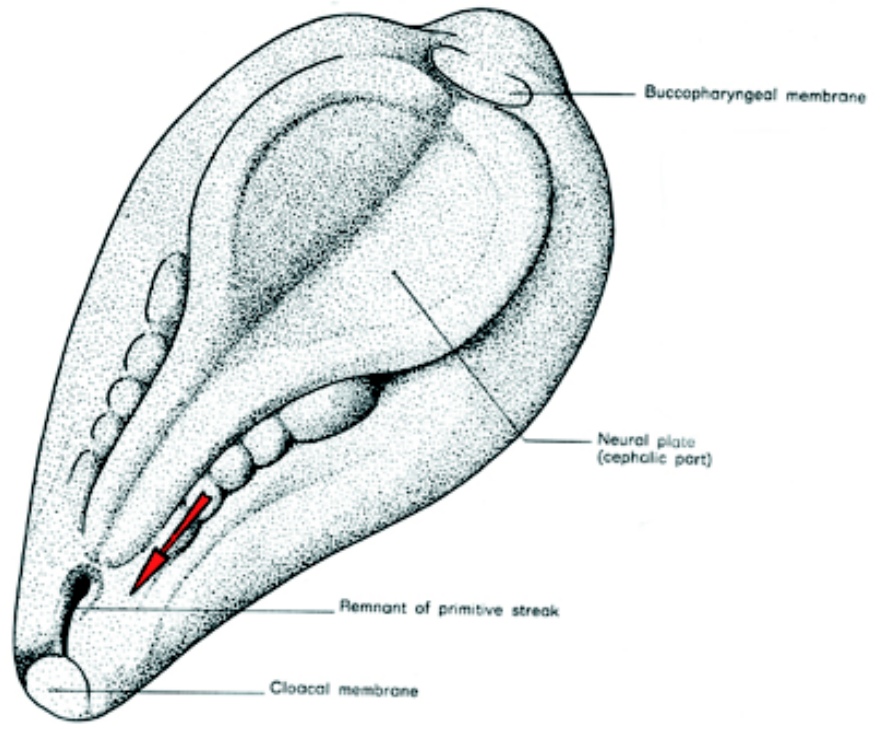


**Fig. 5-2.** Sections through a 17-day embryo showing the differentiation of the mesoderm on either side of the midline. **(A)** Early on day 17, the mesoderm has begun to differentiate into paraxial, intermediate, and lateral plate mesoderm. **(B)** Sagittal cutaway showing the rod-like condensations of paraxial and intermediate mesoderm. The dotted line marks the plane of the two transverse sections. **(C)** Later on day 17, the lateral plate begins to vacuolate to form the rudiment of the intraembryonic coelom.

**Fig. 5-3.** Scanning electron micrograph of an embryo with the ectoderm removed to show somites and, more caudally, the paraxial mesoderm that has not yet segmented. Arrows indicate the region of somitomere formation.



**Fig. 5-4.** Dorsal view of a human embryo of about 3 weeks. The first somite pairs are externally visible, just caudal to the occipital region. The arrow indicates the rostro-caudal sequence of somite development.



also called the segmental plate) begins to condense in a cranial to caudal direction (Fig. 5-3). These condensations will become the somites which are the focus of this lecture.

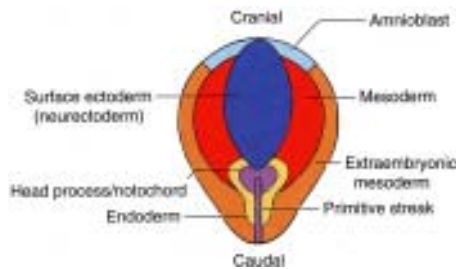
Lateral to the paraxial mesoderm is the intermediate mesoderm. As the embryo begins to fold (see Lecture 3 on embryonic folding) the intermediate mesoderm will lose its connection with the segmental plate and condense to form a solid mass of tissue running most of the length of the embryo. The intermediate mesoderm will give rise to the kidneys (two embryonic forms and the final adult form; see lectures 13/14) and most of the uro-genital tract, including gonadal tissue but excluding the primordial germ cells (see lectures 13/14).

### Somite Formation: Segmentation

One of the mechanisms by which animals generate form and diversity is first to establish a segmental pattern followed by **homeotic transformations** of each segment. Homeotic transformation is the differentiation of initially identical repeating segments into unique structures; the nature of the homeotic transformation is dependent on the expression pattern of homeotic genes (see Fig. 5-9). This strategy is conserved throughout the animal kingdom and vertebrates are recognized as a separate animal phylum because of their most conspicuous segmentation, the vertebral column.

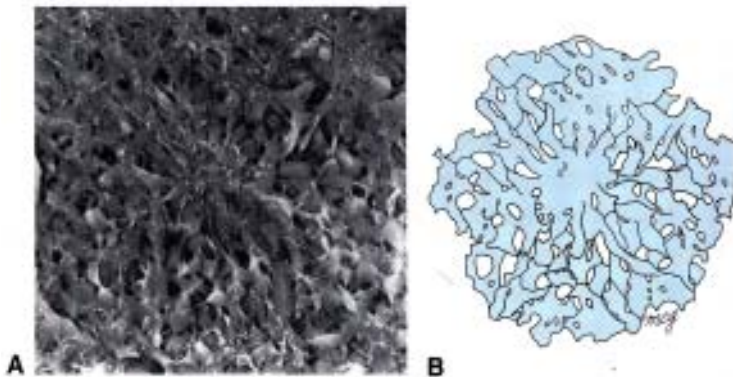
The segmental plate (paraxial mesoderm) is laid down during gastrulation appearing on either side of the midline as the primitive streak regresses. Fate maps demonstrate that **1)** the somite cells arise from epiblast tissue just caudal to the primitive node, and **2)** cells destined to become part of the segmental plate enter the primitive streak during the entire process of gastrulation (Fig. 5-4 and Lecture 2).

As the paraxial mesoderm begins to condense, whorls of cells called somitomeres first appear (Figure 5-6). Most of the somitomeres will develop into epithelial rosettes, the somites, which can be seen clearly through the covering ectoderm (Figs. 5-3, 5-5). The somitic epithelial cells surround a lumen.

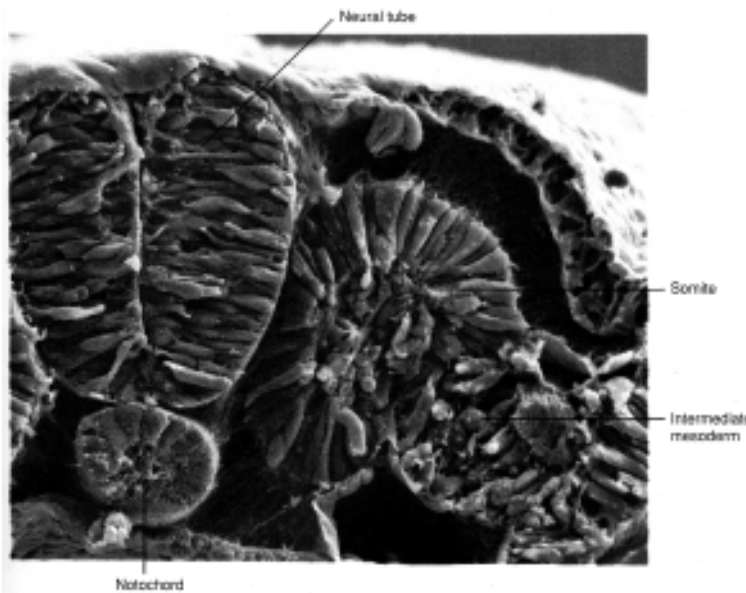


**Fig. 5-5.** Fate map of the epiblast of a mouse/embryo, showing the zones of epiblast that ingress through the primitive streak and form the major structures of the trilaminar germ disc. This map was deduced on the basis of cell lineage studies, in which epiblast cells were injected with tracer molecules and their labeled descendants later located.

They first form in the cranial end of the embryo and appear progressively more caudalward (Fig. 5-4). As the segmental boundaries are established, cells from one somite will not cross into another. We shall see that the somites will decondense (undergo epithelial to mesenchymal transformation) soon after they are formed so that not all somites are present at one time. In humans it is estimated that there are 42-44



**Fig. 5-6.** (A) Scanning electron micrograph and (B) matching sketch of a somitomere. The concentric architecture of these structures is easiest to discern in stereophotographs. (Photo courtesy of Dr. Antone Jacobson.)



**Fig. 5-7.** Scanning electron micrograph of a transversely sectioned embryo showing a somite and the intermediate mesoderm. Note also the notochord and the developing neural tube. (Photo courtesy of Dr. Kathryn Tosney.)

pairs of somites.

Precisely how the plate becomes arranged in repeating (metameric) segments is not well understood (see discussion of wave and clock theory below). The somites will appear even if the node is absent (although no notochord or neural tissue will develop) but will lose their integrity without these other tissues. Hence mesoderm is committed to the somite lineage by the time these cells enter into the presomitic mesoderm.

### **Clock and wavefront model of segmentation: Notching up segments in a Lunatic World**

The clock-like regularity of somitogenesis suggests the presence of a molecular clock. Currently, the most widely

accepted model for an explanation of somitogenesis is a variant of the clock and wavefront model that was originally proposed by Cooke and Zeeman in 1976. (For other models see Neuron 40:11-14, 2003)

#### **Segmentation clock.**

This is a biochemical oscillator in which genes are expressed in a wave like fashion from posterior to anterior in the cells of the presomitic mesoderm (psm; Fig. 5-8). It is characterized by the rhythmic and dynamic expression of such genes as *c-hairy 1* and *lunatic fringe* (among many others). These two genes are part of the *Notch* signaling pathway (see below), suggesting that Notch signaling is at the heart of the oscillations. There are three phases in the oscillations (see Fig. 5-8): I. broad expression in the psm; II a shift in expression to the middle of the psm (wave front moves forward); and a refinement to a strong band across the psm, in the rostral most compartment. Mutations in the oscillating genes lead to disruptions in somite development.

It is thought that signaling through the receptor Notch, via a variety of ligands, is an ancestral, highly conserved pathway for segmentation. What is interesting, conceptually, about Notch signaling is the following: Notch is a transmembrane protein and it pairs with a ligand, also a transmembrane protein, made by a neighboring cell. This interaction makes Notch susceptible to proteolytic cleavage at the plasma membrane. The released cytoplasmic tail migrates to the nucleus where it pairs with inactive transcription factors. This pairing of *Notch* tail and inactive transcription factor leads to transcription factor activation, binding to DNA and alterations in gene transcription.

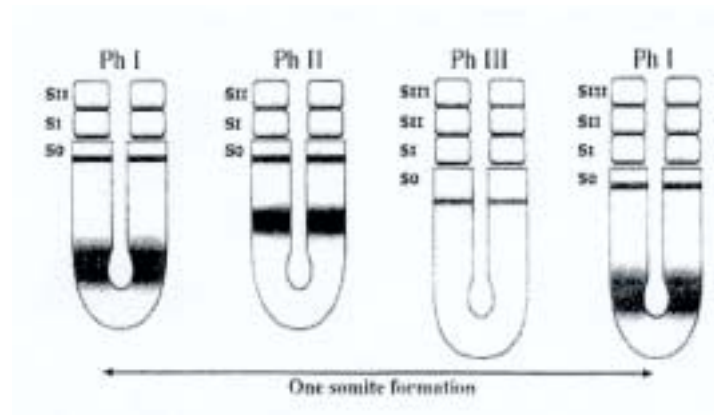
#### **Segmental Identity**

The somites that give rise to each vertebra are not equal even though morphologically they look identical. They carry positional information. For example, if prior to differentiation of the sclerotome you transplanted the thoracic somites which become rib-bearing to the cervical region, these transplanted somites will still form ribs. Hence the original axial position is remembered by the somite and being in a new position along the cranial-caudal axis does not respecify the thoracic segments.

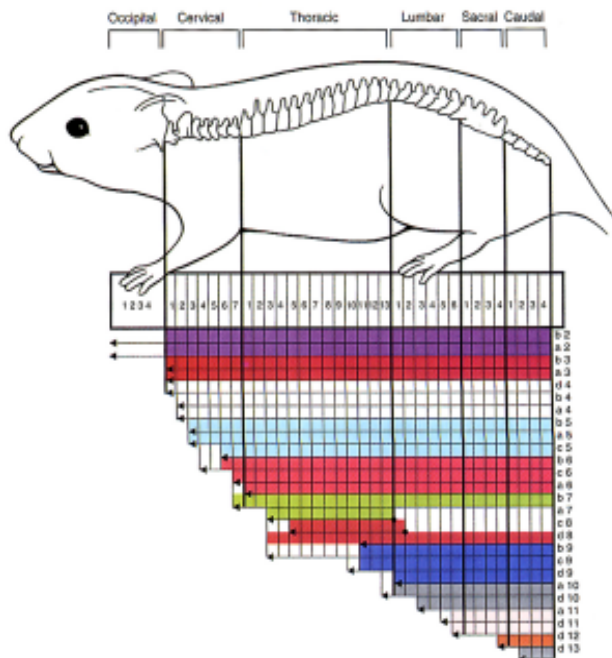
### Homeotic transformations.

The mechanism underlying the positional information encoded in the somites and homeotic transformations is due to the non-identical, overlapping expression of HOX genes with strict anterior boundaries along the cranial-caudal axis (Fig. 5-9). These control positional or segmental specification. Retinoic acid administration during gastrulation alters their expression such that the pattern of HOX gene expression necessary for development of cervical vertebrae, for example, is never established. As shown in Fig. 5-10, mutations leading to loss or gain of function of a HOX gene can respecify segments.

Segmentation implies a repeat pattern of segments. While this is true in general, there are significant patterning variations at different levels (thoracic vertebrae differ from lumbar vertebrae!). These

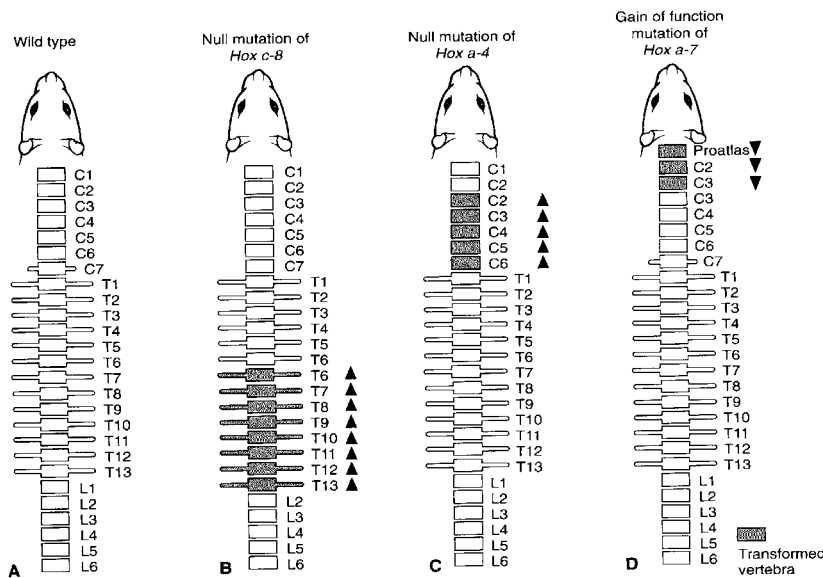


**Fig.5-8.** There are oscillations in gene expression (see text) in the presomitic (paraxial) mesoderm. One wave is illustrated here in which the pre-somite (S0) differentiates into a somite (S1). The wave starts as a broad band (dark area) across the most caudal aspect of the paraxial mesoderm (Ph I). That expression is down-regulated and expression “moves” to the middle of the paraxial mesoderm (Ph II) until it is finally restricted to what is now a narrow band delineating the boundary of the next “segment to be” (S0). This pattern of expression is repeated until all somites are formed.



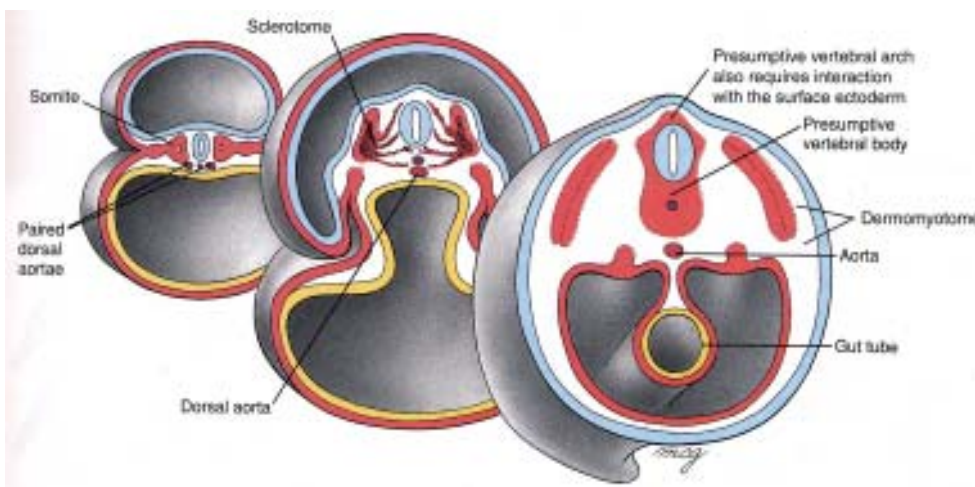
**Fig. 5-9.** Cranial expression boundaries of *Hox* genes in mice parallel their sequence on the chromosomes and sensitivities to retinoic acid (see also Fig. 3-17). The resulting distribution creates specific combinatorial codes that specify development of individual somites or small groups of adjacent somites. It is not possible, however, to determine whether cranial expression boundaries occur precisely between somites or between the recombined caudal and cranial halves of the sclerotomes (prevertebrae) or both, at different times. (From Hunt P, Krumlauf R. 1992. *Hox* codes and positional specification in vertebrate embryonic axes. *Annu Rev Cell Biol* 8:227, with permission.)

patterning variations appear to be under the direction of specific combinatorial codes of Hox genes as illustrated in Fig. 5-8.



**Fig 5-10.** Homeotic transformation of vertebral segments in loss-of-function and gain-of-function *Hox* genes in mice. Null mutations of *Hoxc-8* (compare **B** to **A**) and *Hoxa-4* (compare **C** to **A**) cranialize vertebrae within their normal expression domains. A gain-of-function mutation of *Hoxa-7* caudalizes vertebrae outside their normal expression domain (compare **D** to **A**).

Respecification can also take place. For example, if pregnant mice are treated with retinoic acid, a signaling molecule and powerful teratogen (chemical that causes birth defects, see Lecture 23), during gastrulation (**before somites are formed**), a posterior homeotic transformation occurs. In this type of transformation the same number of somites form but they express more posterior phenotypes (e.g., ribs will appear in cervical regions) and the most anterior phenotype does not appear or is reduced. Retinoic acid exerts similar effects on the developing brain where it causes forebrain to be respecified to hind-brain. The effect of retinoic acid depends on the time of administration as the somites are sensitive to it only during a specific developmental window of time and not all somites are at the same stage in development at any one time.



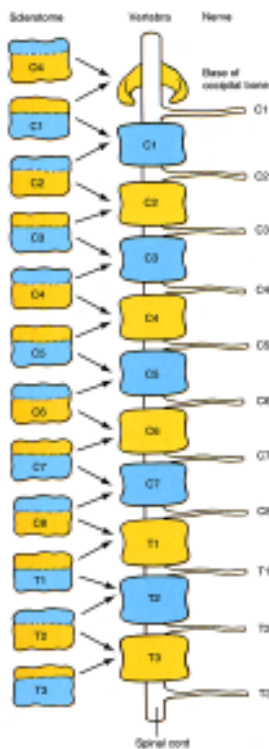
**Fig. 5-11.** Initial subdivision of the somitic mesoderm. The ventromedial and core cells of the somite, constituting the sclerotome, migrate toward the midline of the embryo to enclose the neural tube and notochord, where they subsequently form the vertebral arches and vertebral bodies, respectively. The remaining dorsolateral cells of the somite form the dermamyotome.

## Somite Differentiation: Sclerotome, Myotome, and Dermatome

The first 7 somitomes never condense to form somites. These fuse to form the basal occipital bone (non-segmented). The caudal-most somites also fuse to form the coccyx.

Further differentiation of the somites requires specific changes in gene expression. The initial formation of the epithelial structure of the somite is marked by an increase in cellular adhesion molecules, predominantly E-cadherin, and the addition of the extracellular matrix protein, fibronectin, to the components of the basal lamina. To continue differentiation, the cells must de-epithelialize by down-regulating E-cadherin expression, resume a mesenchymal phenotype, and migrate (Fig. 5-11). It is important to remember that “mesoderm” refers to a germ layer while “mesenchyme” describes a loose embryonic connective tissue which can be of mesodermal OR of neural crest origin.

Each somite becomes subdivided into 2 compartments (Fig. 5-11): the sclerotome and dermamyotome (which will subsequently sub-divided into the myotome and dermatome). The **sclerotome** is derived from the ventromedial aspect of the somite and de-epithelializes first and migrates



**Fig. 5-12.** The mechanism by which the cervical region develops eight cervical nerves but only seven cervical vertebrae. Each somite induces a ventral root to grow out from the spinal cord. When the sclerotomes recombine, however, the cranial half of the first sclerotome fuses with the occipital bone of the skull. In the thoracic, lumbar, and sacral regions, the number of spinal nerves matches the number of vertebrae.

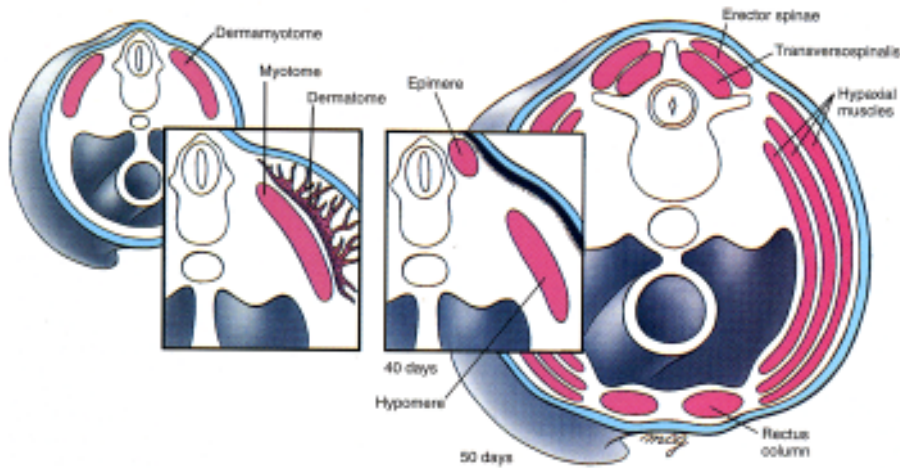
toward the notochord. The cells migrating dorsal to the neural tube will give rise to the vertebral arch while those that migrate ventral to the notochord will differentiate into the vertebral body. Individual vertebral rudiments are actually formed intersegmentally by the fusion of the caudal half of one sclerotome with the cranial aspect of the next (Fig. 5-12).

The cells on the dorsal aspect of the somite which remain compact after the de-epithelialization of the sclerotome are called the **dermamyotome** (Figs. 5-11, 5-13). They will further sub-divide into the dermatome and myotome. Cells of the dermatome are situated nearest the ectoderm and give rise to some of the dermis of the skin; cells of the myotome lie between the sclerotome and dermatome and give rise to axial muscles and muscles (but not bones) of the limb.

Specification within the myotome (Fig. 5-13):

- a. The ventrolateral edge of the dermamyotome are cells with muscle forming potential. They migrate extensively and laterally, including into the limbs if

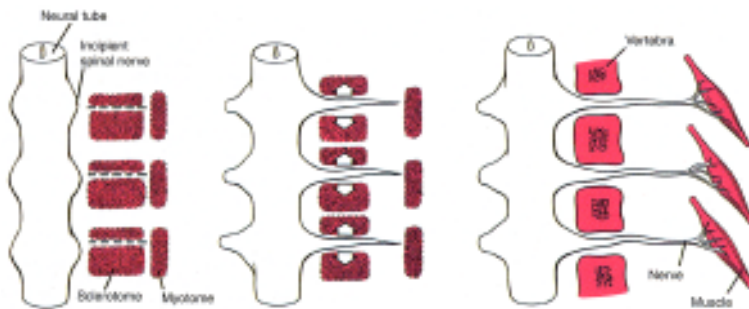




**Fig. 5-13.** Fate of the dermamyotome. Each dermamyotome splits into a dorsolateral dermatome and a myotome. Dermatome cells migrate to the surface ectoderm of the corresponding segmental region, where they collaborate with lateral plate mesoderm to form the dermis. Each myotome splits first into a dorsal epimere and ventral hypomere. The epimere forms deep muscles of the back. In the thoracic region, the hypomere splits into three layers of anterolateral muscles; in the abdominal region, a fourth ventral segment also differentiates and forms the rectus abdominis muscle.

they are at the right axial level; they give rise to the extensors and flexors.

- b.** The dorsomedial edge of the dermamyotome, at the same time, tucks under itself to give rise to elongated cells that span the entire length of (what had been) the somite. This portion of the myotome splits into epimere and hypomere. These give rise to the extensors of the dorsal vertebral column and the intercostal muscles, respectively.



**Fig.5-14.** Recombining of the sclerotomes to form vertebrae. Each sclerotome splits into cranial and caudal segments. As the segmental spinal nerves grow out to innervate the myotomes, the cranial segment of each sclerotome recombines with the caudal segment of the next superior sclerotome to form a vertebral rudiment.

have begun their migration and are still mesenchymal. The nerve exits the neural tube and is confined to the cranial half of the somite, being actively inhibited from entering the caudal half (Fig. 5-14). The migratory path of the neural crest cells (see prior lecture) which will form the dorsal root ganglia and sympathetic chain is also confined to the anterior sclerotome. The sensory axons (neural crest origin) and the motor axons (neural tube origin) are joined into a single nerve bundle (mixed nerve) and the growth/migration arrangement provides a mechanism for this coordination. If the somites are removed,

From recent research we know that the cells from the epiblast that will contribute to the different compartments of the somite are already segregated at the primitive streak stage. They have only a very limited capacity to be interchanged. (Selleck and Stern: *Development* 114, 403-415, 1992).

The segmental pattern established by the somites shapes the development of other nearby tissues like the spinal nerves and neural crest derivatives. Spinal nerves emerge from the developing spinal cord at the stage when the sclerotomal cells

growth of spinal and sensory nerves occurs as does the migration of crest cells but there is no segmentation. The dorsal root ganglia for example will be one continuous mass.

Experimental manipulations have demonstrated that somites are also influenced by the neighboring tissues (see Fig. 5-7). **A.** If the neural tube is removed from an embryo, the vertebrae that develop lack segmentation of the dorsal structures (i.e. the neural arch). **B.** If the notochord is removed, the ventral half of the vertebrae, the vertebral bodies, are non-segmented. In addition, removal of the notochord leads to loss of bilaterality of the somites so that they fuse ventral to the neural tube. **C.** If the notochord and floor plate (specialized cells on the midline of the neural tube) which are normally axial structures are transplanted dorsal to the somite, the formation of muscle is completely inhibited and the myotome differentiates into cartilage instead. These experiments illustrate that the somites and the tissues around them (neural tube, notochord, and floor plate) all create a set of interactive microenvironments influencing each other's development.

The mechanism of somitic segmentation is responsible for the organization of the trunk region. Similar principles apply to the head and neck called branchiomic segmentation. The brain is intrinsically segmented and not dependent on the mesoderm. Branchiomic segmentation will be covered in Lectures 9/10 and in Gross Anatomy.

### **Anomalies of the Vertebral Column**

Many common anomalies, some severe, can be traced to defective development of the somites. One of the most common is scoliosis, affecting up to 10% of women reaching puberty. In scoliosis a portion of the vertebral column is deviated laterally. The defect is often the congenital presence of one or more hemi-vertebrae on the right or left side. Hemi-vertebrae may arise by failure of the cranial and caudal halves of two adjacent somites to form a normal vertebra. If this occurs in the dorsal-ventral plane, the result is either lordosis (anterior deviation of the vertebral column) or kyphosis (posterior deviation). Surgery can correct these defects to some extent.