Paraxial and Intermediate Mesoderm

July 28, 2008
Paraxial and Intermediate Mesoderm Overview

Development of major mesodermal lineages

Somites:
- formation
- specification and differentiation

Mesodermal structures:
- Muscle
- Bone
- Vertebrae
- Tendon
- Kidney
- Primordial germ cells
Chick – Early Development

19-22 h

23-26 h

Primitive streak
Epiblast
Endoderm
Migrating mesodermal cells
Epidermis
Neural plate
Endoderm
Paraxial mesoderm
Notochord
Lateral mesoderm
Chick – Early Development

33-38 h

40-45 h
Mesodermal subdivisions are specified along a mediolateral axis by increasing amounts of **BMPs**
- lateral mesoderm expresses higher BMP4 than midline areas
- different BMP concentrations cause differential expression of the **Forkhead (Fox)** family of transcription factors
  - results in different mesodermal fates
Major Mesoderm Lineages

[Diagram showing the development of mesoderm lineages with labels for Neural tube, Chorda-mesoderm, and Notochord]
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Major somite components:
1. **Sclerotome** – vertebrae, ribs and rib cartilage
2. **Myotome** – musculature of the back, ribs, and limbs
3. **Dermatome** – forms dermis of the back

Minor components:
4. **Syndetome** – tendons
5. cells that generate vascular cells in the dorsal aorta
Somitogenesis

- periodicity
- fissure formation
- epithelialization
- specification
- differentiation

Paraxial mesoderm (presomitic mesoderm)
Regulation of Somite Formation

Somites are formed by a "clock and wave" mechanism:

- FGF expression follows Hensen's node regression from rostral-to-caudal, which triggers...
- an oscillating signal (the "clock"): **Notch**, which stimulates gene expression, (e.g. hairy1), that appears in repeating "waves"
- with each wave of gene expression, another somite is formed

How to construct an "expression clock"

\[
\text{Trigger} \rightarrow \text{Clock} \rightarrow \text{Effector} \\
\text{Clock} \rightarrow \text{Inhibitor (unstable)}
\]

Hypothetical model for somite production

\[
\text{Fgf8 (low?)} \rightarrow \text{Wnt3a} \\
\text{Axin (unstable)} \rightarrow \text{Notch} \\
\text{Lunatic fringe (unstable)} \rightarrow \text{Hairy1} \rightarrow \text{Segmentation}
\]
Regulation of Somite Formation

Notch directs the placement of somite borders
- regulates the periodic expression of *hairy1* (etc.)
  ~ 90 min periodicity (chick)
Regulation of Somite Formation

**a**, Alizarin staining of a corn snake showing 296 vertebrae, including 3 cervical, 219 thoracic, 4 cloacal (distinguishable by their forked lymphapophyses) and 70 caudal. **b**, Time course of corn snake development after egg laying (118-somite embryo on the far left) until the end of somitogenesis (315 somites).

Céline Gomez, Ertuğrul M. Özbudak, Joshua Wunderlich, Diana Baumann, Julian Lewis & Olivier Pourquié Nature 454, 335-339(17 July 2008)
Epithelialization

Mesenchymal mesoderm transforms into hollow epithelial ball

- cells polarize: sub-apical surface (inward); basal membrane (outside)

- tight junctions form between basal lamina

- synthesize extracellular matrix proteins; e.g. fibronectin, N-cadherin (see above)

Epithelialization promoted by:
- fibronectin (ECM organizing protein)
- N-cadherin (adhesion protein)
Specification and Differentiation

Somites look identical, but will form different structures; e.g.
- cervical, thoracic, lumbar vertebrae

Each somite forms a specific type of vertebrae; not interchangeable
- tissue specification occurs early – prior to somitogenesis
- somite tissue specified by:
  - notochord (Shh)
  - neural tube floor plate (Shh)
  - neural tube roof plate (Wnts)
  - epidermal ectoderm (BMPs)
  - lateral plate mesoderm (Fgfs)

Somite identity specified according to **Hox gene expression**
- once identity is specified, somites retain their patterns of Hox gene expression pattern, even if transplanted
**Somite Development**

**Sclerotome** - cartilage of vertebrae and part of rib

**Dermamyotome** - remaining portion of the somite contains precursors for:
- **Dermatome** – dermis (mesenchymal connective tissue of the skin)
- **Myotome** – muscle
**Primaxial** (epaxial) **myotome** – intercostal muscles of the ribs; deep muscles of the back

**Abaxial** (hypaxial) **myotome** – body wall, limbs, tongue
**Determination of the Somites**

**Sclerotome** – Shh (high) from the notochord and floor plate
- sclerotome cells secrete Pax1 (transcription factor)
- cartilage/vertebrae formation

**Dermatome** – neurotrophin-3, Wnt1 from roof plate (Shh antagonist)
Determination of the Somites

**Myotome:**
- primaxial – Shh (low), Wnt1, Wnt3
- abaxial – Wnt (epidermis), BMP4, Fgf5 (lateral plate mesoderm)

**Notochord** – degenerates through apoptosis (mostly; remnants remain as **nucleus pulposus**
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Somites:
- formation
- specification and differentiation

Mesodermal structures:
Muscle
Bone
Vertebrae
Tendon
Kidney
Primordial germ cells
Muscle

MyoD – definitive muscle marker
Alignment mediated by membrane glycoproteins, cadherins
Fusion mediated by metalloproteinases; e.g. meltrin

Note - stem cells can regenerate muscle after injury
- satellite, mesenchymal SCs
Bone

The skeleton is generated from:
- somites → vertebrae
- lateral plate mesoderm → limb bones
- neural crest cells → brachial arch, craniofacial bones

Ossification types

**Intramembranous** – direct conversion – mesenchyme to bone

**Endochondral** – mesenchyme to cartilage to bone

Mesenchymal cells commit to become cartilage

Committed mesenchyme cells condense into nodules

**Chondrocytes** proliferate;
- form cartilage model;
- secrete cartilage-specific extracellular matrix (ECM)

Chondrocytes stop dividing; increase volume (hypertrophy)

ECM shifts: ↑mineralization

secrete **angiogenesis factor** - VEGF
Endochondral Bone Formation

Reserve \rightarrow \text{Proliferating} \rightarrow \text{Prehypertrophic} \rightarrow \text{Hypertrophic} \rightarrow \text{Endochondral bone}
Bone/Cartilage Differentiation

(B)

Mesenchymal cells → Prechondrocyte → Reserve chondrocyte → Proliferating chondrocyte → Prehypertrophic chondrocyte → Hypertrophic chondrocyte → VEGF

- BMPs, FGFs
- Wnt
- BMPs

- Parathyroid hormone-related hormone

<table>
<thead>
<tr>
<th>Pax1</th>
<th>NCAM</th>
<th>N-cadherin</th>
<th>Sox9, Agrim, collagen 2</th>
<th>Runx2, Dlx5/6</th>
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- Twist
- Runx2
- Wnt

Pre-osteoblast → Osteoblast → Osteocyte

(bone formation)
Endochondral Bone, continued

Blood vessels invade the cartilage model; hypertrophic chondrocytes die; replaced by **osteoblasts** (sclerotome)

ECM mineralizes (CaPO₄)

New bone material added peripherally from the internal surface of the **periosteum**

**Osteoclasts** (lateral plate mesoderm) hollow internal region - bone marrow cavity
Vertebral Formation

Notochord induces mesenchyme to attract sclerotome cells
- differentiates into cartilage (endochondral bone formation)
Sclerotomes split into rostral and caudal segments
Re-segmentation enables muscles to coordinate movement
Tendon

Syndetome is induced by myotome cells (above) Fgf8 inducing scleraxis in first row of sclerotome cells (below)

Scleraxis transcription is blocked by sclerotome cartilage precursors
Tendon

Sclerotome
Myotome

Dermatome

Sclerotome
Syndetome
Myotome

Myotome

FGF

Sclerotome

Shh

Pea3, Erm

Sox9
Sox5, Sox6

Cartilage

Tendon

Myotome

Sclerotome

Tendons

Rib

Tendons

Intercostal muscle

Vertebrae

Neural tube

Vertebral progenitors

Anterior

Muscle progenitors

Posterior

Note – ant, post.
Major Mesoderm Lineages

BMP

Lim1
Pax2
Pax8

Intermediate mesoderm

Kidney
Gonads
Development of the Vertebrate Kidney

Pronephric
- fish
- amphibian larvae

Wolffian duct

Mesonephric (function - ?)

Nephrogenic cord

Cloaca
Pronephros - Mesonephros
Development of the Vertebrate Kidney

- Pronephric
  - fish
  - amphibian larvae

- Mesonephric
  (function - ?)

- Metanephric (amniotes)

**Pronephros**
- Nephric duct
- Mesonephros
- Nephrogenic cord
- Cloaca

**Wolffian duct**
- Gonad
- Mesonephros
- Metanephrogenic mesenchyme
- Ureteric bud
- Nephric bud

**Metanephric (amniotes)**
Metanephric Kidney

Reciprocal inductions between the urereric bud and metanephrogenic mesenchyme result in formation of the nephron – the functional unit of the mammalian kidney.

Mesonephric tubules and duct become:
- efferent ducts
- epididymus
- vas deferens

reproductive system

Mesonephric tubules
Gonad
Nephric duct
Metanephrogenic mesenchyme
Ureter
Primordial Germ Cells

**PGCs**

**Amphibians** – germ plasm at vegetal pole
- maneuvered during cleavage to endoderm at floor of blastocoel
- migrate along gut to genital ridges

**Mammals** – migrate into endoderm from posterior primitive streak
- migrate along hindgut & mesentery to the gonadal rudiment

**Reptiles, birds** – originate in epiblast
- migrate to hypoblast (germinal crescent)
- distributed through blood stream
- enter gonadal rudiment