vizsga minimum követelmények
exam test basic requirements

BEGINNING OF DEVELOPMENTAL BIOLOGY. ORGANIZATION CENTERS, SPEMANN ORGANISER AND ITS MOLECULARBACKGROUND.

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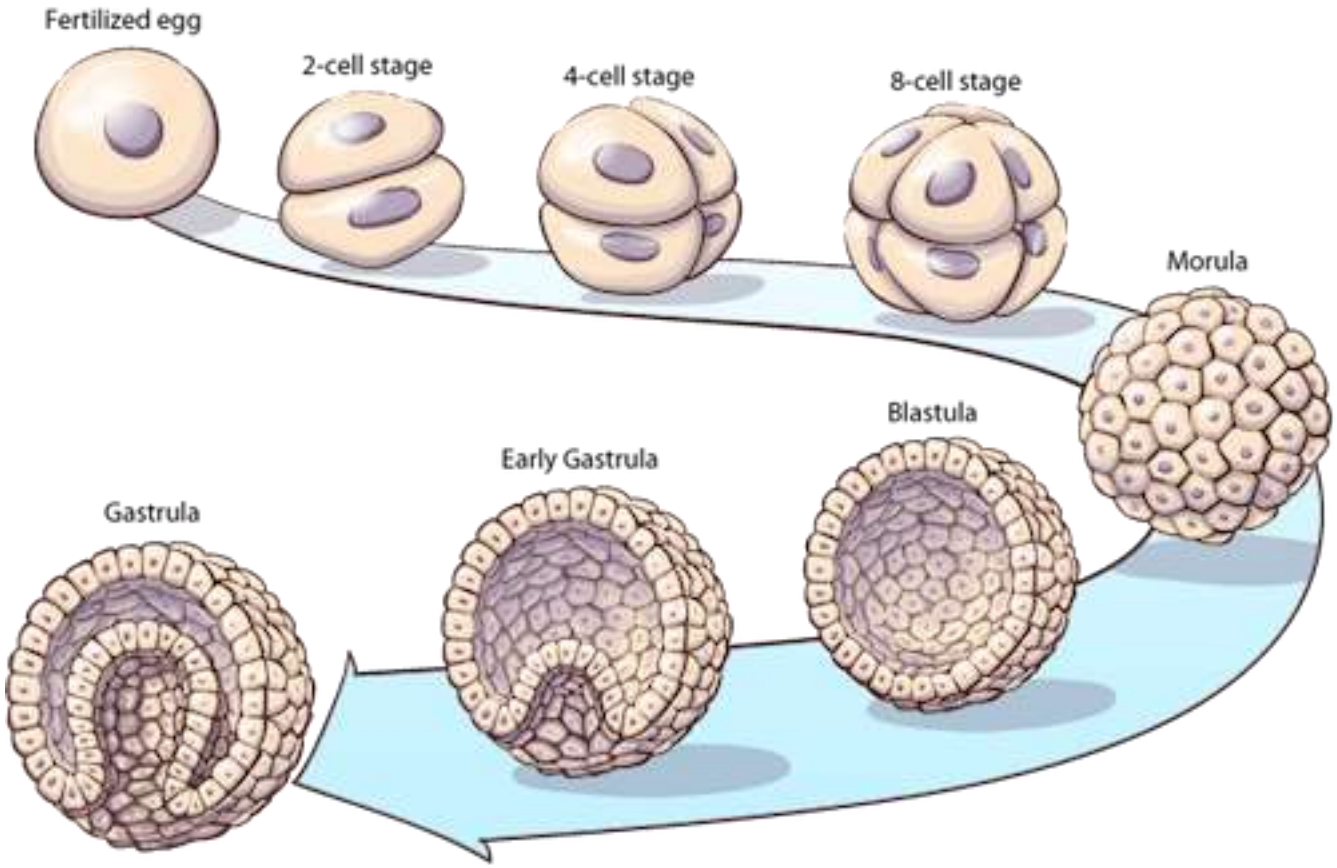
Developmental Biology I. 2017

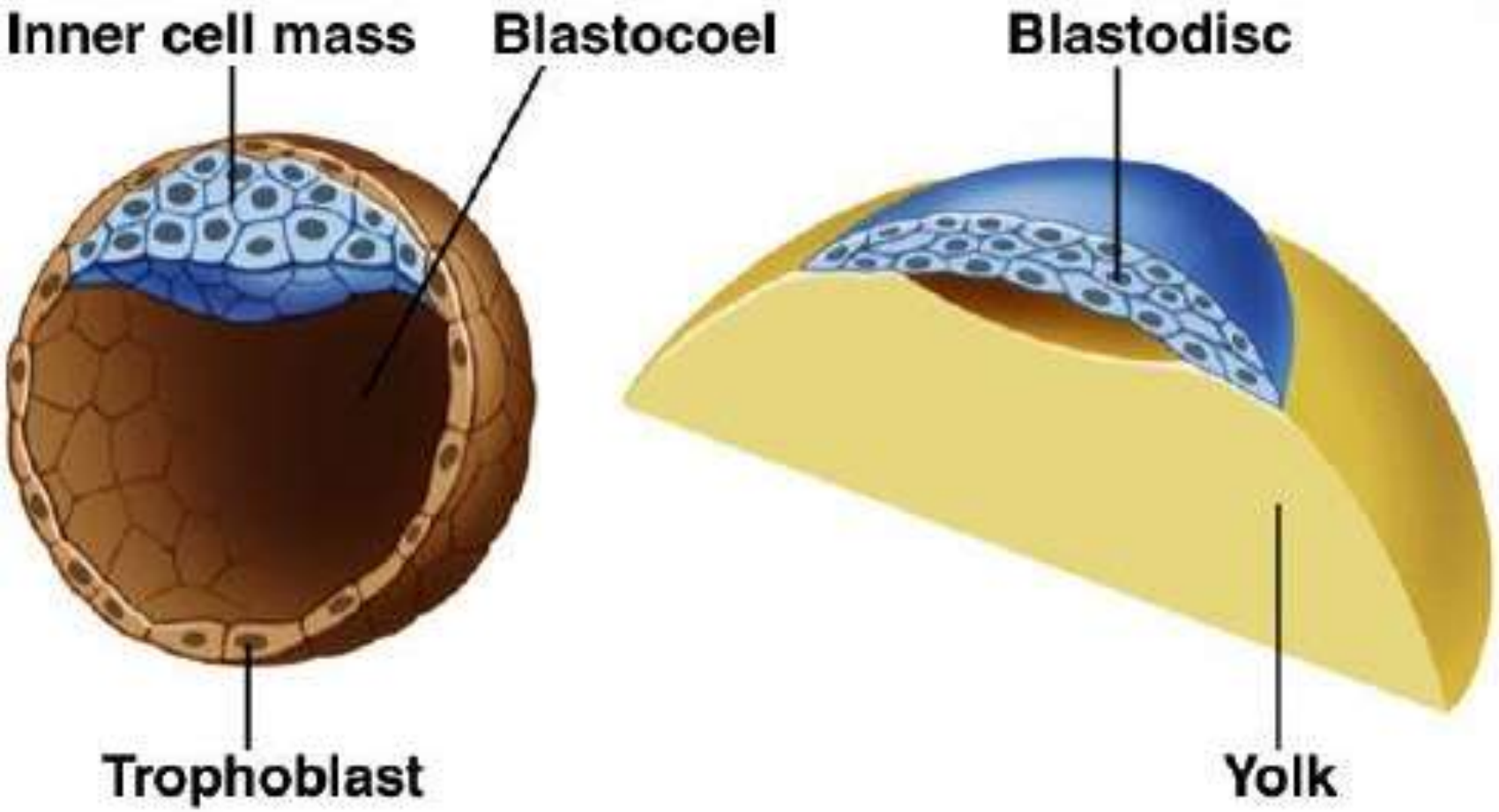
A central question in developmental biology is how form and pattern emerge from the simple beginnings of a fertilized egg. How and when do individual cells and tissues decide which developmental route to take? Are cell fates somehow predetermined or do cells and tissues interact with one another to orchestrate developmental processes?

Egg, mulberry, vesicle



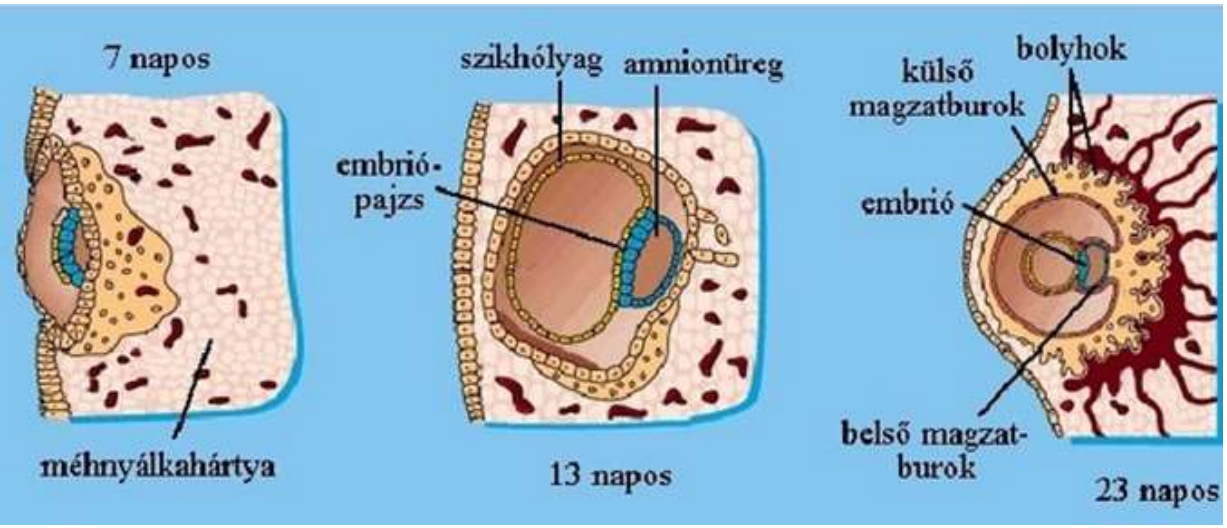
Cell Cleavage
Process by which the number of cells in a developing embryo is multiplied through cell division.

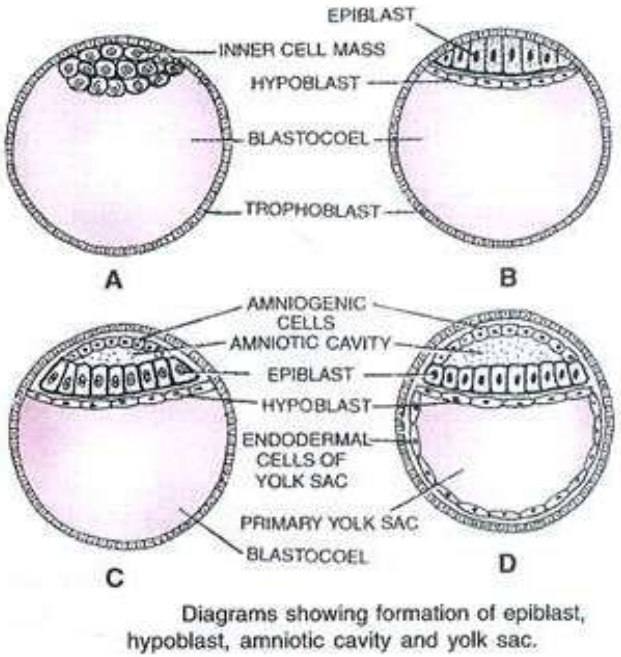
Blastula of Mammals and Birds



http://www.gastrulation.org/Movie9_3.mov

Let's start embryogenesis!





Diagrams showing formation of epiblast, hypoblast, amniotic cavity and yolk sac.

Formation of Embryonic Disc:

We have seen that early blastocyst consists of inner cell mass and trophoblast. The inner cell mass contains cells called stem cells which have the potency to give rise to all tissues and organs. The cells of the inner cell mass differentiate into two layers around 8 days after fertilization, a hypoblast and epiblast.

The hypoblast (primitive endoderm) is a layer of columnar cells and epiblast (primitive ectoderm) is a layer of cuboidal cells. The cells of the hypoblast and epiblast together form a two layered embryonic disc.

Formation of Amniotic Cavity:

A space appears between epiblast and trophoblast, called amniotic cavity filled with amniotic fluid. The roof of this cavity is formed by amniogenic cells derived from the trophoblast, while its floor is formed by the epiblast

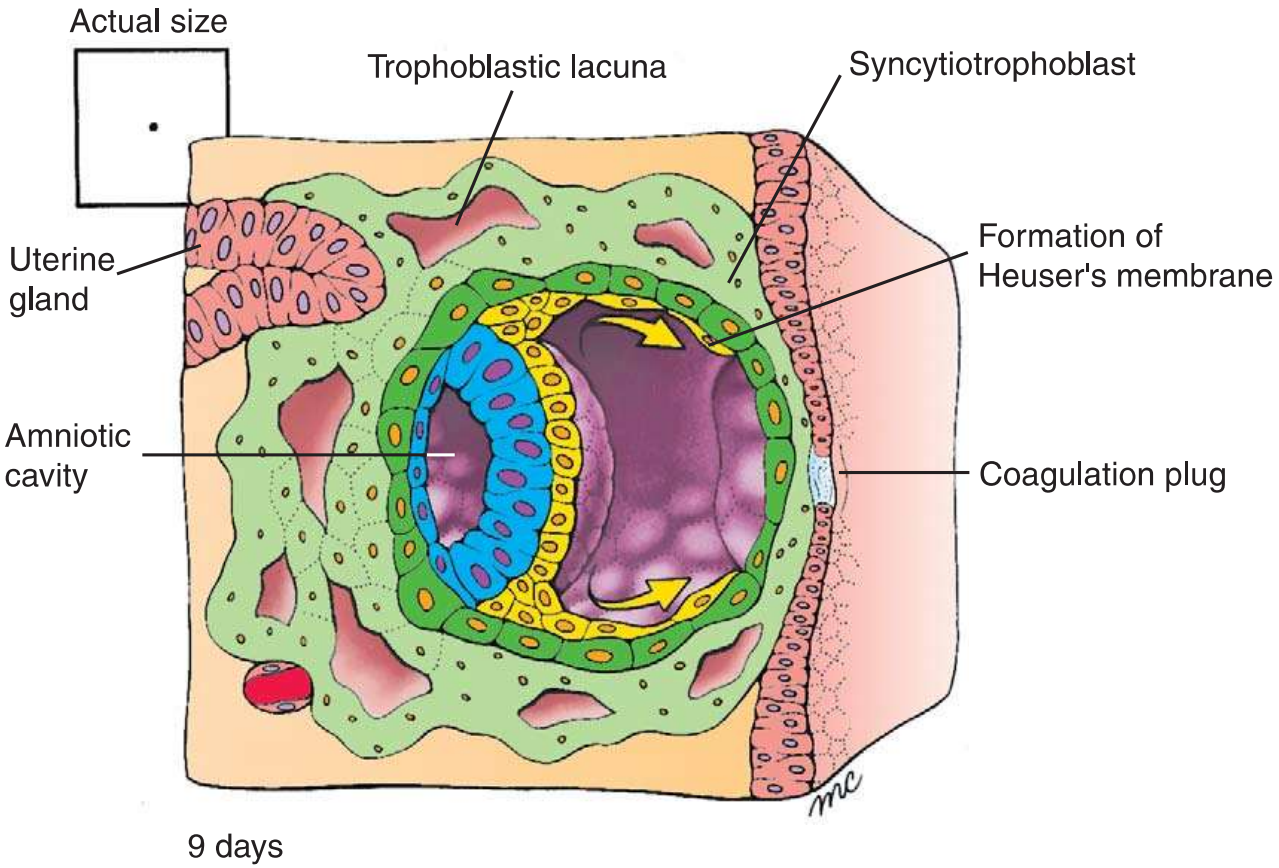


Figure 2-3. By 9 days, the embryo is completely implanted in the uterine endometrium. The amniotic cavity is expanding, and cells from the hypoblas have begun to migrate to form Heuser's membrane. Trophoblastic lacunae form in the syncytiotrophoblast, which now completely surrounds the embryo The point of implantation is marked by a transient coagulation plug in the endometrial surface.

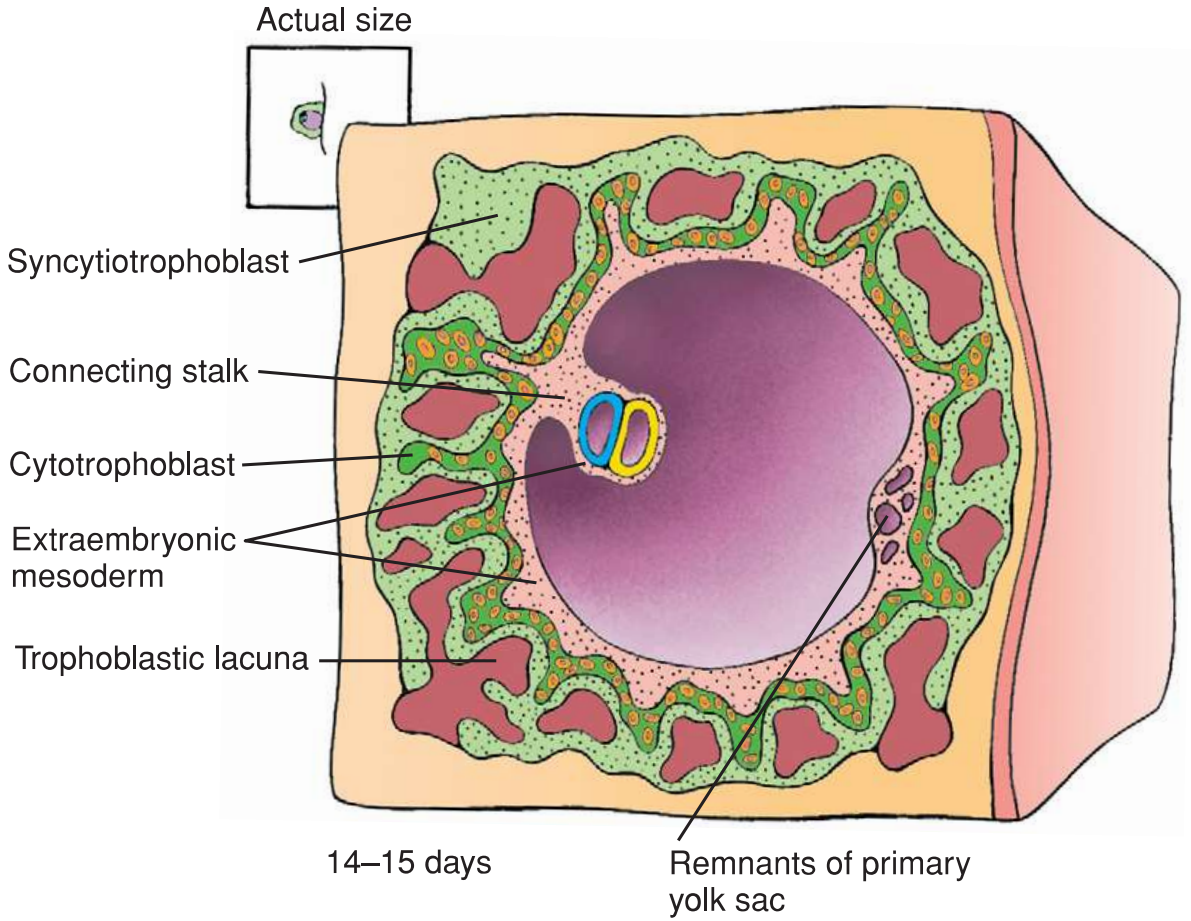


Figure 2-6. By the end of the second week, the definitive yolk sac loses contact with the remnants of the primary yolk sac, and the bilaminar embryonic disc with its dorsal amnion and ventral yolk sac is suspended in the chorionic cavity by a thick connecting stalk.

"It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life."

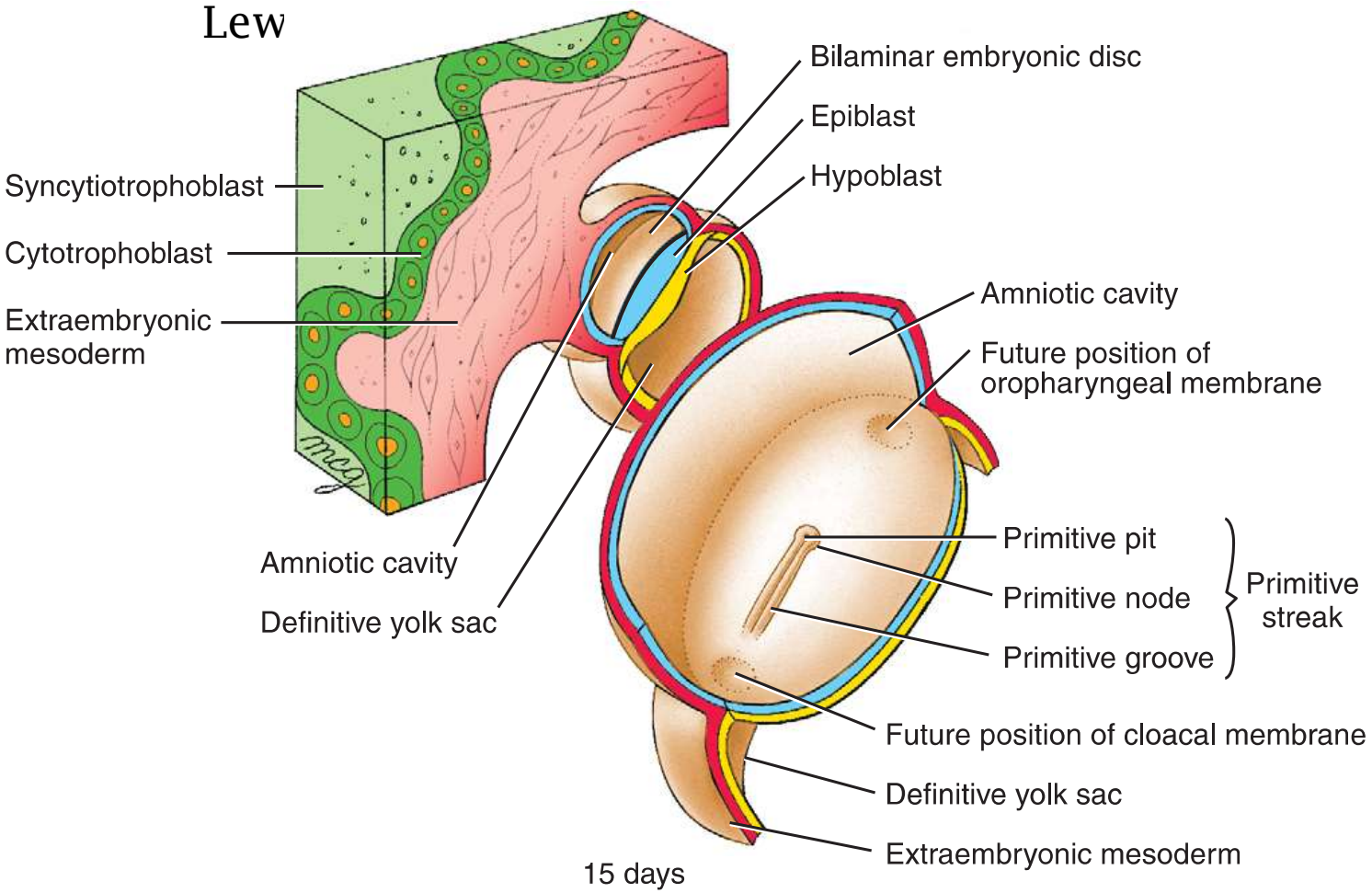
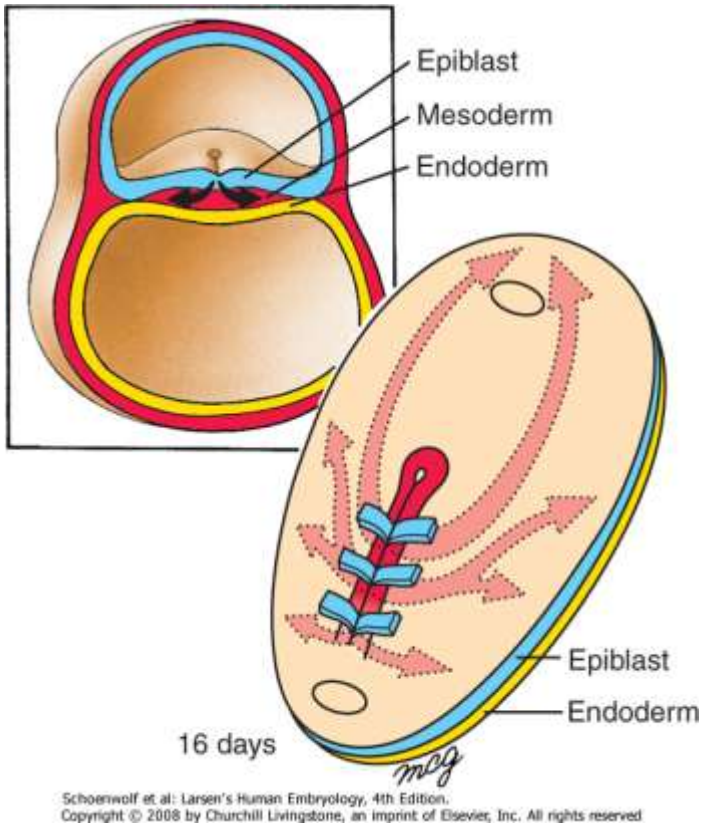
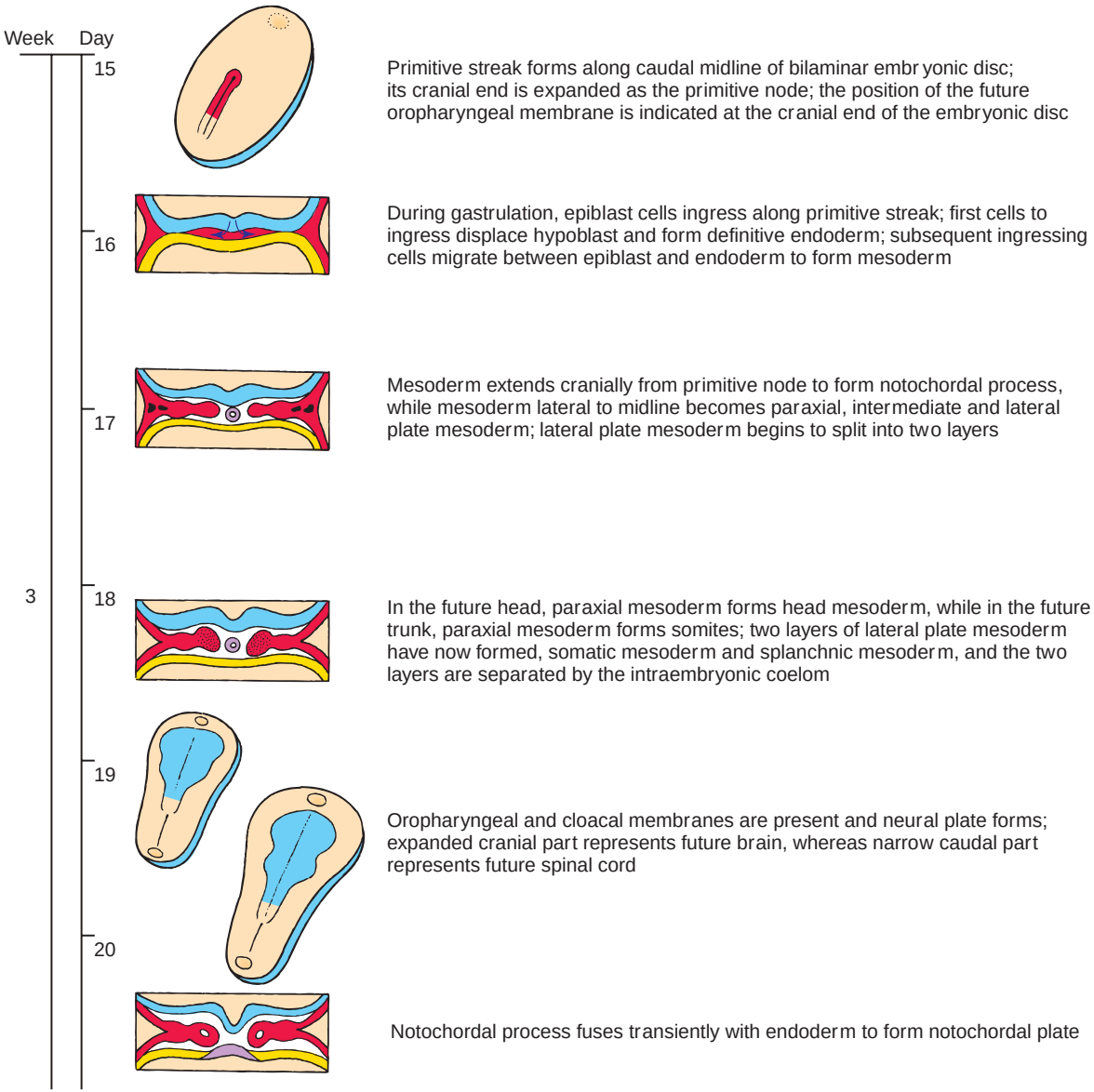


Figure 3-1. View of dorsal surface of bilaminar embryonic disc through sectioned amnion and yolk sac. Inset at upper left shows relation of the embryo to the wall of the chorionic cavity. The primitive streak, now 1 day old, occupies 50% of the length of the embryonic disc. The future positions of oropharyngeal and cloacal membranes are indicated.

The first major event of the 3rd week, **gastrulation,** commences with the formation of a longitudinal midline structure, the primitive streak, in the epiblast near the caudal end of the bilaminar embryonic disc.

The cranial end of the **primitive streak** is expanded as the **primitive node**; it contains a circular depression called the **primitive pit**, which is continuous caudally down the midline of the primitive streak with a trough-like depression called the **primitive groove**. The primitive pit and groove represent areas where cells are leaving the primitive streak and moving into the interior of the embryonic disc. Some of these cells invade the hypoblast, displacing the original hypoblast cells and replacing them with a layer of **definitive endoderm**. Others migrate bilaterally from the primitive streak and then cranially or laterally between endoderm and epiblast and coalesce to form the **intraembryonic mesoderm.** After gastrulation is complete, the epiblast is called the ectoderm. Thus, during gastrulation the **three primary germ layers** form: **the ectoderm, mesoderm, and endoderm**. Germ layers are the primitive building blocks for formation of organ rudiments.





Formation of the primitive streak also defines for the first time all major **body axes.** These consist of the **cranial-caudal**(or head-tail) **axis, dorsal-ventral** (or back-belly) **axis**, the **medial-lateral axis** and the **left-right axis.** Before the flat embryonic disc folds up into a three-dimensional tube-within-a-tube body plan, these axes remain incompletely delimited

In addition, gradients of secreted molecules (FGFs, Wnts, retinoic acid) have been postulated to act in a planar way along the AP axis to define more posterior values

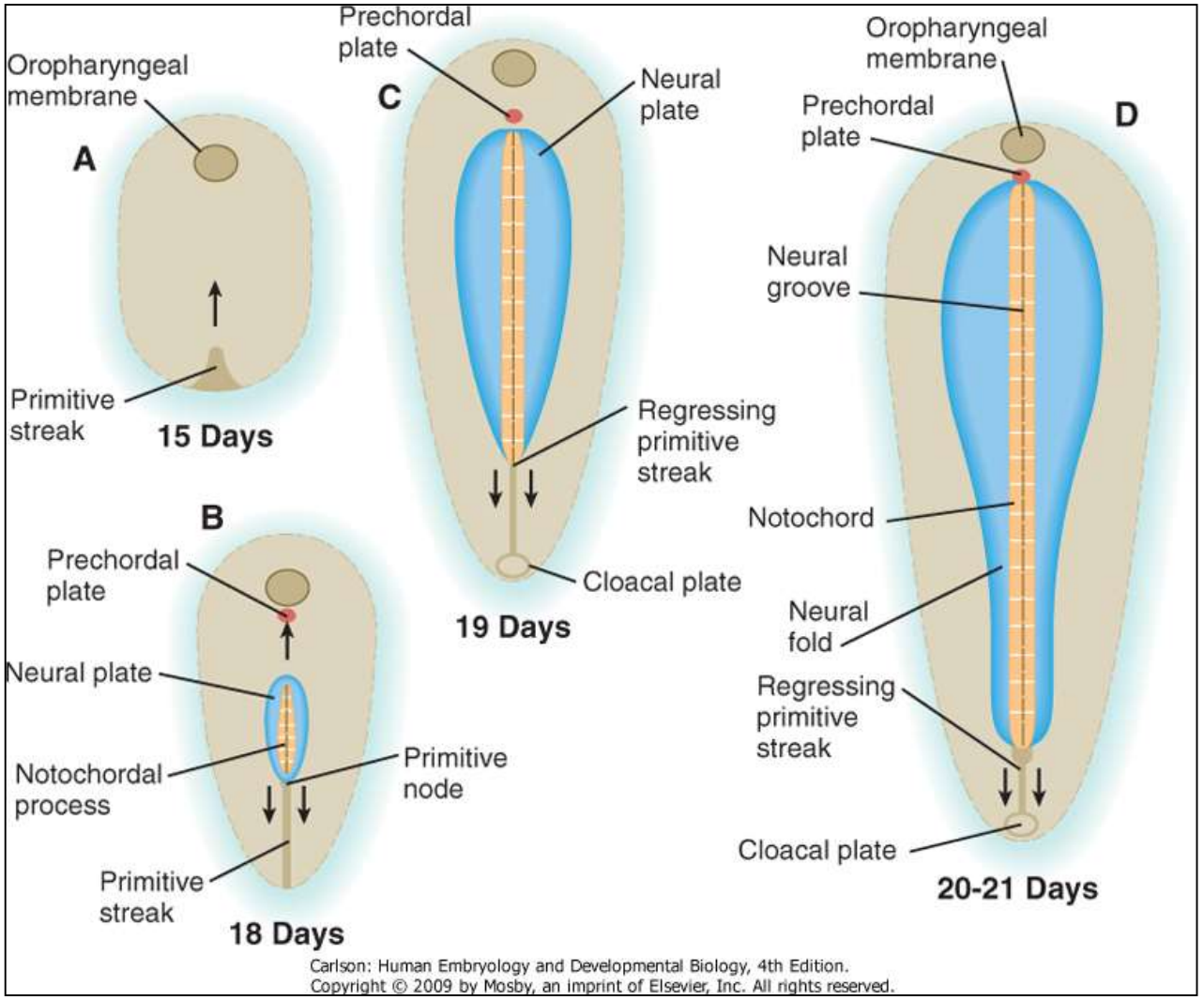


Figure 5-12 Relationships between the neural plate and primitive streak. A, Day 15. B, Day 18. C, Day 19. D, Days 20 to 21.

Neurulation

During gastrulation, a major inductive event occurs in the embryo: **neural induction.** In this process, the primitive node induces the overlying ectoderm to thicken as the **neural plate**, the earliest rudiment of the central nervous system. During subsequent development the neural plate will fold up into a **neural tube. Neural crest cells** arise from the lateral edges of the neural plate during formation of the neural tube. Also during subsequent development, the definitive endoderm will fold to form three subdivision of the primitive gut: **foregut, midgut**, and **hindgut.** The cranial midline endoderm, just cranial to the tip of the extending notochord, forms a thickened area called the **prechordal plate.** It contributes to the **oropharyngeal membrane** during later development and is an important signaling center for patterning the overlying neural plate. With the formation of endodermal, mesodermal, and ectodermal subdivisions during gastrulation, the stage is set by the end of the 3rd week for **formation of the tube-within-a-tube body plan** and subsequent **organogenesis**, the processes by which primitive organ rudiments are established and subsequently differentiated to form all major organ systems.

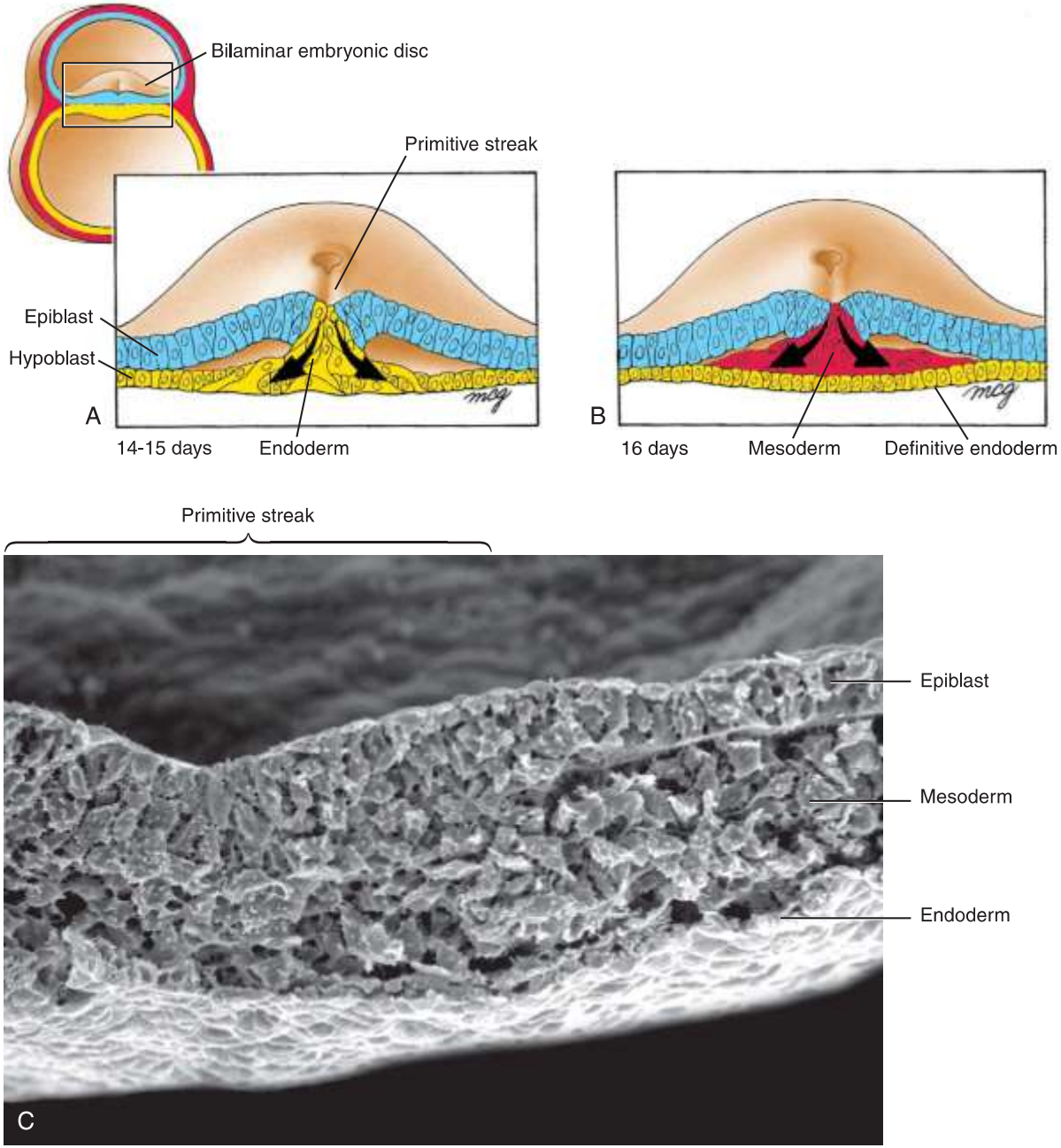
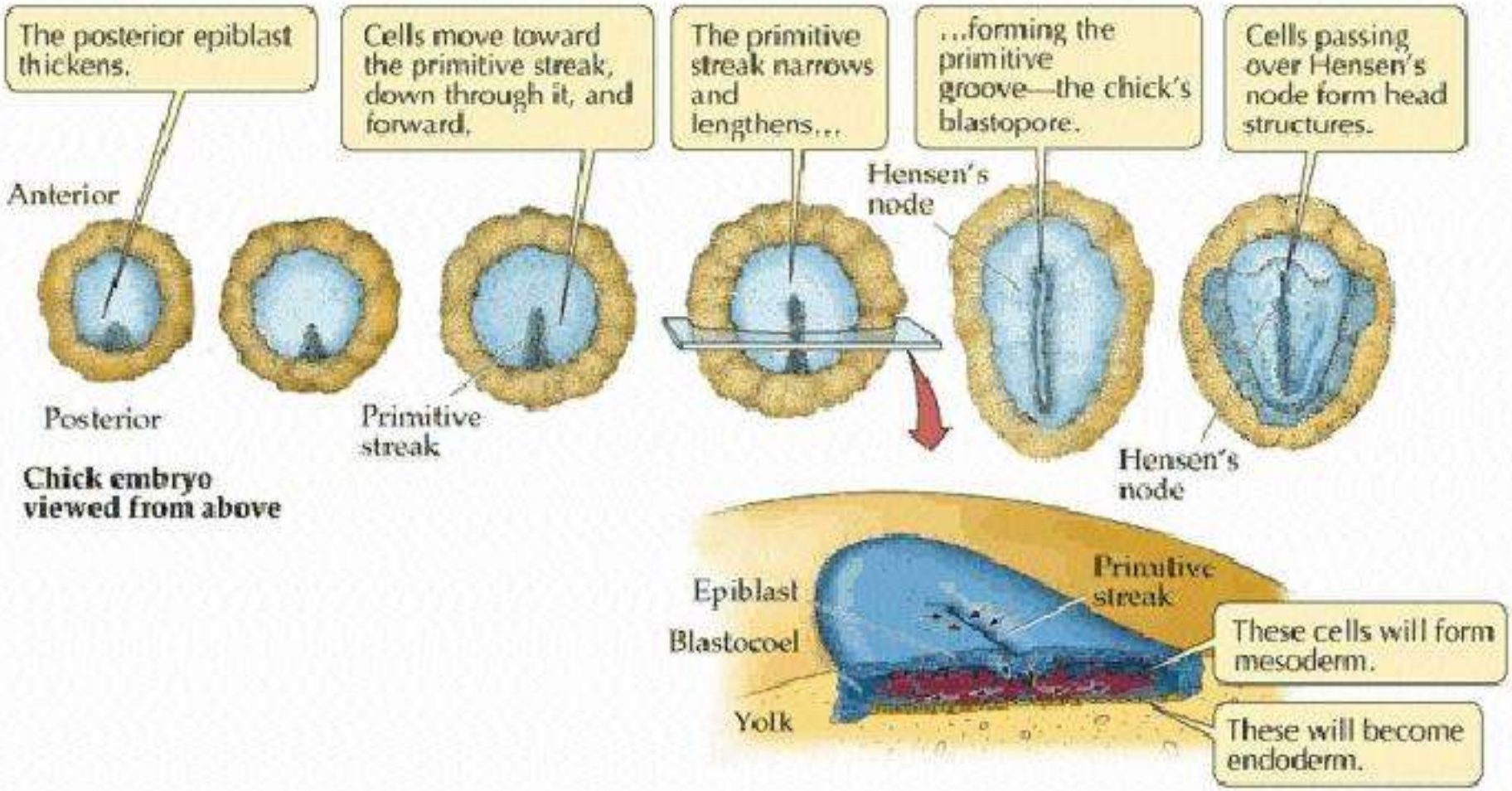
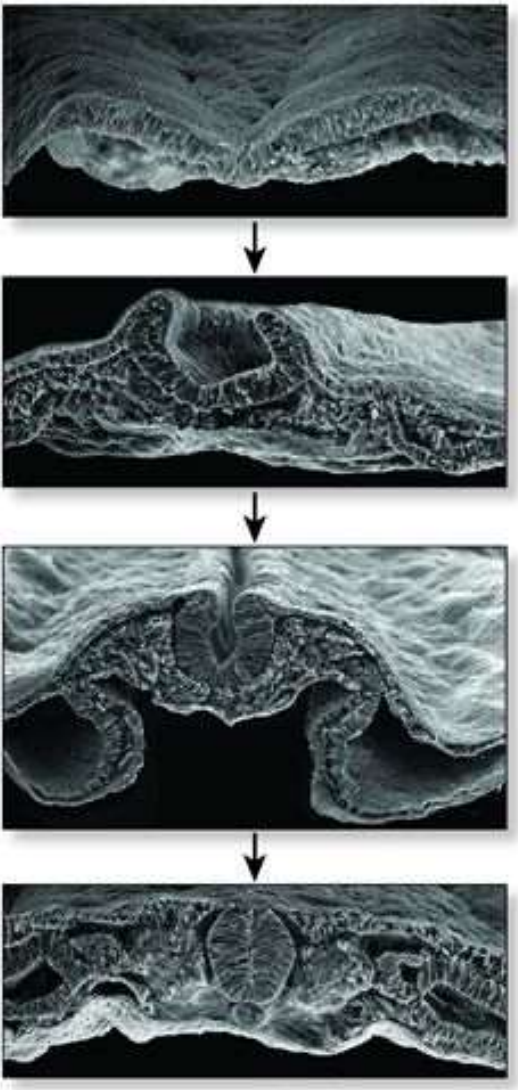
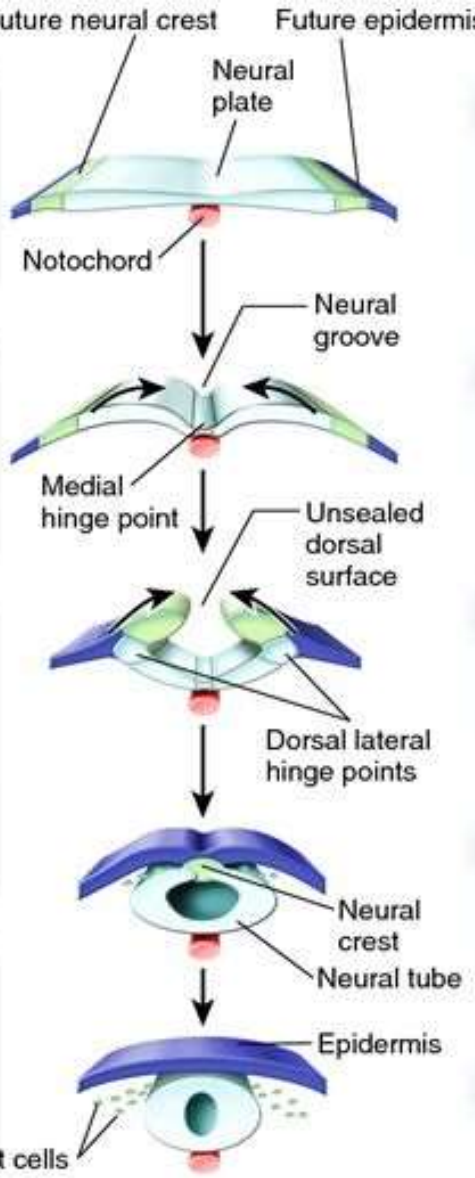


Figure 3-6. Embryonic discs sectioned through the region of primitive streak, showing ingression of epiblast cells during gastrulation. *A*, On days 14 and 15, ingressing epiblast cells displace hypoblast and form definitive endoderm. *B*, Epiblast that ingresses on day 16 migrates between endoderm and epiblast layers to form intraembryonic mesoderm. *C*, Scanning electron micrograph of a cross section through the chick primitive streak.


The posterior epiblast thickens.
Cells move toward the primitive streak, down through it, and forward.
The primitive streak narrows and lengthens...
...forming the primitive groove—the chick's blastopore.
Cells passing over Hensen's node form head structures.
Anterior
Posterior
Primitive streak
Hensen's node
Hensen's node
Chick embryo viewed from above
Epiblast
Blastocoel
Yolk
Primitive streak
These cells will form mesoderm.
These will become endoderm.
Cross section through chick embryo





1 **Thickening and elongation:**
Ectoderm over the notochord thickens to form the neural plate, which elongates. Adjacent regions will form a neural crest and epidermis.

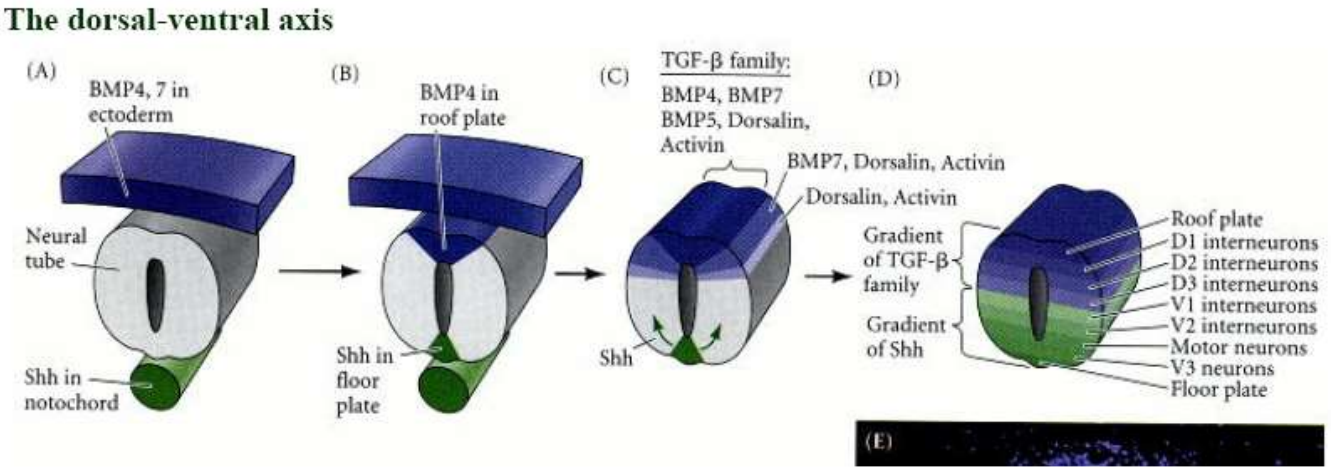
2 **Folding:**
Cells along the medial hinge point undergo apical constriction, causing the formation of a neural groove.

3 **Convergence:**
Two dorsal lateral hinge points undergo apical constriction to produce a tubelike structure.

4 **Fusion:**
The dorsal-most cells on each side of the neural tube begin to fuse. Epidermis also fuses dorsal to the neural tube.

5 Neural crest cells migrate away as fusion is completed.

Dorsalising effect: TGF-β family



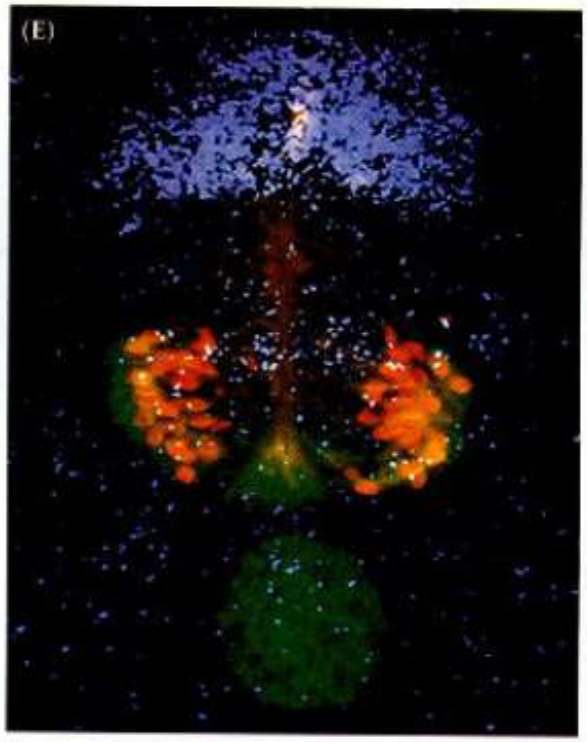
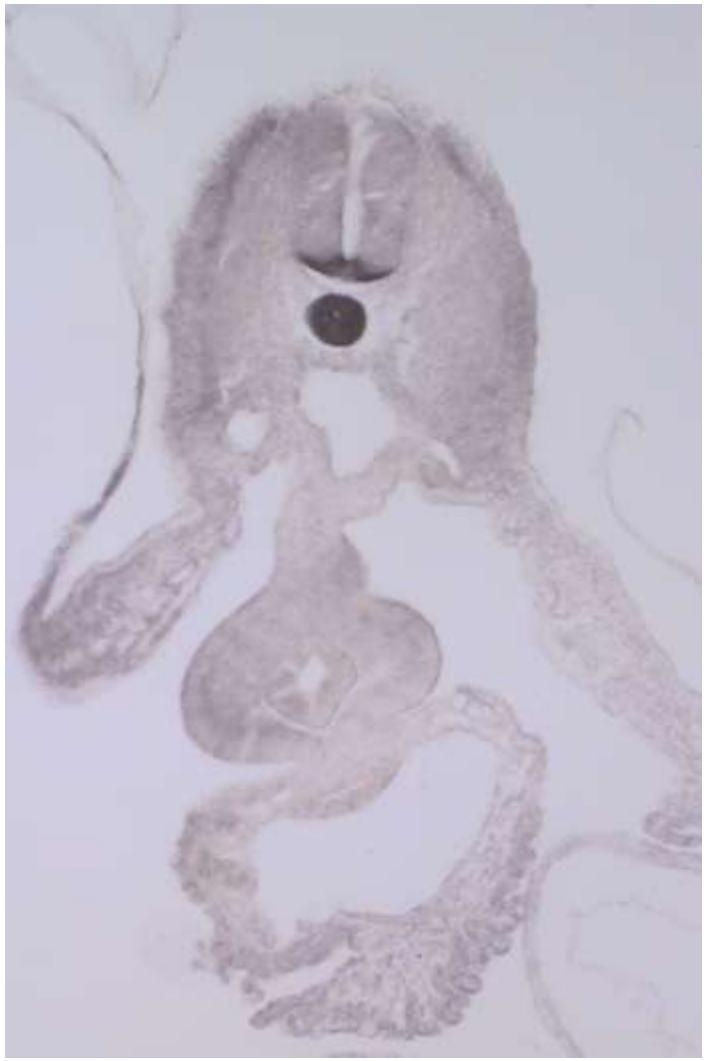
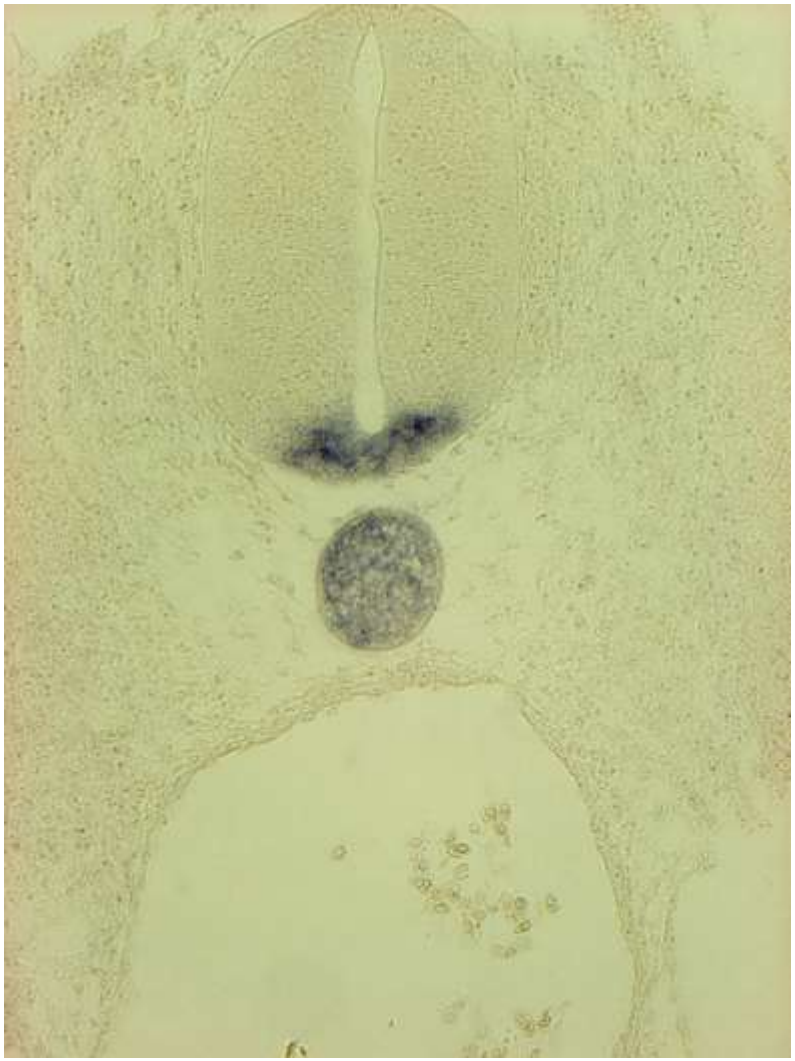


Figure 12.13
Dorsal-ventral specification of the neural tube. (A) The newly formed neural tube is influenced by two signaling centers. The roof of the neural tube is exposed to BMP4 and BMP7 from the epidermis, and the floor of the neural tube is exposed to Sonic hedgehog protein from the notochord. (B) Secondary signaling centers are established within the neural tube. BMP4 is expressed and secreted from the roof plate cells; Sonic hedgehog is expressed and secreted from the floor plate cells. (C) BMP4 establishes a nested cascade of TGF-β-related factors, spreading ventrally into the neural tube from the roof plate. Sonic hedgehog diffuses dorsally as a gradient from the floor plate cells. (D) The neurons of the spinal cord are given their identities by their exposure to these gradients of paracrine factors. The amount and type of paracrine factors present cause different transcription factors to be activated in the nuclei of these cells, depending on their position in the neural tube. (E) Chick neural tube, showing areas of Sonic hedgehog (green) and Dorsalin expression (blue). Motor neurons induced by a particular concentration of Sonic hedgehog are stained orange/yellow. (Photograph courtesy of T. M. Jessell.)

Ventralising effect: Shh





Induction and competence

inducer —signals→ **responder**

Cell shape changes
Changes in mitotic rate
Cell fate changes

In the case of neural induction:

Spemann organiser notochord → ectoderm → neuroectoderm

About the events before neurulation

In the early gastrula only the early blastoporal lip develops autonomically.

Mit besten Grüßen.
H. S.

Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren

Von

H. Spemann und Hilde Mangold

Mit 25 Textabbildungen

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Julius Springer
1924

Induction of Embryonic Primordia by Implantation of Organizers from a Different Species

by

HANS SPEMANN and HILDE MANGOLD (Née Proescholdt)

With 25 illustrations
(Submitted 1 June 1923)

CONTENTS

I. Introduction

In a *Triton* embryo, at the beginning of gastrulation, the different areas are not equivalent with respect to their determination. It is possible to exchange by transplantation parts of the ectoderm at some distance above the blastopore that in the course of further development would have become neural plate and parts that would have become epidermis, without disturbing normal development by this operation. This is feasible not only between embryos of the same age and of the same species but also between embryos of somewhat different age and even between embryos of different species (Spemann 1918, 1921). For instance, presumptive epidermis of *Triton cristatus* transplanted into the forebrain region of *Triton taeniatus* can become brain; and presumptive brain of *Triton taeniatus* transplanted into the epidermal region of *Triton cristatus* can become epidermis. Both pieces develop according to their new position; however they have the species characteristics with which they are endowed according to their origin. O. Mangold (1922, 1923) has extended these findings and has shown that prospective epidermis can furnish not only neural plate but even organs of mesodermal origin, such as somites and pronephric tubules. It follows from these experimental facts, on the one hand, that the exchangeable pieces are still relatively indifferent with respect to their future fate; and, on the other hand, that influences of some sort must prevail in the different regions of the embryo that determine the later fate of those pieces that are at first indifferent.

Notes added by the [W.R.] Editorial Office: 1. The serial number of each experiment, e.g. Um 25, refers to two embryos (a and b), between which transplants were exchanged. Thus "a" usually refers to the donor, "b" to the host embryo. 2. It is worthwhile noting that all figures in this paper were hand-drawn by Hilde Mangold. The drawings of histological sections are based on photographic paper prints. On these, each nucleus and cell border was traced with Indian ink. Thereafter, the silver halogenide grains were removed chemically, after which the drawing stood out on the white background. This method was described in Spemann (1918, p. 345).

[Abbreviations used in this paper: Bl, blastopore; Ch, chorda; ncr, notochord; pr, perichordium; pr. Abd, primary neural tube; sc. Ch, secondary notochord; sc. D, secondary mesoderm; sc. Lab, secondary cavum; sc. Abd, secondary neural tube; sc. Abs, secondary mesoderm; sc. Pros, secondary prosencephalic duct; sc. Ur, secondary somite; Ur-Bl, Urmund (meaning "primitive mouth" or blastopore) followed by the serial number "X" of the experiment.]

They concluded that the dorsal lip acts not only as a neural inducer but also as an „organizer" of the entire body axis. As a result of these experiments, this region of the embryo is known as the Spemann organizer.

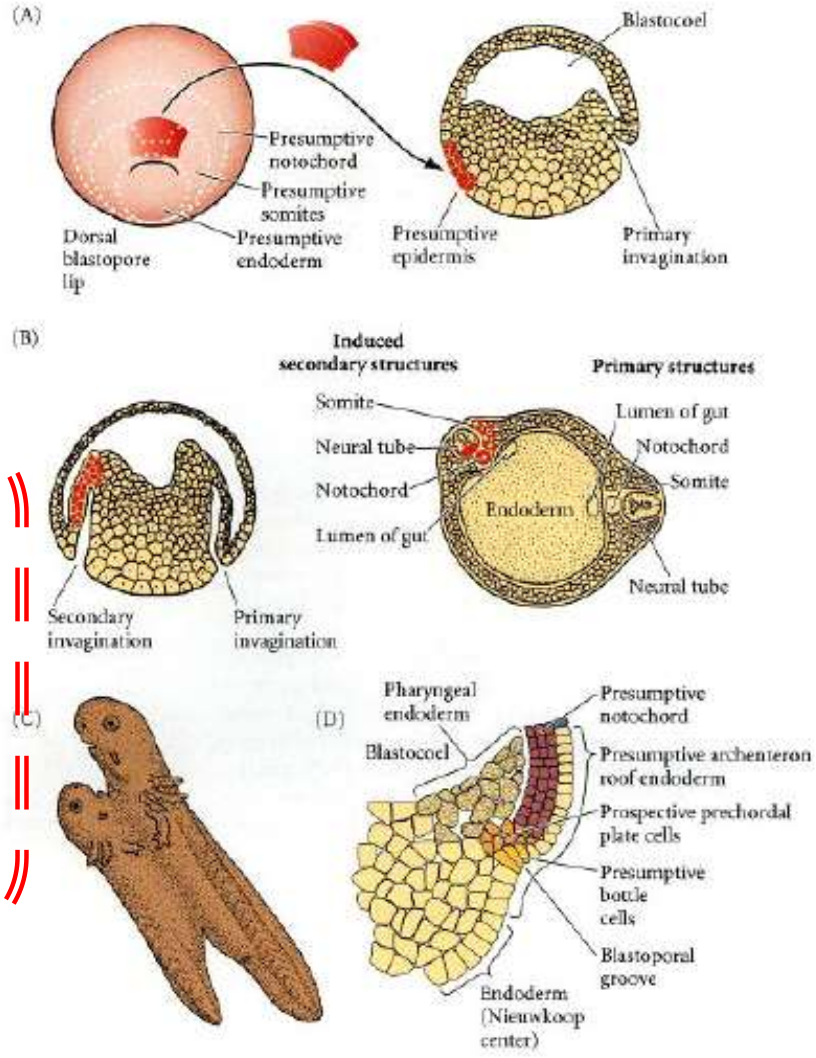
Hilde Mangold and Hans Spemann

A SPEMANN-MANGOLD ORGANISER

Spemann investigated how cell fates were determined during embryogenesis. Spemann was particularly interested in exploring the mechanism of neural plate induction. The neural plate is the embryonic structure that gives rise to the central nervous system during development. To explore neural plate induction, Spemann first performed a transplant experiment that was nearly identical to the later organizer experiment.

Spemann transplanted the blastopore lip from one newt gastrula into another, and noticed a second notochord that developed at the site of transplantation.
(Spemann a blastoporus ajak szövetet transzplantálta az egyik tarajos gőte gasztrulából egy másikba, és megfigyelte, hogy a transzplantáció helyén egy második chorda dorsalis alakult ki.)

However, the newts were of the same species and it was difficult to determine whether the host tissue or transplanted tissue was acting to create the second nervous system.



Spemann also believed the cells at the blastopore lip became determined in their fate first, and that fixed determination then spread outward from that blastopore lip across the ectoderm. Spemann was mistaken, however, as the organizer experiment would later demonstrate. Cells at the blastopore lip are not ectoderm, but mesoderm. The mesoderm cells of the blastopore lip invaginate over the course of gastrulation and are subjacent to the ectoderm.

Mangold, with the task of conducting a cross-species transplant of blastopore lips between different newt species. The tissue color was different between species, allowing Mangold to see whether the features that developed were from transplanted or host tissue. Mangold used the microtools developed by Spemann to excise the blastopore lip of the unpigmented Triturus cristatus egg, and transplant it under the ectoderm of a pigmented Triturus taeniatus newt egg. The transplanted blastopore lip differentiated into a notochord and somites, while the ectoderm of the host tissue that was sitting above the transplanted mesoderm differentiated into a neural plate. The neural plate then went on to form neural arches and a completely separate central nervous system. The ultimate result was what appeared to be two embryos conjoined at the gut.

THE IMPORTANCE OF THE ORGANIZER EXPERIMENT IS THE DISCOVERY THAT A PART OF THE MESODERM INFLUENCES THE ECTODERM AS THE ECTODERM DIFFERENTIATES INTO CENTRAL NERVOUS SYSTEM TISSUE.

The techniques used to discover the Spemann-Mangold organizer had limitations. The technique for transplanting the organizer involved surgery at a cellular scale, and it demanded great precision. When possible, transplanting was difficult, and many things could not be transferred under the surface of the ectodermic layer because it is only one cell thick in many places. The fact that many materials could not be transplanted kept scientists from testing the inductive capacity of other cellular materials on the embryo. The Einsteck method circumvented that limitation. Spemann and Otto Mangold, Hilde Mangold's husband, developed the technique. While each claimed credit for the novel technique, the exact origin of its invention is unknown. Spemann proposed that both may have discussed the idea conversationally, and that each investigator felt justified in claiming credit for the idea.

The Einsteck method consists of using the glass and hair microsurgical tools developed by Spemann to plant material inside of the blastocoels of a developing embryo in either the
blastula or early gastrula stage. This technique insures that material need not fuse with or adhere to the ectodermic layer. It simply passes through the ectoderm into the cavity beneath, where it can affect the embryo. The insertion wound also heals quickly, leaving the foreign material within the developing embryo to affect change.

Spemann's co-researcher, Hanns Bruno Geinitz transplanted a Spemann-Mangold organizer into developing blastocoels using the Einsteck method, which induced embryos like the ones obtained in Spemann and Mangold's original experiments. Geinitz expanded on the original organizer experiment by transferring organizers from frogs and toads into salamander gastrulae. Geinitz called this type of transfer xenoplastic because it involved transplant of cells from a different genus, as opposed to heteroplastic, which involved crossspecies transfers like those used by Spemann and Mangold.

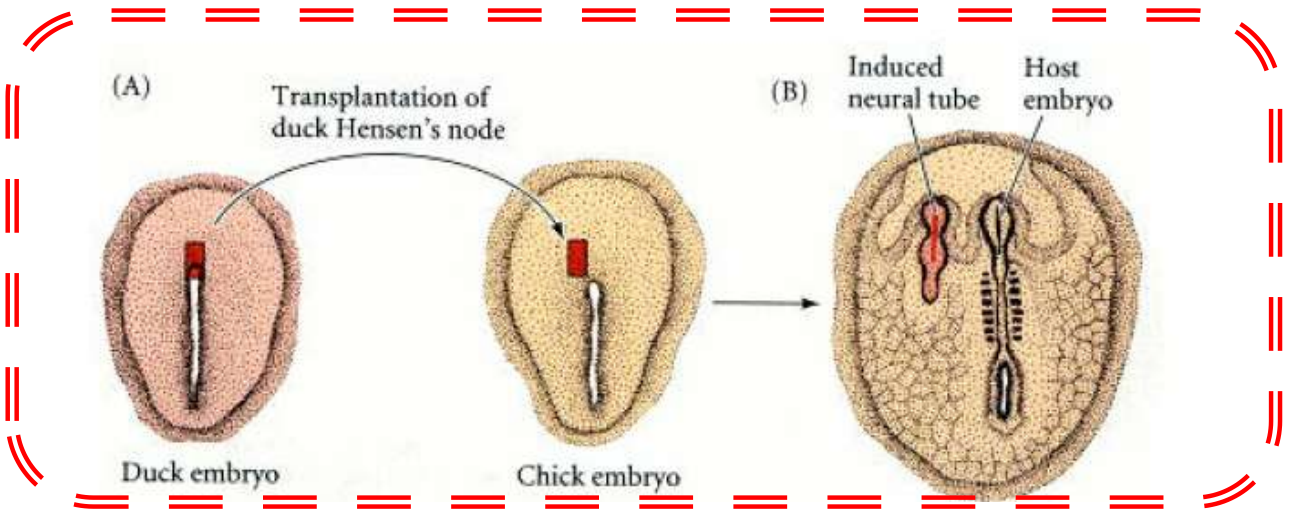
Although it was initially proposed that the Spemann-Mangold organizer induced differentiation of the central nervous system, more recent mechanistic examinations have revealed more complicated genetic interactions. Embryologists have determined that the presence of the Spemann-Mangold organizer impedes signaling to the overlying ectoderm. Instead of becoming skin cells, the organizer-affected ectoderm cells become central nervous system tissue. In 1992, Richard M. Harland and William C. Smith discovered a protein integral to cellular induction at the Spemann-Mangold Organizer.

As the protein was essential for neural development and eventually the formation of the head, the scientists called it noggin.

The developing blastula secretes bone morphogenetic protein-4 (BMP-4) from the ventral side of the embryo, opposite the organizer. BMP-4 diffuses throughout the blastocoel and induces skin cells where it binds to ectodermal cells. However, the organizer blocks BMP-4 from binding to the surrounding ectoderm by secreting the proteins chordin and noggin. Chordin and noggin bind to BMP-4 in the organizer-affected area to prevent BMP-4 from binding to ectoderm receptors. Instead of becoming skin cells, the ectodermal cells in the area of the organizer take the default path of becoming central nervous system tissues.

A fejlődő blastula az organizátorral ellentétes oldalon, az embryo ventralis oldalán BMP-4 molekulát szekretál. A BMP-4 a blastocoelben diffundál, és ahol az ectoderma sejtekhez kötődik, ott epidermis sejtek képződését indukálja. Az organizátor chordin és noggin fehérjéket szintetizál, és ezekkel megakadályozza, hogy a BMP-4 az organizátort körülvevő sejtekhez kötődjön. A chordin és a noggin megköti a BMP-4-et az organizátor által befolyásolt területeken, így megakadályozzák a BMP-4 kötődését az ectoderma sejtek receptoraihoz. Epidermis sejtekké alakulás helyett az organizátor környezetében levő sejtek az "eredeti" ("default") programot követve központi idegrendszeri szövetekké alakulnak.

Conserved role of the organiser in birds



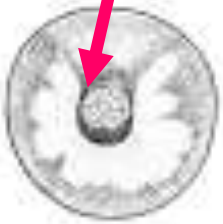
The mechanisms for neural induction in the chick, however, probably differ from those of the frog, and the antagonism of the BMP signal does not appear to be sufficient for neural induction. In chick embryos, BMPs do not inhibit neural induction, nor does ectopic expression of chordin in the non-neural epiblast cause neural induction (see Streit and Stern 1999).

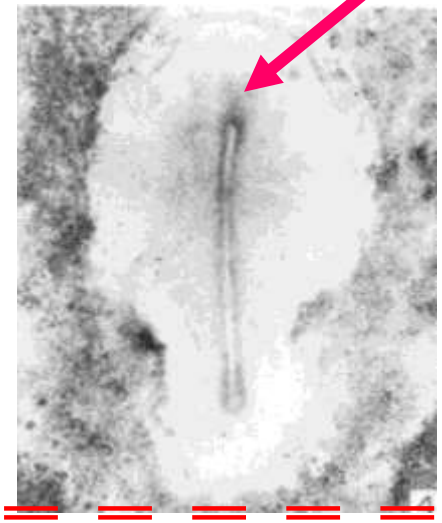
(A)

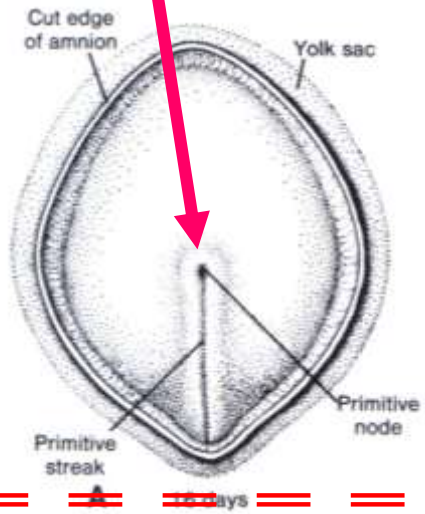
Rather, fibroblast growth factors (FGFs) appear to generate neuronal phenotypes in the epiblast cells. Fibroblast growth factors are produced in Hensen's node and the primitive streak, and beads containing certain FGFs can induce trunk and hindbrain neuronal expression in the epiblast cells (Alvarez et al. 1998; Storey et al. 1998). The factor or factors regulating anterior neuron production remain unknown, but recent evidence (Diaz and Schoenwolf 1990; Darnell et al. 1999) suggests that the anterior visceral endoderm is providing these signals.

Spemann organiser homologue structures in higher vertebrate
Spemann organizátorral homológ struktúrák magasabbrendű gerincesekben

Spemann Mangold organizer/ Hensen's node (csomó)/ Primitive node (csomó)







Prechorda, notochord, endoderm of the pharynx, dorsoanterior endoderm, floor plate

First, self-differentiation of the organizer generates a variety of mesodermal and endodermal tissues, including head mesoderm, notochord, and pharyngeal endoderm.

Second, the organizer performs morphogenetic movements and in addition induces them in adjacent cells (e.g. convergence and extension in the presumptive notochord and in the somitic mesoderm). The timing of mesodermal and endodermal internalization also depends on signals from the organizer.

Third, the organizer secretes signals which affect all three germ layers of the developing embryo. Most of these signals have been found to antagonize ventralizing signals like BMPs, Wnts, and Nodals .

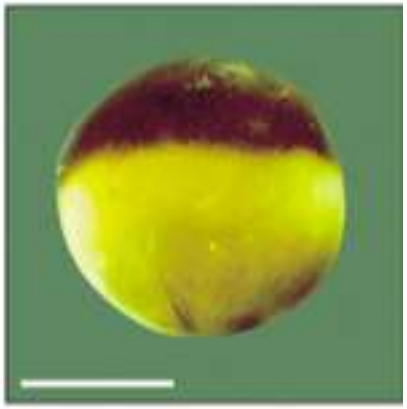
What induce the organiser?

The role of **NIEUWKOOP CENTER**

Pieter Nieuwkoop and his colleagues proposed an alternative model. They postulated that, neurectoderm of an anterior character is induced via an initial "activation" step (i.e. the actual neural induction or neuralization). In a subsequent "transformation" step this anterior neurectoderm is gradually modified to make more posterior regions of the central nervous system

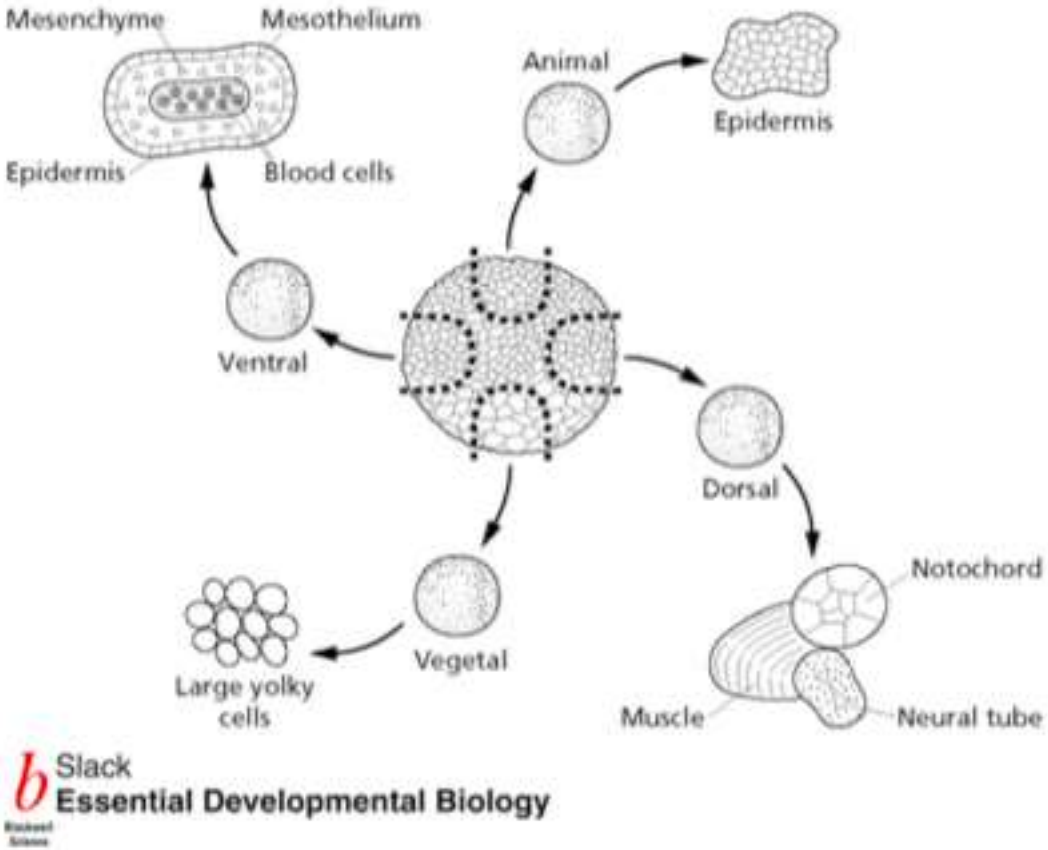
Dorsal vegetal cells are needed to induce dorsal mesoderm in Xenopus

In higher vertebrate it is the **A**nterior **V**isceral **E**ndoderm (**AVE**)



Animal and vegetal explants can be isolated from early blastulae and cultured in a simple salt solution for several days, where they will differentiate into recognisable tissues. Animal explants, called animal caps, will only form epidermis, even though they will also form neural tissue if left in the embryo. Vegetal explants don't differentiate but will express genes that are specific to the endoderm, but not genes that are specific to the ectoderm or mesoderm.

Mesoderm is formed in the marginal zone of *Xenopus* blastulae as a result of inductive signals released by vegetal blastomeres, a process known as mesoderm-induction. This was first shown by Peter Nieuwkoop (1969), who isolated animal and vegetal explants from axolotl mid-blastulae and found that they formed ectoderm and endoderm, respectively, when incubated alone (see section 1.3). However, they also formed mesoderm (notochord, muscle, pronephros) when combined. Since all the mesoderm was derived from the animal cap, Nieuwkoop concluded that it was induced by the vegetal hemisphere. Mesoderm was not induced when explants were isolated from early gastrulae, indicating that mesoderm-induction occurs during blastula stages.



This demonstrates that ectoderm and endoderm are specified by maternal components inherited from the egg, but that neural tissue requires continued interactions with the embryo. Explants from the dorsal marginal zone differentiate dorsal mesoderm (e.g. notochord), while ventral and lateral explants differentiate ventral mesoderm. However, it is impossible to isolate pure mesoderm and marginal zone explants always include ectoderm and endoderm. Therefore, a role for inductive signalling in mesoderm formation, between endoderm and ectoderm, cannot be excluded.

In both mouse and chick suggest that **Tgfβ** and **Wnt1 family members induce the primitive streak.**

In mouse, Wnt3 and its downstream target Brachyury (a T-box containing transcription factor) are expressed in both the future cranial and caudal prestreak epiblast. During subsequent development, **Wnt3 is downregulated cranially**, by signals from a specialized region of extraembryonic endoderm called the anterior visceral endoderm, and **upregulated caudally**.

Loss-of-function mutations of **genes** expressed by **the anterior visceral endoderm** (e.g., Cerl, Lefty1—both inhibitors of Tgfβ and Wnt signaling) result in **formation of extra primitive streaks**.

Moreover, embryos with **loss-of-function mutations of Nodal** (or its cofactor Cripto) fail to **form a primitive**
streak.

Egér és csirke kísérletek alapján is feltételezhető, hogy **Tgfβ és Wnt1 molekulacsalád tagjai indukálják a primitív csík kialakulását.**

Egérben, a Wnt3, és annak célmolekulája, a Brachyury (T-box-ot tartalmazó transzkripciós faktor) expresszálódnak abban a későbbi cranialis és caudalis epiblastban, amely a primitív csíkot képezi. A későbbi fejlődés során a **Wnt3 craniálisan downregulálódik** , specializálódott extraembryonalis endoderma, **Anterior Visceralis Endodermá**nak nevezett terület által szekretált molekulák hatására **Wnt3 caudálisan upregulálódik**.

Anterior Visceralis Endoderma által expresszált gének (Cerl, Lefty1—mindkettő a Tgfβ és Wnt signaling inhibítorai) **funkció-vesztő mutációi extra primitive csík-képződéshez vezetnek**.

A **Nodal** (vagy annak a cofactora, a Cripto **funkció-vesztő mutációja esetén primitív csík nem képződik.**.

Function of the Organizer:
he ability to cause the neural plate to become the neural tu

The Functions of the Organizer

While the Nieuwkoop center cells remain endodermal, the cells of the organizer become the dorsal mesoderm and migrate underneath the dorsal ectoderm. There, the dorsal mesoderm induces the central nervous system to form. The properties of the organizer tissue can be divided into five major functions:

1. The ability to become dorsal mesoderm (prechordal plate, chordamesoderm, etc.)

2. The ability to dorsalize the surrounding mesoderm into lateral mesoderm (when it would otherwise form ventral mesoderm)

3. The ability to dorsalize the ectoderm into neural ectoderm

4. The ability to initiate the movements of gastrulation

5. The ability to cause the neural plate (the induced neural ectoderm) to become the neural tube

Table 10.2. Proteins expressed solely or almost exclusively in the organizer (partial list)

Nuclear proteins	Secreted proteins
XLim1	Chordin
Xnot	Dickkopf
Otx2	ADMP
XFD1	Frzb
XANF1	Noggin
Goosecoid	Follistatin
HNF3 β -related proteins (e.g., Forkhead, Pintallavis)	Sonic hedgehog
	Cerberus
	Nodal-related proteins (several)

Main references:

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Gilbert: Developmental Biology

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