## Amniotes

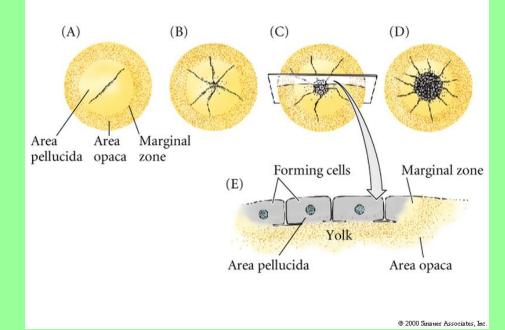
- Birds, reptiles and mammals
- Extraembryonic adaptations for terrestrial life
  - yolk sac
  - allantois
  - amnion
  - chorion
- In birds and reptiles, development takes place on substratum of yolk
  - flattened

# Early Chick Development

- Meroblastic
- Discoidal
- Forms blastoderm on top of yolk
- Forms subgerminal space
- From top, see an area pellucida and area opaca
- Marginal zone in between

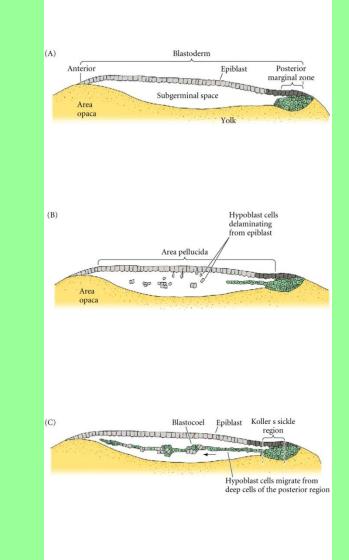
## Chick Blastula

- Single layer
   blastoderm becomes
   multi-cell thick over
   cavity: subgerminal
   space
- Then becomes one cell thick area pellucida



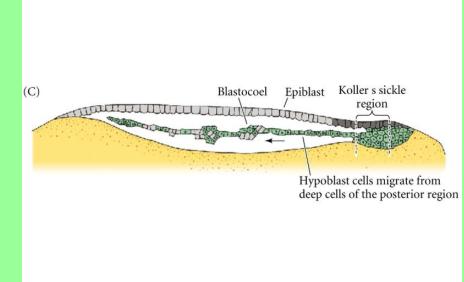
# Chick Blastoderm

- Area opaca remains thick
- In between, posterior marginal zone
- Pellucida cells delaminate and migrate into subgerminal cavity
- Others migrate from marginal zone (Kohler's sickle)
  - form 2º hypoblast
- Space is blastocoele



## Chick Blastoderm Fates

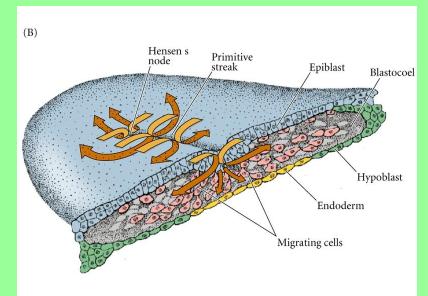
- Epiblast forms all 3 germ layers
- Hypoblast contributes to yolk sac



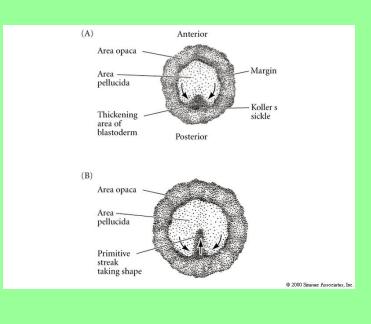
© 2000 Sinauer Associates, Inc

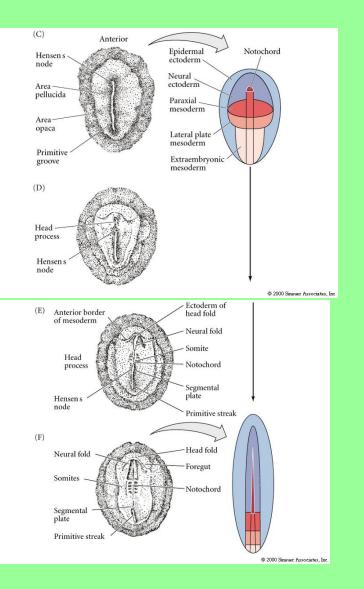
## What is the Primitive Streak?

- In birds, reptiles and mammals
- By migration of cells into blastocoele
- Elongation towards future head
- Defines axes
  - start at posterior
  - ingression is from dorsal
  - separates left and right



#### Primitive Streak Movements

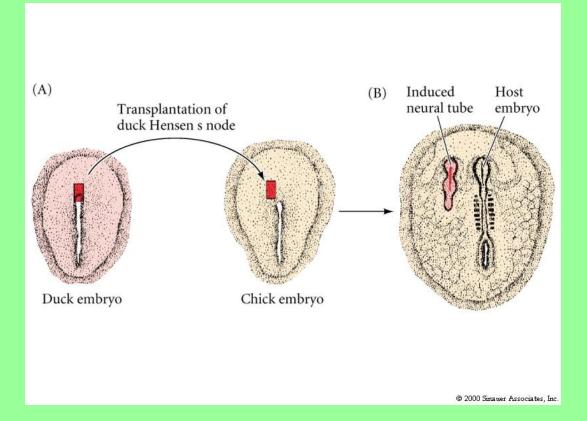




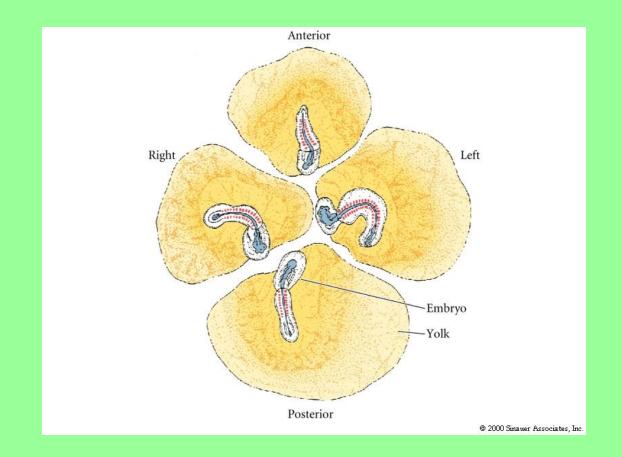
### What is Hensen's Node?

- The primitive groove of the primitive streak
  - Equivalent to blastopore
  - Cells form most endoderm and mesoderm
- Thickening at anterior end (Hensen's node)
  - equivalent to dorsal lip of blastopore
  - cells ingressing form foregut, head mesoderm, notochord
- Primitive streak regresses to posterior followed by Hensen's node laying down notochord

# Transplantation of Hensen's Node Induces 2º Axis



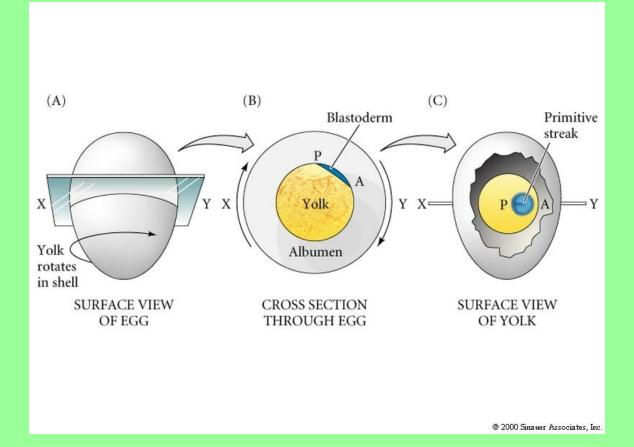
# **Chick Regulation**



### How Does A-P Axis Form?

- Gravity shifts yolk
  - upper portion of blastoderm becomes posterior, lower anterior
- Posterior marginal zone PMZ = Nieuwkoop center
  - inhibits rest of marginal zone
- Rest is capable of making primitive streak if separated from PMZ

#### A-P Axis in Chick



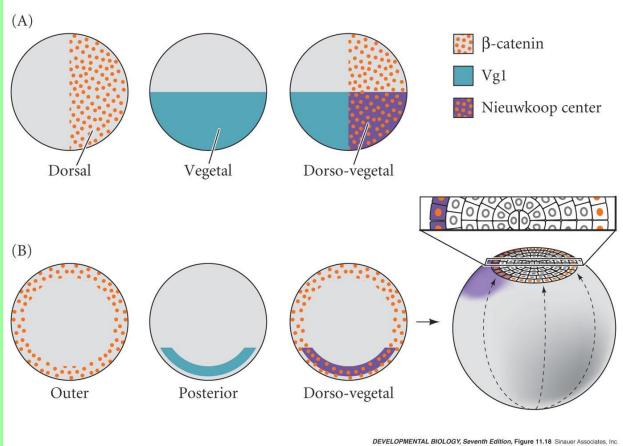
## How Does DV Axis Form?

- pH distinguishes
  - epiblast facing albumin = dorsal
  - yolk side = ventral
  - upper albumin pH 9.5
  - subgerminal cavity pH 6.5
- Also potential difference: upper is negative – dorsal
- Reverse DV by either pH or electrical potential reversal

# How Does Mesodermal Inducer Form?

- PMZ = Nieuwkoop center
  - $-\beta$ -catenin in rim of blastoderm
  - Veg1 in presumptive posterior
  - Overlap equals center
- Transplant to anterior makes primitive streak and Hensen's node forms from surrounding cells
- *Nodal* and *veg1* express first in Kohler's sickle then throughout primitive streak

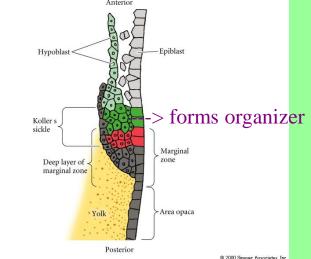
### Amphibian vs Chick



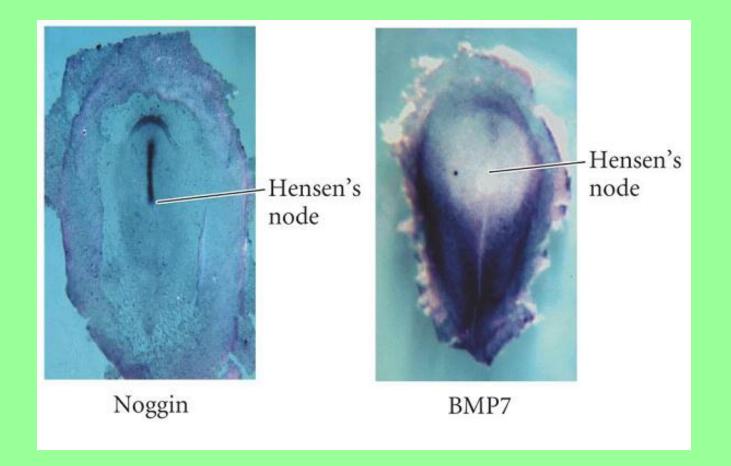
© 2003 All rights reserved.

# Hensen's Node (Organizer)

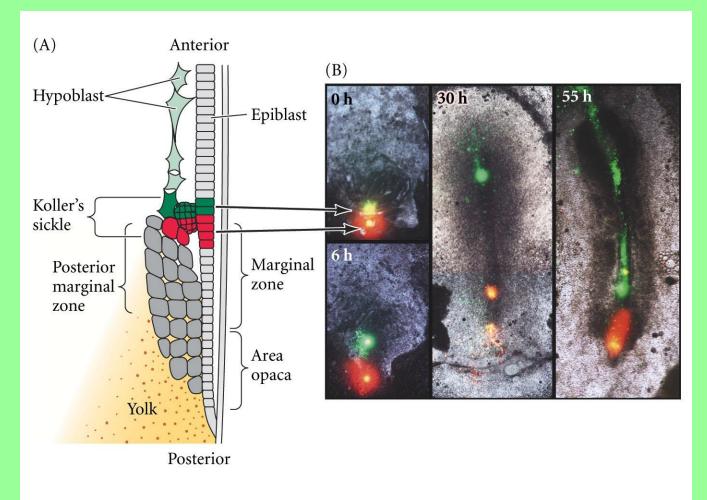
- Forms just anterior to Nieuwkoop center
- *Chordin* and *sonic hedgehog* expressed in most anterior primitive streak and Hensen's node
- Then makes *noggin*, more *chordin*, *nodal* which antagonize BMPs
  - dorsalize
- Also need FGF



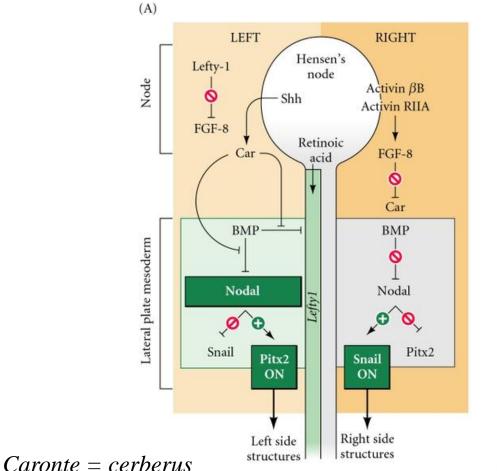
## Noggin vs BMP



#### Hensen's Node Movement



## Left-Right Signal Pathway





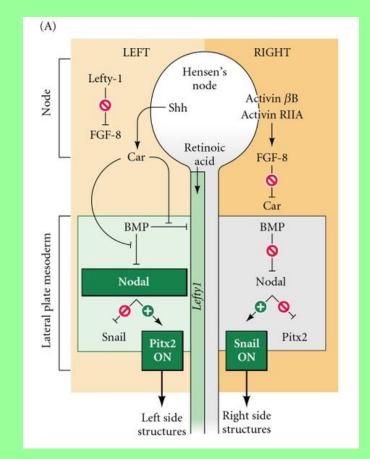
#### Nodal mRNA

© 2000 Sinauer Associates, Inc.

# Left-Right Asymmetry in Chick

- Regulated by Nodal (paracrine signaling molecule) and Pitx2 (transcription factor)
- Activin expression on right blocks Shh which starts cascade on left
- On left Lefty-1 blocks FGF-8

   Sets up a wall
- On right FGF-8 is active



#### **Evolutionary Conservation So Far**

- Fish, amphibians and birds all show similarities in
  - vegetal endodermal factors
  - $-\beta$ -catenin to mark Nieuwkoop center
  - organizer that makes factors that antagonize
     BMPs
- Adaptations alter anatomy