Patterning the Vertebrate Body Plan II: Mesoderm & Early Nervous System

Vertebrate embryos are similar at the phylotypic stage. The embryo has undergone gastrulation, and the main axial structure (somites, notochord and neural tube), are well developed and already show signs of regionalization both A/P and D/V axes.

Patterning turns mesoderm into repeated structures of skeleton and trunk muscles. Dorsal mesoderm is internalized during gastrulation to eventually become the notochord and the somites. Notochord is transient and becomes part of the spinal column.

Somite are formed along the antero-posterior axis. In the chick, mesoderm forms anterior to the regressing node of the primitive streak (Hensen’s node).

Pre-somitic mesoderm is the region between the last formed somite and the regressing node. This region will become 4 or 5 somites which form simultaneously as pairs on either side of the notochord. No signal specifying A/P position or timing is involved. Direction was A → P. (Fig. 4.2)

The position of somites were related with early stage of gastrulation. Transplant and reverse the pre-somatic mesoderm does not influence of temporal order of somitogenesis.
The segmental plate mesoderm is determined by its position along the anterior-posterior axis before somitogenesis.

Somites Form Progressively
Side of the anterior primitive streak cell → epiblast → move, in gastrulation → form somitogenic stem cell around Hensen’s node → leave node and form pre-somitic mesoderm (Fig 4.3)

Somites are formed along the antero-posterior axis
Somite form was A/P dependently and begin during gastrulation. Anterior somites form cervical vertebrate. Posterior ones form ribbed thoracic vertebrate. Somite form was according to its (pre-somitic mesoderm) original position (depend on fate). (Fig 4.5) Develop in a temporal and spatial order. Rearranging pre-somitic mesoderm will not change timing. The pattern is laid down earlier by an A/P axis signal.

Somites are formed along the antero-posterior axis

Neural tube and somites seen by SEM (scanning electron microscopy)
When surface ectoderm is peeled away
The Positional Identity of Pre-Somitic Mesoderm is Determined
Will development to thoracic somites

Neural tube
somitomere
Paraxial mesoderm → rounding → somitomere → compacted and bound → separate from presomitic paraxial mesoderm → somites
Somite development is specific gene expression dependent

Gastrulation and neurulation in the chick embryo

Gastrulation → Notochord → neurulation → somatic →

The major lineages of the mesoderm

The mechanisms are complex.
The current view is the clock and wavefront model.
This model invokes:

- Cyclical patterns of gene expression
- A morphogen gradient

The important components of somitogenesis are,
(1) Periodicity, (2) fissure formation, (3) Epithelialization, (4) specification, and (5) differentiation
Somite formation correlates with the wavelike expression of the hairy1 gene in the chick.

Cyclical Waves of Gene Expression

![Diagram of cyclical waves of gene expression](image)

Cyclical waves of c-Hairy (a transcription factor) expression in somitic mesoderm.

Oscillations in Notch and Wnt gene expression are at the core of the clock.

Cyclical Waves of Gene Expression

![Diagram of cyclical waves of gene expression](image)

Pre-Somite Mesoderm

Expression of hairy, Notch, Delta all confined to posterior of each somite

A Moving Morphogen Gradient In the Clock and Wavefront Model

The node is a source of Fgf 8 and Wnt, morphogens for somite formation.


A Moving Morphogen Gradient In the Clock and Wavefront Model

![Diagram of morphogen gradient](image)

A

The node


Fig. 4.4 Notch-Delta signaling pathway

Mutation of Notch-Delta, no somites formation
The formation of somites was timing and position specific
Notch signaling involved in somite fissioning

Notch→somite boundary formation

Notch protein itself and its ligands Dll1 and Dll3 are involved in somite fissioning. In human, individuals with scoliosis or vertebral dysplasia have numerous vertebral rib defects that have been linked to mutations of the Dll gene.
Integration of Clock and Wavefront


Blue FGF8 → LOW → did not express hairy → FGF8 high → + notch interaction → boundary formation → white region formation

Possible scheme for the regulation of the clock through which an Fgf8 gradient regulates a Wnt oscillating clock, which in turn controls a Notch clock

High → Axin inhibit Wnt3a → Notch did not activate → Hairy x
Low → Axin did not inhibit Wnt3a → Notch activate → Hairy expression

Somitogenesis

Regulating Notch activity at the somite boundaries
Hairy expression pattern reflects notch activity
Multiple regulatory interactions to establish and control Notch in somite

Gastrulation and neurulation in the chick embryo

Mesoderm becomes notochord and somites
The fate of somite cells depends upon adjacent tissue signals
In chick-quail trans-species grafts (with distinctive nuclei), somite fate maps have been constructed. (Fig. 4.6)

The major lineages of the mesoderm

Circulatory body cavity
Cartilage       skeletal      dermis
Circulatory system
Sclerotome        Myotome

The fate of different somite
Dorsal region → dermomyotome → myotome → muscle cell
Medial region → axial and back muscle
Ventral region → sclerotome → migrate to notochord → develop into vertebrate and ribs
The somites need notochord and neural tube signal regulates
No neural tube and notochord, somites → apoptosis
Notochord induces sclerotome cells (develops into cartilage) and suppresses the formation of dermomyotome. Fig. 4.8
Notochord/neural tube are induce of cartilage. Lateral plate mesoderm regulated the lateral part of the dermomyotome. Fig. 4.9

Mesoderm becomes notochord and somites

The patterning of somites differentiation

From notochord transplantation experiments, an additional notochord induces unsegmented pre-somatic mesoderm to produce greatly increased amount of cartilage.

Neural tube (ventral side: the floor plate) induces cartilage: sclerotome
Lateral plate mesoderm and the ectoderm induce the dermomyotome.

Signals that may pattern the somites are secreted signalling proteins that may include:

1) Sonic hedgehog which may specify the ventral somites.
2) BMP-4 which may specify the lateral somites.
3) Wnt family proteins which may specify the dorsal somites.
Many signal modulated the somites differentiation

From dorsal neural tube, ectoderm, lateral

From Notochord, floor plate of the neural tube

Sonic Hedgehog protein

Wnt

Fig. 4.9

A model for patterning of somite differentiation. Notochord and the floor plate of the neural tube → sonic hedgehog protein (diffusible signal) → somites → sclerotome

Dorsal neural tube and ectoderm → signal → somite → dermomyotome

Lateral plate mesoderm

The major lineages of the mesoderm

Circulatory body cavity

Cartilage      skeletal       dermis

Circulatory system

Scler Myo
tome Cartilage skeletal demis

Diagram of a transverse section through the trunk of a chick embryo
Mesoderm becomes notochord and somites

Regulation of the Pax homeobox genes (transcription factors)

Pax genes are regulated by signals from the notochord and neural tube to control the somitic cell fate. Notochord/neural → signal → pre-somatic cell → regulate Pax gene expression

Pax3 is expressed early in all cells that will form somites.

Pax3 is modulated by BMP-4 and Wnt to confine it to muscle precursors.

Pax3 is further down-regulated in back muscle precursors but remains active in future limb muscle cells.

In mice, Splotch (Pax3-minus) mutants lack limb muscles.

Defined by the presence of a conserved paired-box that codes for a 128-amino-acid paired domain, a DNA binding domain

9 Pax genes: Pax-1 to -9

Positional somites along the A/P axis specified by homeobox (HOX) genes (invertebrate or vertebrate)

Patterning along the A/P axis in all vertebrate involves the expression of a set of genes (HOX) that specify positional identity along the axis.

Homeobox genes: (Box 4A)

Homebox about 180 base pair (DNA) → 60 amino acids (homeodomain) → transcription factor (DNA binding protein) → bind to DNA (helix-turn-helix DNA binding motif) → turn on gene

Homebox gene encode a large family of transcription factors. The transcription factor also called homeodomain proteins.

Share a similar 60 amino acid DNA binding homeodomain which is encoded by 180 basepair homebox sequence.

Homeotic transformation: One mutation → replace by adjacent another gene (structure) → develop by another gene; It means mutation causes transformation of one body part into another body part.

Homeodoamin protein → activate → batteries of gene → specify the particular properties of each segment

There are four separate clusters of Hox genes (subset of the homeobox genes) in all vertebrates.

Hox genes start to be expressed in mesoderm cells at an early stage of gastrulation when they begin to leave the primitive streak.

Hox genes: anterior genes are expressed first. The posterior pattern develops later; Clearly defined patterns of Hox gene expression are most easily seen in the mesoderm and neural tube, after somites formation and neurulation.

helix-turn-helix DNA binding motif

Box 4A Homeobox genes

Paralogy groups

Orthology groups
Paralogy group
- are the Hox complexes
- natural clusters of Hox genes on a chromosome
- order relates to the duplication that occurred within a chromosomal region

Orthology group
- appropriate (pseudo-orthologs) gene in the four different hox complexes
- genes with maximum sequence identity
  orthology – same gene in a different species

Hox gene clusters
Hox genes (Hox gene clusters) are a subset of the homeobox genes encoding transcription factors, homeodomain.
Might have arisen by rounds of duplication of ancestral genes (to form a cluster), followed by further duplication of that cluster in mammals.
Paralogous group are composed of the most similar members of each cluster, in the same chromosome. Mouse paralogous subgroup=13
Partially overlapping zones of expression which vary in the anterior extent of their expression define distinct regions.
Various genes respond to the combination of gene products expressed.
Homeotic genes are involved in specifying regional identity along the A/P axis.
How Hox gene can specify the identity of axis? (Fig.4.10)

Homeobox is a highly-conserved, 180 bp DNA sequence only DNA has a homeobox.
homeodomain is the protein product of the homeobox and proteins encoded by homeobox genes are homeodomain proteins.
homeotic genes act in a cell autonomous manner
- an essential gene encoding a body part or segment
- a gene whose mutant allele causes replacement of a certain body part with a body part normally found elsewhere
- best described by homeotic mutants (transformation)
  - the mutation causes transformation of one body part into another body part
  - only one mutant → affect phenotype
all homeotic genes are homeobox genes; but not all homeobox genes are homeotic genes
The homeotic genes in the HOM (Drosophila) and HOX (vertebrate) complexes are all homeodomain transcription factors.

Gene activity can provide positional values
The pattern of expression defines four distinct regions, coded for by the expression of different combinations of genes. Different gene expression produced different interaction → specification of A/P.
Hox genes pattern the A/P axis

The differences between vertebrae (i.e. anterior - attach to skull; cervical; thoracic - have ribs; lumbar, sacral and caudal) clearly demonstrate that identity of somites differ along the A/P axis.

Hox genes are expressed along the A/P axis in mouse.

First, anterior Hox genes expressed in early gastrulation as mesoderm begins to leave the primitive streak.

The "anterior" gene are expressed first. As the posterior pattern develop latter.

More posterior Hox genes turn on as development continues.

Defined patterns of Hox gene expression are seen in:
1) mesoderm (after somite formation) and
2) neural tube (neurulation).

Fig. 4.11 For different antibody to stain
The arrowheads indicated the anterior boundary of expression of each gene within the neural tube

Hox gene expression is co-linear

Hoxa1 has its most anterior expression in the posterior head.
Hoxa11 has its most anterior expression in the sacral (lower back) region.

Hox gene expression is co-linear (from different chromosome) as order of genes on the chromosome (per cluster) reflects the order of spatial and temporal expression along the A/P axis.

Hox gene are involved in controlling regional identity. (Fig 4.13)

Combination of Hox gene → provide positional identity.

Hox gene expression is conserved between mouse and chick.

Different Hox gene → regulated specific tissue (region)
d12 and d13 may switched

Hox gene expression is conserved between mouse and chick.

Different Hox gene, control the same somites
Gene Physiological function: 1. transgenic mice
2. gene knock out

Transgenic: 1. Injection DNA (interesting gene) to the fertilized eggs
2. Alter or add a gene to the genome of embryonic cell (EC cell)

EC cell: Gene transfection insert randomly by homologous recombination → mutant cell (inactive gene; gene knock out). It radonly, insert to blastocyst, usually altered the gene function but not knock gene.

Why use EC cell: 1. less umber cell → effect all body
2. can produced germ cells

EC cell micro-injection

The lox-Cre recombination system can knock out genes

loxP inserted on each side of target gene →
produced a chimera mice; Chimera mice mating with another chimera mice (has recombinase Cre) →
produced lop X plus Cre chimera mice

loxP + cre → specific knock out
loxP + normal → nothing happen
No lloxP → nothing happen

Altering Hox gene expression alters axial patterning

In mice, “gene knock-out” experiments produce mutants. There is redundancy, where a missing gene can be at least partially compensated for the expression of related genes. Paralogous genes from another Hox complex may compensate for gene loss. Posterior prevalence: mutation affects the anterior extent of gene expression. Homeotic transformations result from Hox gene loss. For replace by another Hox gene expression → different and specific tissue Loss leads to cells assuming a “more anterior value” i.e. Hoxc8 mutant mice have extra ribs. Loss one Hox gene → more anterior tissue expressed Abnormal expression of Hox genes in anterior regions lead to tissues becoming more like posterior positioned tissues.
Homeotic transformation (Fig.4.14)

Loss of Hox gene, the conversion of one body part into another.

Its absence gives them a more anterior positional value, and they develop accordingly.

Retinoic acid can alter positional value

Retinoic acid is a derivative of vitamin A. It has very important role in signaling vertebrate development. In early development, retinoic acid can cause homeotic transformation of the vertebrate.

It can diffuse across plasma membranes to bind protein receptors and form an active transcription factor. (steroid molecule)

RA → cross membrane → bind receptor complex → transcription factor → turn on gene

Retinoic acid interferes with the normal expression of Hox genes. Later, it can alter positional development in limb development.

Neural induction and the role of organizer

Neural induction:
Form ectoderm
During gastrulation → ectoderm lying along the dorsal midline → form neural plate → neural tube → brain/spinal cord
Nervous system must be linked to the mesoderm patterning

Organizer-primary embryonic induction
Amphibians: spemann organizer
Zebrafish: shield
bird/chick: Hensen’s node
Mouse: equivalent node region

Organizer vs. variety animal

Mouse: equivalent node region
Amphibians: spemann organizer
Zebrafish: shield
The development of mouse embryo

Primary neurulation: neural tube formation in the chick embryo

MHP: medial neural hinge point

DLHP: dorsolateral hinge point

Neural crest cells

Neural crest cells migrate away from the neural tube to develop into...
1) skull (bone)
2) sensory and autonomic nervous systems
3) pigment cells

Somites are formed after gastrulation along the antero-posterior axis.

Primary neurulation
Neural crest cells

- Neural crest cells migrate away from the neural tube to develop into...
  1) skull (bone)
  2) sensory and autonomic nervous systems
  3) pigment cells

- Somites are formed after gastrulation along the antero-posterior axis.

Dorsal-Ventral Specification of the Neural Tube

Shh: Sonic Hedgehog

Cascade of Inductions Initiated by the Notochord in the Ventral Neural Tube

Heart, kidney, Gaunard, gut muscle, Blood forming tissue
The role of Spemann organizer: axis development (A/P; D/V)

Spemann organizer is temporally dependent.

Spemann organizer as primary embryonic induction.
Spemann Organizer (dorsal blastopore lip) grafted to the ventral side of the marginal zone results in a twinned embryo. Fig. 4.15

The second embryo can have head, trunk and, sometimes, a tail but will be joined to primary embryo along the axis.
Blastopore transplants vary with time (timing dependent).

In different gastrulation stage, transplantation of organizer
Early gastrula induces a full 2nd embryo. (completed body axis and CNS, but only older organizer induce-posterior structure)
Mid-gastrula induces trunk and tail (no head).
Late gastrula induces only tail.
Different stage of embryo, Spemann organizer plays different induction of axis. May different gene expression at different stage in organizer.

Fig. 4.15 Transplantation of organizer in different gastrulation stage

Organizer can induce many region (Fig. 4.17) is timing dependent
Early stage: head
Mid stage: no head, other yes
Late stage: tail

In early gastrulation stage, the inducting region as determined form the results of transplantation of small pieces of tissue form the dorsal marginal zone.

Xenopus and mouse share a number of genes that are expressed in the organizer:
1) Brachyury
2) FGF (fibroblast growth factor)
Control of Hox genes is unknown

Fig. 4.18 Genes expressed in the spemann organizer.
Neural plate is induced in the ectoderm (by mesoderm; positional specification).

Dorsal lip transplantation experiments demonstrate that a nervous system can be induced from ectoderm. (Fig. 4.19)

The nervous system of Xenopus is induced during gastrulation.

Specific position → induced to neural system; not dependent on cell fate

Different region develops different structure.

The formation of the nervous system is dependent on an inductive signal (is induced), it also positional specification. The specific gene turn on (specific time and position) → induced neural plate.

Neural plate is induced in the ectoderm by secreted proteins (specific region → secreted specific protein → to ectoderm → formed neural plate).

Organizer can secret many neural induction protein.

- Chordin, noggin, cerberus are BMP-4 antagonists. (BMP-4 → mesoderm)
  - BMP-4, secreted growth factor, inhibits cells from forming neural tissue.
  - Inhibition of BMP-4 allows neural tissue formation.
  - noggin (secreted by the organizer) inhibits BMP-4 and acts to dorsalize the mesoderm. noggin also induces neural tissue (neural induction factor). BMP is ventral induction.
  - chordin expressed by the future neural plate cells of the organizer. It can bind BMP-4 and preventing its action.
  - Both noggin and chordin directly bind BMP-4 and inactive it to allow the induction of neural tissue.
  - Knock-out noggin and chordin in zebrafish, no neural plate developing

Others proteins: FGF (induce), Wnt (block)
Cortical cytoplasmic rotation causes translocation of the dishevel protein to the dorsal side of the embryo.
- Dishevel inhibits GSK-3 (glycogen synthase kinase 3)
- β-catenin is not degraded in blastomeres of the future dorsal side
- β-catenin enters the nuclei and binds with Tcf3 to form a transcription factor
- β-catenin/Tcf3 complex activates genes such as siamois (drosalize cause factor)
- Siamois along with factors stimulated by the VegT and Vg1 (nodal) → cumulate to activated goosecoid+ gene in the cell of the blastopore lip
- Goosecoid+ turns on the expression of genes that code for dorsalizing molecules like noggin and chordin
- The organizer is now functional, directly dorsalization.

The hypothesis of induction of the organizer in the dorsal mesoderm.

Neural plate is induced by signaling mechanism(s) from mesoderm (positional specification)
Nervous system can be patterned by signals from the mesoderm. It is positional specificity in this induction. Fig. 4.20
In newt neurula, mesoderm transplantation into younger newt embryos, anterior explants induce head and brain.
Posterior explants induce trunk & spinal cord.
Neural plate explants induce specific neural structures (depending upon position) when transplanted beneath the ectoderm of a gastrula.

Induction of the nervous system by the mesoderm is position specific
Different region of mesoderm → induced different neural tissue

Neural tissue formation, depend on the development of axis. Hox gene expression regulated the formation of somites, but it can not detected in the anterior-most neural tissue.
Hox gene expression vs. somites development

Different HOX gene → different somite → induced different neural tissue

Gastrulation (HOX gene turn) → somite → neural tissue
How the mesoderm signal induced neural patterning

(Model: The two signal model of neural patterning)

Signal 1 from the mesoderm induces ectoderm to become anterior neural tissue. Directly turn on the formation of neural tissue. (chordin and noggin are good candidates)

Signal 2 turns part of this into posterior neural tissue in a graded manner. Regulated the neural patterning (FGF, Wnt & retinoic acid are candidates).

Fig. 4.21 Models of neural patterning by induction

They are related with Hox gene expression

Not only mesoderm but also Hensen’s node can induce gene expression characteristic of neural tissue.

Mouse and chick grafts of the primitive streak (ie node or Hensen's node) can also induce neural tissue.

This model differs from another model which suggests that there may exist a number of region-specific inducer molecules. Hensen’s node (chick) can induce neural gene expression in Xenopus ectoderm.

This (Fig.4.22) demonstrates the evolutionary conservation of neural induction signals and confirm similarity of Hensen's node and Spemann Organizer. Specific region may has specific induced-signal.

Early nodes induce anterior neuron structures.
Later nodes induce posterior neuron structures.
The nodes can specify different A/P positional values over time.
The capacity to produce signals that generate anterior structure is lost.

Neural induction is time, position, mesoderm and Hensen’s dependent
Neural plate signals travel within the neural plate (did not cross mesoderm)

The signal (planar: route from the mesoderm to the overlying ectoderm, whereas the other is) being generated within the neural plate itself and traveling within the ectodermal sheet.

Fig. 4.23 Induction and patterning of the nervous system involves planar signal originating with in the ectoderm.

Specific gene ( engrailed-2 and krox-20) are only expressed in the neural tissue.

N-CAM, a neural cell-cell adhesion protein, neurogenic factors and other neural specific proteins can be expressed in the ectoderm in the correct A/P order in exogastrula which suggests that the inducing signal can travel a relatively long distance through the tissues and did not across the mesoderm.

Hindbrain rhombomers restrict cell lineage

(The hindbrain is segmented into rhombomeres by boundaries of cell-lineage restriction.)

Posterior head and hindbrain development requires segmentation of the anterior neural tube, it along A/P axis. It did not occur elsewhere along the spinal cord. Other spinal region, the pattern of dorsal root ganglia and ventral motor nerves is imposed by the somites.

Segmentation events in the 3 day chick embryo's posterior head include...

1) somite formation from mesoderm on either side of notochord,
2) the hindbrain (rhombocephalon) is divided into 8 rhombomeres, and
3) the lateral mesoderm forms the branchial arches.

(Note: spinal cord is segmented into dorsal root ganglia and ventral motor nerves by the somites)

Rhombomeres: the region do not cross form one side of a boundary to the other. It formed by cell-lineage restriction. Fig. 4.25

Rhombomeres share some adhesive property that prevent mixing with those of adjacent cell. Fig. 4.26

Division of the hindbrain into rhombomeres: a unique identity and develop
Early human brain development
Regional specification in the developing brain

Rhombomeres formation → different development
Why?
For different function development

Fig. 4.24

Fig. 4.25 Lineage restriction in rhombomere

Cells in each rhombomere may be under the control of the same genes, and that the rhombomere is a developmental unit.

Ephrin and its receptor separately express, thus preventing cell mixing at the rhombomere. It means that cells in each rhombomere may be under the control of the same genes, and that the rhombomere is a developmental unit.

Development of posterior head involves interactions

Neural crest cells innervate the face and neck to form the segmental cranial nerves.

Neural tube forms segmentally arranged the segmental cranial nerves. Neural crest cells also give rise to peripheral nerves and bones including jaw (from the first branchial arch) and the bony parts of the ear (from the second arch).

Eight rhombomeres form by constricting the freshly closed neural tube into eight evenly spaced sections.

Lineage restriction occurs with cells and their descendants remaining in their ‘parent’ rhombomere.

Cell movement restriction depends on adhesive properties.
Within each rhombomere, the cells are under the control of the same genes and act as a developmental unit.
Neural crest cells have positional values from different rhombomeres.
Different gene expression produces different neural crest cell → different tissue development.

Chick neural crest cells can be labeled and their fate mapped.
The cranial neural crest cells migrate out from the rhombomeres of the dorsal region of the hindbrain.
Branchial arch 1 (formed jawbones, ear bone face bones) is populated by cells from rhombomere 2.
Branchial arch 2 (formed neck cartilage, middle ear bone) by rhombomere 4 cells.
Branchial arch 3 (thymus, parathyroid, thyroid gland) by rhombomere 6 cells.
Neural crest cells from rhombomeres 3 and 5 die by apoptosis (programmed cell death).
Transplantation of rhombomere 2 cells to where rhombomere 4 cells should be results in formation of a second jaw. Lineage restriction Rhombomeres developed independently, produces different neural crest. Different neural crest can develop to different tissues.

Hindbrain Hox gene expression

Mouse embryonic hindbrain Hox gene expression is well defined and correlates well with the segmental pattern.
Paralogous group 1 (Hoxa1, b1 etc.) is expressed anterior to paralogou 2 (Hoxa2, b2 etc.) followed by paralog 3. Fig. 4.27
Control of gene expression is complex (i.e. Hoxb2 is controlled in rhombomere 3 and 5 by one enhancer which is activated by Krox-20 and in r4 by another enhancer).
Hoxa2 knock-out mice show head skeletal defects that are consistent with a homedetic transformation of the inner ear (2nd branchial arch) to jaw (1st arch).
(By neurula stage, the embryo is divided into regulating organ-forming regions.)

Fig. 4.27 Expression of Hox genes in the branchial region of the head.

Fig 4.28 Gene expression in the hindbrain.
The embryo is patterned by the neural stage into organ-forming regions. At gastrulation state, body plane had established. At the neurula stage, the regions of embryo have become determined, however it can be regulated.

Vertebrate embryos are similar at the phylotypic stage:
- Somites, notochord and neural tube show A/P organization.
- Patterning turns mesoderm into repeated structures of skeleton and trunk muscles.
- Dorsal mesoderm is internalized during gastrulation to eventually become the notochord and the somites.
- Notochord is transient and becomes part of spinal column.
- With neuralation, ectoderm overlaying the notochord forms the neural tube to give rise to brain and spinal cord.
- Somites, mesoderm in blocks flanking notochord, become:
  1) the vertebrate and ribs
  2) muscles of the trunk and limbs and
  3) contribute to the dermis.
Primary Neurulation: Neural Tube Formation in the Chick Embryo

Secondary Neurulation in the Caudal Region of a 25-Somite Chick Embryo

Three Views of Neurulation in an Amphibian Embryo
Neural crest cells migrate away from the neural tube to develop into...
1) skull (bone)
2) sensory and autonomic nervous systems
3) pigment cells
4) muscle

Dorsal-Ventral Specification of the Neural Tube

Neural crest cells

Heart, kidney
Gut muscle
Blood forming tissues
Cascade of Inductions Initiated by the Notochord in the Ventral Neural Tube

In the chick, mesoderm forms anterior to the regressing node of the primitive streak.

Pre-somatic mesoderm is the region between the last formed somite and the regressing node.

This region will become 4 or 5 somites which form simultaneously as pairs on either side of the notochord.

The position of somites along A/P axis determines fate.

Anterior somites form cervical vertebrate.

Posterior ones form ribbed thoracic vertebrate.

The connection between the proteins oscillations and somatic formation is not clear; maybe Delta-Notch signaling pathway.

The pre-somatic mesoderm has a positional identity before somatic formation.

Ephrin and Its Receptor Constitute a Possible Cut Site for Somite Formation
Mesoderm becomes notochord and somites

- The fate of somite cells depends upon adjacent tissue signals.
- In chick-quail trans-species grafts (with distinctive nuclei), somite fate maps have been constructed.
- The dorsal and lateral region of somites form the dermomyotome (which expresses Pax3). This is made up of the myotome (forms muscle cells) and the dermatome (an epithelial sheet that forms dermis).
- Cells from the medial region of the somite (which express MyoD) form axial & back muscles.
- Lateral region of somites form abdominal and limb muscles.
- Ventral medial region of the somite contain sclerotome cells (future cartilage which express Pax1) and migrate to surround the notochord and form the vertebrate.
- Notochord induces sclerotome cells.

The Major Lineages of the Amniote Mesoderm

- From notochord transplantation experiments, an additional notochord induces unsegmented pre-somatic mesoderm to produce greatly increased amount of cartilage.
- Neural tube (ventral side: the floor plate) induces cartilage.
- Lateral plate mesoderm and the ectoderm induce the dermomyotome.
- Signals that may pattern the somites are secreted signaling proteins that may include: 1) Sonic hedgehog which may specify the ventral somites. 2) BMP-4 which may specify the lateral somites. 3) Wnt family proteins which may specify the dorsal somites.
Major Postulated Interactions in the Patterning of the Somite

Mesoderm and homeobox genes

- Regulation of the Pax homeobox genes (transcription factors)
- Pax genes are regulated by signals from the notochord and neural tube to control the somitic cell fate.
- Pax3 is expressed early in all cells that will form somites.
- Pax3 is modulated by BMP-4 and Wnt to confine it to muscle precursors.
- Pax3 is further down-regulated in back muscle precursors but remains active in future limb muscle cells.
- In mice, Splotch (Pax3-minus) mutants lack limb muscles.
- Homeobox genes:
  - Encode a large family of transcription factors.
  - Share a similar 60 amino acid DNA binding homeodomain which is encoded by 180 basepair homeobox sequence.
  - Homotic transformation is often observed in mutants of genes that have this domain.
- Identified first in Drosophila (Bithorax and Antennapedia complexes) as a “split cluster”.
- There are four separate clusters of Hox genes (subset of the homeobox genes) in vertebrates.

Hox gene clusters

- Hox genes (Hox gene clusters) are a subset of the homeobox genes encoding of transcription factors.
- Might have arisen by rounds of duplication of ancestral genes (to form a cluster), followed by further duplication of that cluster in mammals.
- Paralogous group are composed of the most similar members of each cluster.
- Partially overlapping zones of expression which vary in the anterior extent of their expression define distinct regions.
- Various genes respond to the combination of gene products expressed.
- Most homeobox genes are not Hox genes (i.e. Pax genes)

Hox genes pattern the A/P axis

- The differences between vertebrates (i.e. anterior-attach to skull; cervical; thoracic have ribs; lumbar, sacral and caudal) clearly demonstrate that identity of somites differ along the A/P axis.
- Hox genes are expressed along the A/P axis in mouse.
- First, anterior Hox genes expressed in early gastrulation as mesoderm begins to leave the primitive streak.
- More posterior Hox genes turn on as development continues.
- Defined patterns of Hox gene expression are seen in:
  1) mesoderm (after somite formation) and
  2) neural tube (neuralation).
- Most anterior somites express Hoxa1 and Hoxb1 only.
- Posterior regions express all Hox genes.
- The anterior head, forebrain and midbrain do not express Hox genes but have other homeobox genes (pitx & otx).
Hox gene expression is co-linear

- Hoxa1 has its most anterior expression in the posterior head.
- Hoxa11 has its most anterior expression in the sacral (lower back) region.
- Hox gene expression is co-linear as order of genes on the chromosome (per cluster) reflects the order of spatial and temporal expression along the A/P axis.
- Hox gene expression is conserved between mouse and chick.

Technique: Insertional mutagenesis (gene knock-out)

- A targeting vector is constructed that has the central (functional) region of a gene replaced with a drug resistance gene.
- This is transfected into ES cells and selected by drug exposure.
- By homologous recombination, a fraction of the transformants will have one copy of the original gene replaced with the altered (non-functional) form.
- These cells are injected into the inner cell mass of a blastocyst.
- Resultant chimeric mice give rise to heterozygous mutants which can be bred to generate mutant homozygotes.

Altering Hox gene expression alters axial patterning

- In mice, “gene knock-out” experiments produce mutants.
- There is redundancy, where a missing gene can be at least partially compensated for the expression of related genes.
- Paralogous genes from another Hox complex may compensate for gene loss.
- Posterior prevalence: mutation affects the anterior extent of gene expression.
- Homeotic transformations result from Hox gene loss.
- Loss leads to cells assuming a “more anterior value” i.e. Hoxc8 mutant mice have extra ribs.
- Abnormal expression of Hox genes in anterior regions lead to tissues becoming more like posterior positioned tissues.

Retinoic acid can alter positional value

- Retinoic acid is a derivative of vitamin A.
- It has very important role in signaling vertebrate development.
- In early development, retinoic acid can cause homeotic transformation of the vertebrate.
- It can diffuse across plasma membranes to bind protein receptors and form an active transcription factor.
- Retinoic acid interferes with the normal expression of Hox genes.
- Later, it can alter positional development in limb development.
The Spemann Organizer is temporally dependent

- Spemann Organizer (dorsal blastopore lip) grafted to the ventral side of the marginal zone results in a twinned embryo.
- The second embryo can have head, trunk and, sometimes, a tail but will be joined to primary embryo along the axis.
- Blastopore transplants vary with time.
- Early gastrula induces a full 2nd embryo.
- Mid-gastrula induces trunk and tail (no head).
- Late gastrula induces only tail.

The Spemann Organizer has conserved functions

- As gastrulation proceeds, the A/P axis become specified and as it progresses the blastopore lip can only induce more posterior structures.
- Hensen's node (the chick Organizer), at the anterior of the primitive streak contributes to notochord and somites and can induce another axis (more difficult than frogs)
- Xenopus and mouse share a number of genes that are expressed in the organizer:
  1) Brachyury
  2) FGF (fibroblast growth factor)
- Control of Hox genes is unknown

Neural plate is induced by mesoderm and in the ectoderm

- Dorsal lip transplantation experiments demonstrate that a nervous system can be induced from ectoderm.
- BMP-4, secreted growth factor, inhibits cells from forming neural tissue.
- Inhibition of BMP-4 allows neural tissue formation.
- noggin (secreted by the organizer) inhibits BMP-4 and acts to dorsalize the mesoderm.
- noggin also induces neural tissue.
- chordin expressed by the future neural plate cells of the organizer.
- Both noggin and chordin directly bind BMP-4 and inactive it to allow the induction of neural tissue.

Neural plate is induced by signaling mechanism(s)

- Nervous system can be patterned by signals from the mesoderm
- In newt neurula, mesoderm transplantation into younger newt embryos, anterior explants induce head and brain.
- Posterior explants induce trunk & spinal cord.
- Neural plate explants induce specific neural structures (depending upon position) when transplanted beneath the ectoderm of a gastrula.
- Otx (orthodenticle in Drosophila), Emc are expressed in anterior to hindbrain
Model: The two signal model of neural patterning

- Signal 1 from the mesoderm induces ectoderm to become anterior neural tissue. (chordin and noggin are good candidates)
- Signal 2 turns part of this into posterior neural tissue in a graded manner (FGF, Wnts & retinoic acid are candidates).
- Mouse and chick grafts of the primitive streak (ie node or Hensen's node) can also induce neural tissue.
- This model differs from another model which suggests that there may exist a number of region-specific inducer molecules.

Hensen's node is the chick organizer

- Hensen's node (chick) can induce neural gene expression in Xenopus ectoderm.
- This demonstrates the evolutionary conservation of neural induction signals and confirm similarity of Hensen's node and Spemann Organizer.
- Early nodes induce anterior structures.
- Later nodes induce posterior structures.
- The nodes can specify different A/P positional values over time.
- The capacity to produce signals that generate anterior structure is lost.

Neural plate signals travel within the neural plate

- Mesoderm does not have to lie in contact with ectoderm to induce it.
- N-CAM, a neural cell-cell adhesion protein, neurogenic factors and other neural specific proteins can be expressed in the ectoderm in the correct A/P order in exogastrula which suggests that the inducing signal can travel a relatively long distance through the tissues.

Hindbrain rhombomeres restrict cell lineage

- Posterior head and hindbrain development requires segmentation of the anterior neural tube.
- Segmentation events in the 3 day chick embryo's posterior head include...
  1) somite formation from mesoderm on either side of notochord, 2) the hindbrain (rhombencephalon) is divided into 8 rhombomeres, and 3) the lateral mesoderm forming the branchial arches.
- Neural crest cells also give rise to peripheral nerves and some of the bones including the first branchial arches.
- Eight rhombomeres form by constraining the freshly closed neural tube into eight evenly spaced sections.
- Lineage restriction occurs with cells and their descendants remaining in their 'parent' rhombomere.
- Cell movement restriction depends on adhesive properties.
- Within each rhombomere, the cells are under the control of the same genes and yet are a segmentation unit.

Rearranges of chick embryo cranial nerves and segmental boundaries in spatial and temporal order
Positional Identity of the rhombomere and neural crest

• Chick neural crest cells can be labeled and their fate mapped.
• The cranial neural crest cells migrate out of the rhombomeres of the dorsal region of the hindbrain.
• Branchial arch 1 is populated by cells from rhombomere 2.
• Branchial arch 2 by rhombomere 4 cells.
• Branchial arch 3 by rhombomere 5 cells.
• Neural crest cells from rhombomeres 3 and 5 die by apoptosis (programmed cell death).
• Transplantation of rhombomere 2 cells to where rhombomere 4 cells should be results in formation of a second jaw.
• Mouse embryonic hindbrain Hox gene expression is well defined and correlates well with the segmental pattern.
• Paralogous group 1 (Hoxa1, b1, etc.) is expressed anterior to paralog 2 (Hoxa2, b2, etc.) followed by paralog 3.
• Control of gene expression is complex (i.e., Hoxa2 is controlled in rhombomeres 3 and 5 by one enhancer which is activated by Krox-20 and in r4 by another enhancer).
• Hoxa2 knock-out mice show head skeletal defects that are consistent with a homeotic transformation of the inner ear (2nd branchial arch) to jaw (1st arch).
• (By neurula stage, the embryo is divided into regulating organ-forming regions.)