FATE MAPS

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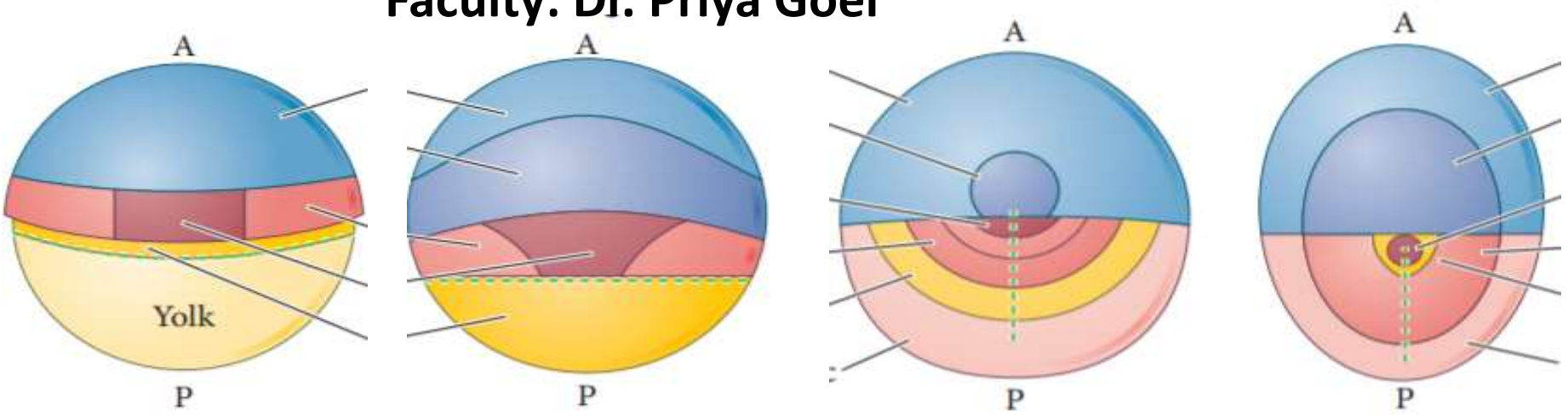
Faculty: Dr. Priya Goel



FATE MAPS

- **Fate map** is a diagrammatic representation of the prospective fate of each part of an embryo at an early stage of development
- Embryonic regions with a distinct fate are called **Primordia / Rudiments**
- **Fate maps change over time** –as- cells multiply & move relative to each other
- Thus, fate maps indicate fates at a closely following stage
- A series of fate maps at consecutive stages shows the progression of different cells or regions through longer periods of development
- **Significance**:
  - Essential tools in most embryological experiments
  - Help to trace cell lineage by following the fate of parts of early embryo during development*
  - Helps understand the mechanism of morphogenetic movements during gastrulation

Different ways of establishing fate maps

- **Observing living embryos with few cells - under the microscope – augmented with histological sections & time-lapse motion pictures**
- **Large/opaque embryos can be mapped by labeling specific cells**
- ***Mammalian embryos are among the most difficult to map (since they develop inside another organism), and researchers are actively constructing, refining, and arguing about the fate maps of mammalian embryos.***
- **Characteristics of a good label**
  - **should not spread to neighbouring cells**
  - **readily detectable at later stages**
  - **should not interfere with normal cell development**

LABELING METHODS

NATURAL MARKERS

- **natural colour differences in the cytoplasm of early blastomeres**
- fertilized eggs of Ascidians e.g. *Ciona,* Tunicate (Sea squirt) *Styela partita* (Cynthia)
- **E.G.Conklin (1905)**: followed the fate of each blastomere
  - cells containing clear cytoplasm → ectoderm
  - Dark grey yolky → endoderm
  - Light grey → notochord & neural plate
  - Yellow → mesoderm

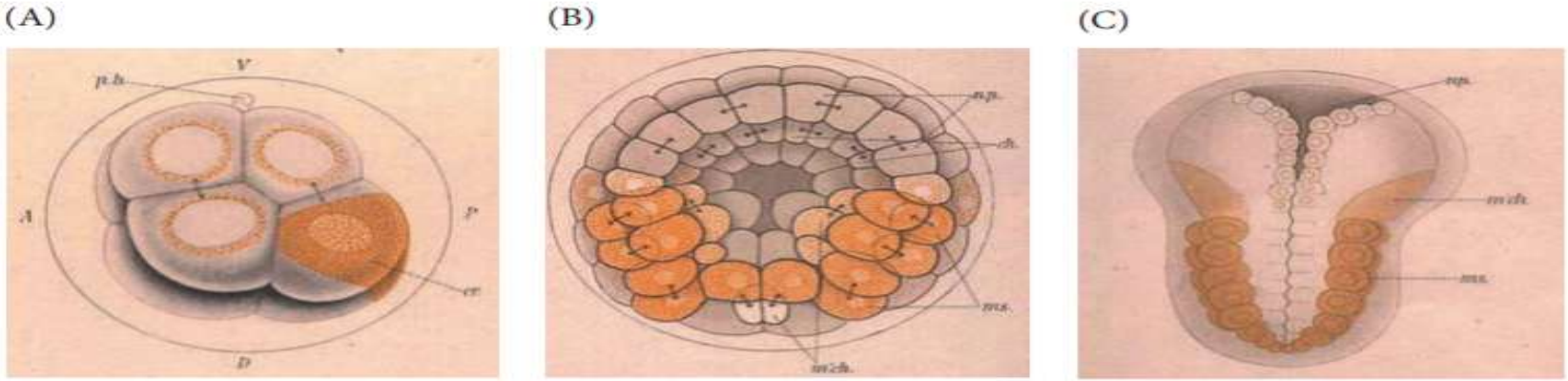


FIGURE 1.12 The fates of individual cells. Edwin Conklin mapped the fates of early cells of the tunicate *Styela partita*, using the fact that in embryos of this species many of the cells can be identified by their different colored cytoplasms. Yellow cytoplasm marks the cells that form the trunk muscles. (A) At the 8-cell stage, two of the eight blastomeres contain this yellow cytoplasm. (B) Early gastrula stage, showing the yellow cytoplasm in the precursors of the trunk musculature. (C) Early larval stage, showing the yellow cytoplasm in the newly formed trunk muscles. (From Conklin 1905.)

ARTIFICIAL METHODS

- **VITAL DYES: Vogt (1925)**
  - stain cells but do not kill them: **Nile Blue Sulphate, Neutral red, Bismark brown, Janus green**
  - spread dye mixed with agar / cellophane on the slide → dried → pressed against a chosen area of blastula for a short period → stain diffuses to blastomeres → stained cells' movements are followed within the embryo
  - Several areas can be marked separately simultaneously & their fate traced
  - **But become diluted with each cell division**

- **CARBON PARTICLES:**
  - **N. Spratt (1946) – fate map of chick:** C-particles stick on the cell surface & used as markers to trace the cell fate
  - **William W. Ballard (1981) – modified technique:** injected C-particles or chalk particles inside the particular region of teleost embryo for tracing its fate
  - **But become diluted with each cell division**

- **RADIOACTIVE MARKERS: C14, P32, H3 in Chick embryo**
  - **Cells with these markers are studied by autoradiography to trace their fate**
  - **Tritiated thymidine labels the nuclei when incorporated into the DNA of cells**
  - **A region of interest is cut from host embryo → replaced by radioactive graft from donor embryo**
  - **Limitation: become diluted with each cell division; exposure to radioactivity**
- **HISTOCHEMICAL STAINS**: Enzyme specific staining of embryonic cells → can be visualized by adding appropriate substrate for its enz.activity
  - e.g. enz. **Horseradish peroxidase (HRP)**

- **FLUORESCENT DYES:**
  - fluorescent dyes **conjugated** to large, metabolically inert **carrier molecules**, that will not cross cell membranes or pass through gap junctions → **microinjected** into one or more cell, all descendants of the injected cell are labeled distinctly under a fluorescent microscope
  - e.g. **fluorescently labeled DEXTRAN: Fluorescein Dextran Amine (FDA) & Rhodamine Dextran Amine (RDA)**
  - e.g. **fluorescent carbocyanine dyes: dialkyl indocarbocyanine (DiI) & dialkyl oxocarbocyanine (DiO)** are lipophilic membrane stains that diffuse in the cell → produce red & green emissions resp.
  - **Microinjection of stable mRNAs encoding fluorescent proteins**: long lasting label

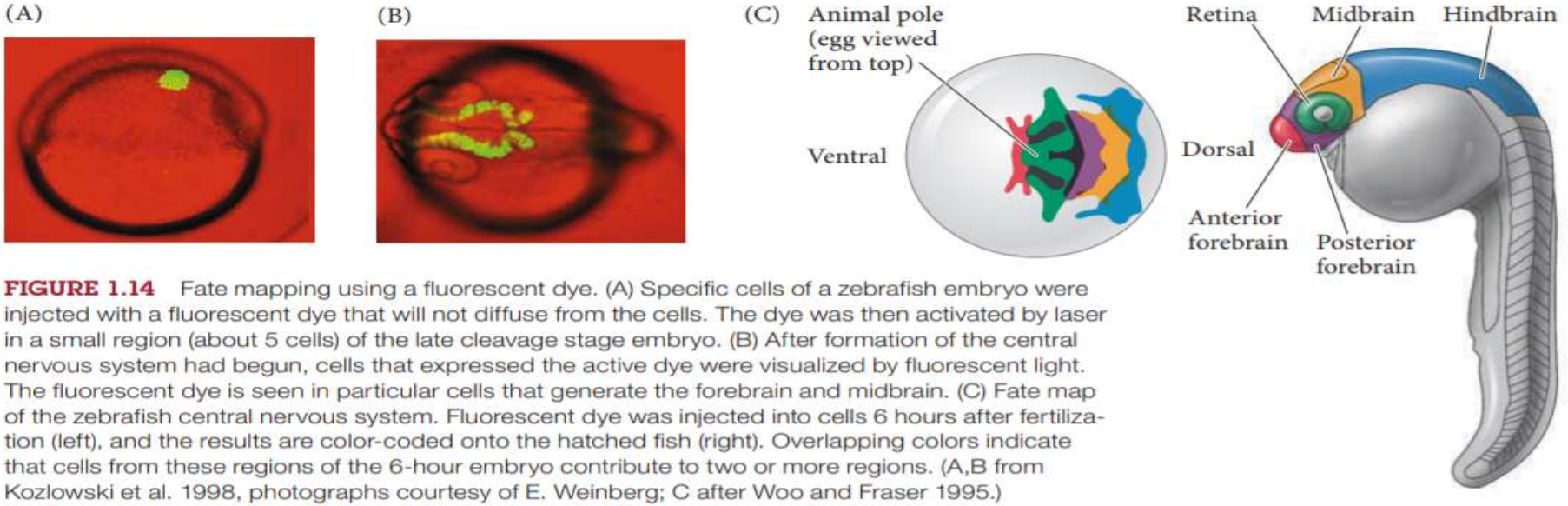
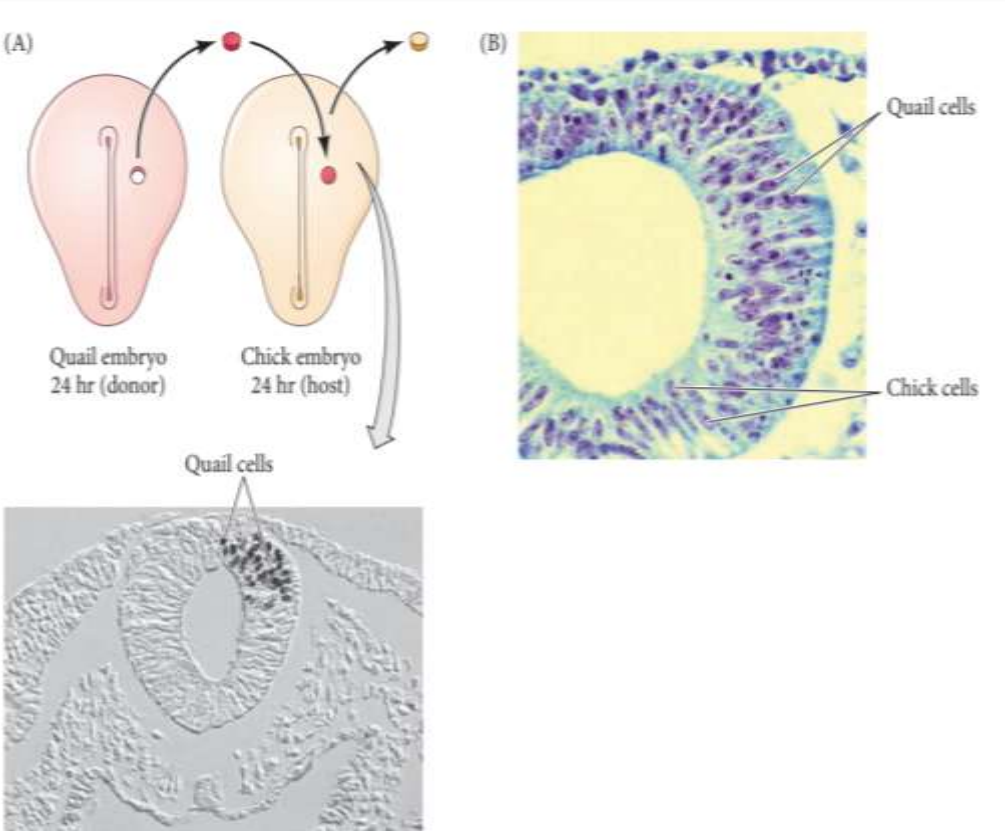


FIGURE 1.14 Fate mapping using a fluorescent dye. (A) Specific cells of a zebrafish embryo were injected with a fluorescent dye that will not diffuse from the cells. The dye was then activated by laser in a small region (about 5 cells) of the late cleavage stage embryo. (B) After formation of the central nervous system had begun, cells that expressed the active dye were visualized by fluorescent light. The fluorescent dye is seen in particular cells that generate the forebrain and midbrain. (C) Fate map of the zebrafish central nervous system. Fluorescent dye was injected into cells 6 hours after fertilization (left), and the results are color-coded onto the hatched fish (right). Overlapping colors indicate that cells from these regions of the 6-hour embryo contribute to two or more regions. (A,B from Kozlowski et al. 1998, photographs courtesy of E. Weinberg; C after Woo and Fraser 1995.)

- **GENETIC MARKERS**
  - One way of permanently marking cells and following their fates is to create embryos in which the same organism contains cells with different genetic constitutions.
  - **Advantages**: donot spread to neighboring cells, If stably expressed, are inherited by the descendants of the marked cell
  - **Limitations**: Low efficiency of introducing the gene in the cell
  - e.g. used to create **Chimeric Embryos:** by **Xenoplastic transplantation of** embryonic grafts from animal of interest having different genetic constitution but similar development pattern

e.g. **Chick-quail chimeras** are made by grafting embryonic quail cells inside a chick embryo while the chick is still in the egg.

- Chicks and quail embryos develop in a similar manner (especially during the early stages), and the grafted quail cells become integrated into the chick embryo and participate in the construction of the various organs
- The chick that hatches will have quail cells in particular sites, depending on where the graft was placed.



Quail cells also differ from chick cells in several important ways, including the species-specific proteins that form the immune system.

There are quail-specific proteins can be used to find individual quail cells, even when they are “hidden” within a large population of chick cells

By seeing where these cells migrate, fine-structure maps of the chick brain and skeletal system have been produced

Chimeras dramatically confirmed the extensive migrations of the neural crest cells during vertebrate development

- Mary Rawles (1940) showed that the pigment cells (melanocytes) of the chick originate in the neural crest, a transient band of cells that joins the neural tube to the epidermis.
  - When she transplanted small regions of neural crest-containing tissue from a pigmented strain of chickens into a similar position in an embryo from an unpigmented strain of chickens, the migrating pigment cells entered the epidermis and later entered the feathers

Chick resulting from transplantation of a trunk neural crest region from an embryo of a pigmented strain of chickens into the same region of an embryo of an unpigmented strain. The neural crest cells that gave rise to the pigment migrated into the wing epidermis and feathers

- **Ris (1941) used similar techniques to show that, although almost all of the external pigment of the chick embryo came from the migrating neural crest cells**
  - **the pigment of the retina formed in the retina itself and was not dependent on migrating neural crest cells.**
  - **This pattern was confirmed in the chick-quail chimeras, in which the quail neural crest cells produced their own pigment and pattern in the chick feathers.**

Transgenic DNA chimeras

- most animals - difficult to meld a chimera from two species.
- Hence, better to transplant cells from a genetically modified organism (retrovirus) → the genetic modification can then be traced only to those cells that express it
- **Retrovirus marking:** incorporating retrovirus engineered reporter gene into DNA of host cells → expression of reporter gene → histochemical/fluorescent marking of gene products
- Expression of host DNA to express **Green Fluorescent Protein (GFP)**
  - naturally occuring in some jellyfishes
  - When the infected embryonic cells are transplanted into a wild-type host, only the donor cells will express GFP
- **BRAINBOW: a recent technique used to tag neurons in brain**
  - fluorescent proteins are used to tag different proteins in the cell → an array of colours are generated by different expression of distinct fluorescent proteins

Experiment: Fate mapping with transgenic DNA showed that the neural crest is critical in making the gut neurons

- **Freem *et al* (2012)** used transgenic techniques to study the migration of neural crest cells to the gut of **chick embryos**, where they form the neurons that coordinate peristalsis—the muscular contractions of the gut necessary to eliminate solid waste.
- The parents of the GFP-labeled chick embryo were infected with a replication-deficient virus that carried an active gene for GFP
- This virus was inherited by the chick embryo and expressed in every cell → glowed green when placed under ultraviolet light
- They then transplanted the neural tube and neural crest of a GFP-transgenic embryo into a similar region of a normal chick embryo
- A day later, they could see GFP-labeled cells migrating into the stomach region and by 7 days, the entire gut showed GFP staining up to the anterior region of the hindgut

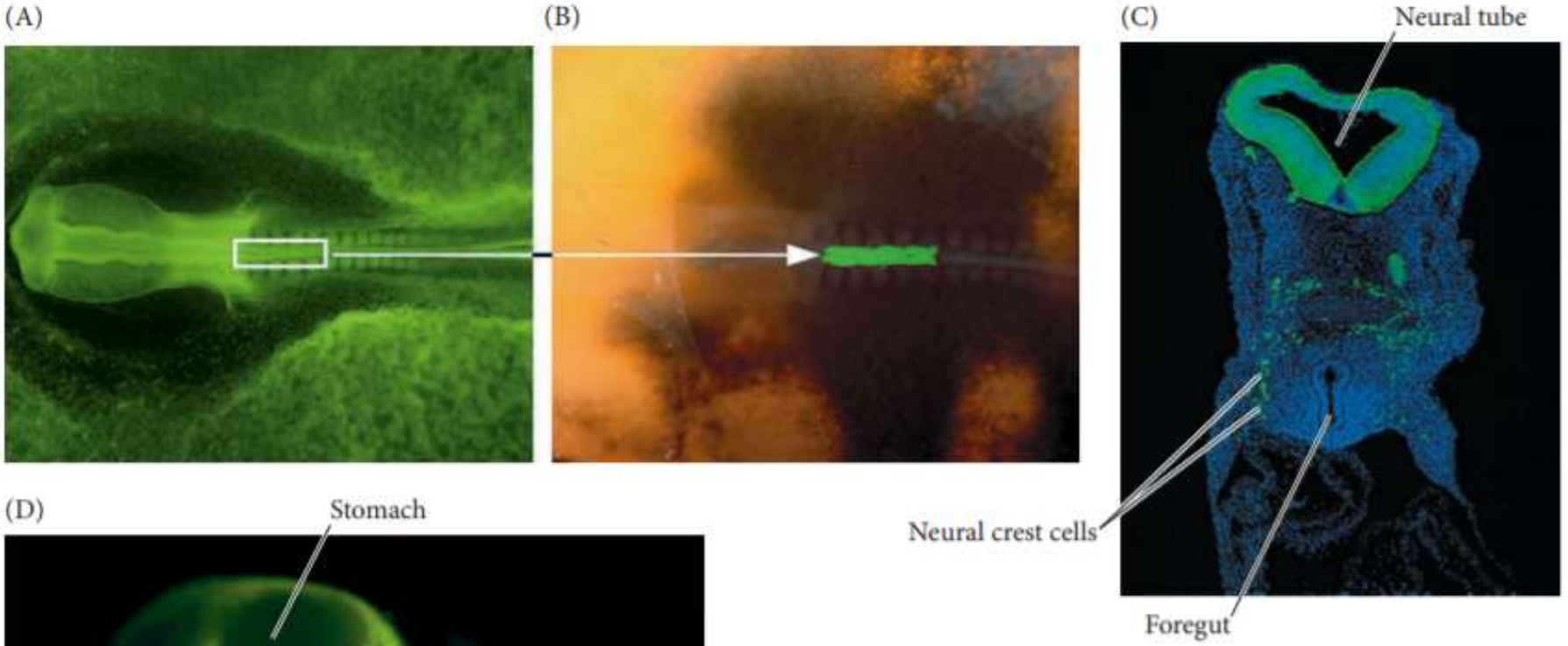
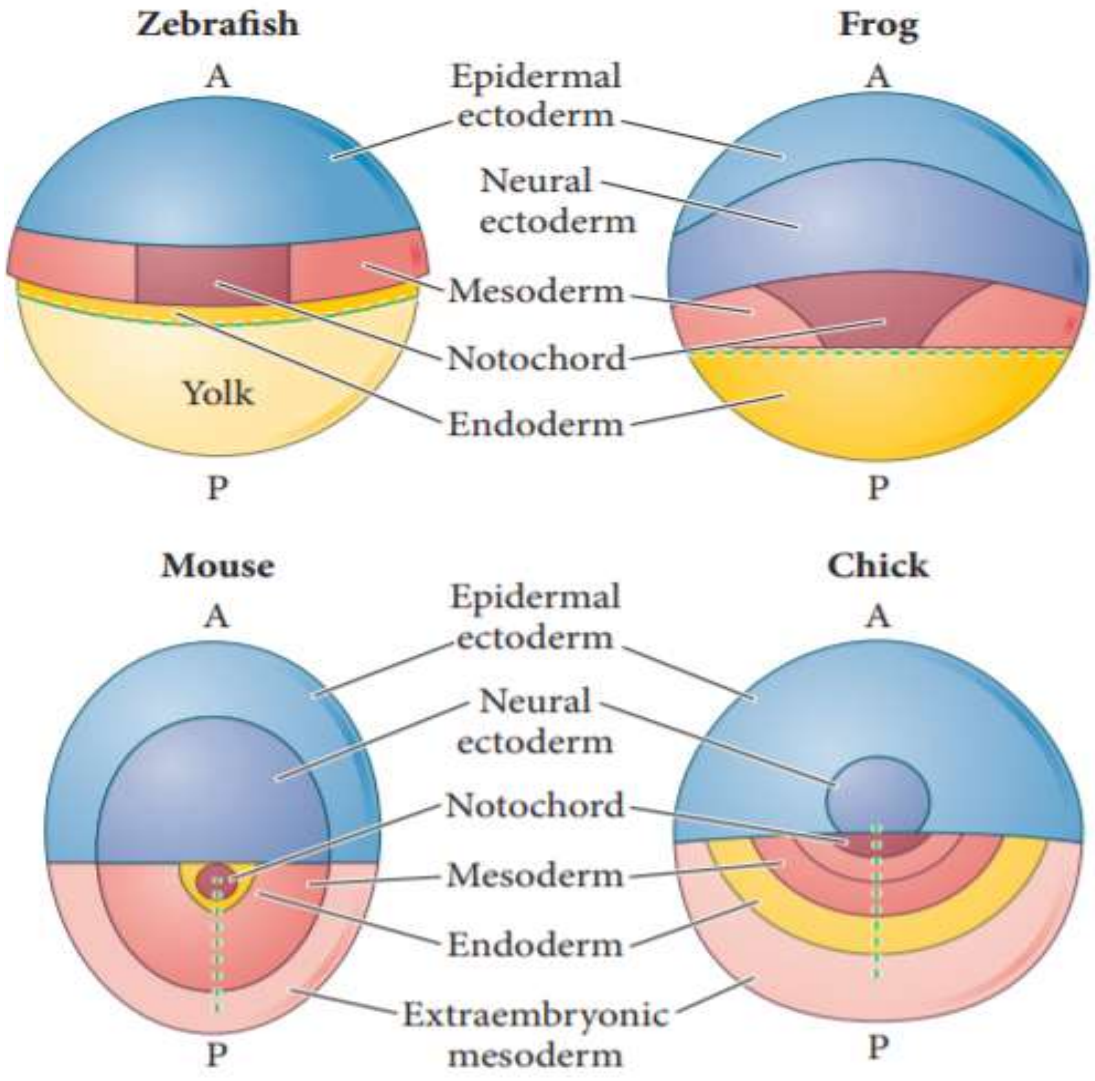


FIGURE 1.16 Fate mapping with transgenic DNA shows that the neural crest is critical in making the gut neurons. (A) A chick embryo containing an active gene for green fluorescent protein expresses GFP in every cell. The brain is forming on the left side of the embryo, and the bulges from the forebrain (which will become the retinas) are contacting the head ectoderm to initiate eye formation. (B) The region of the neural tube and neural crest in the presumptive neck region (rectangle in A) is excised and transplanted into a similar position in an unlabeled wild-type embryo. One can see it by its green fluorescence. (C) A day later, one can see the neural crest cells migrating from the neural tube to the stomach region. (D) In 4 more days, the neural crest cells have spread in the gut from the esophagus to the anterior end of the hindgut. (From Freem et al. 2012; photographs courtesy of A. Burns.)



FATE MAPS OF VERTEBRATES AT THE EARLY GASTRULA STAGE:

All are dorsal surface views (looking "down" on the embryo at what will become its back). Despite the different appearances of the adult animals, fate maps of these four vertebrates show numerous similarities among the embryos. The cells that will form the notochord occupy a central dorsal position, while the precursors of the neural system lie immediately anterior to it. The neural ectoderm is surrounded by less dorsal ectoderm, which will form the epidermis of the skin. A indicates the anterior end of the embryo, P the posterior end. The dashed green lines indicate the site of ingression—the path cells will follow as they migrate from the exterior to the interior of the embryo.

EXAMPLES (DIY)

- **Fate map of Amphioxus**
- **Fate map of frog *Xenopus laevis***
- **Fate map of chick**
- **Fate map of mouse**