Bio 127 - Section II
Early Development and Cell Fate Determination

I. Fertilization and Cleavage

II. Specification and Gastrulation

III. Organizing Power and Axis Formation

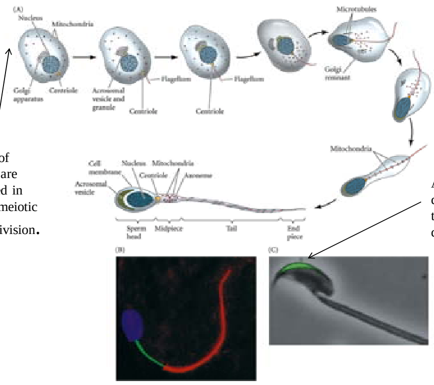
A. Some Really Big Ideas....

1. Species Specificity Must be Maintained
2. A Single Sperm is all You Need (or Want)
3. Genetic Fusion Signals Major Changes
4. The Egg is Built for Early Development

B. The Structures of the Gametes

- The sperm is built for species identification and high speed DNA delivery
- The egg is built to receive only one sperm and to store everything needed to get started down the path to development

Structure and formation of a sperm cell

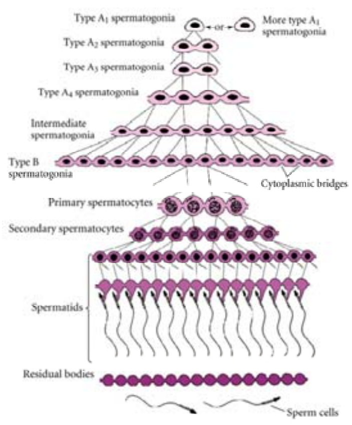


Four of these are formed in each meiotic cell division.

Acrosome has digestive enzymes to get past egg defenses.

DEVELOPMENTAL BIOLOGY, 9e, Figure 4.2

You may remember...



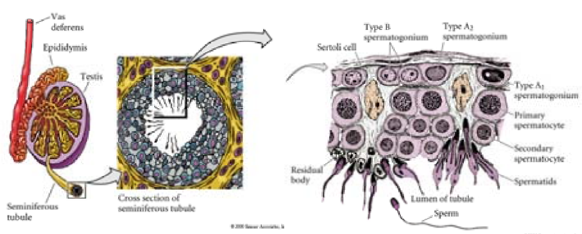
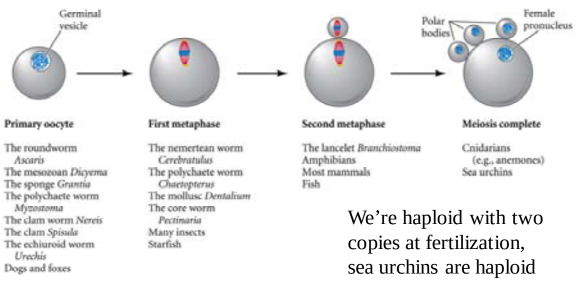


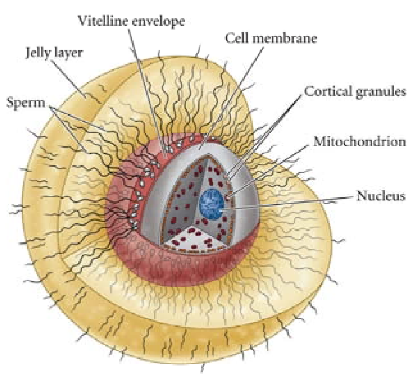
Figure 4.5 Stages of egg maturation at the time of sperm entry in different animal species



We're haploid with two copies at fertilization, sea urchins are haploid with a single copy. We extrude two more polar bodies afterward.

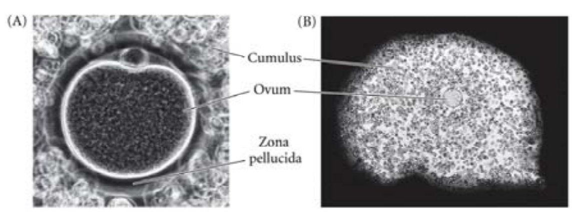
***DEVELOPMENTAL BIOLOGY*, 9e, Figure 4.5**

Structure of sea urchin eggs



***DEVELOPMENTAL BIOLOGY*, 9e, Figure 4.4**

Structure of mammalian eggs

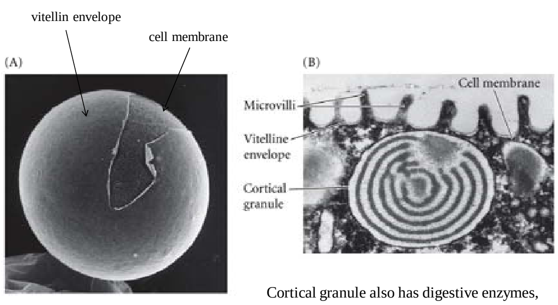


***DEVELOPMENTAL BIOLOGY*, 9e, Figure 4.7**

Remember, the egg is complexly organized....

- The sea urchin egg is 10,000 times the volume of the sperm (ostrich eggs – wow!)
  - Nutrition
  - mRNA
  - Transcription factors
  - Ribosomes and tRNA
  - Secretable paracrine factors
  - Protection: DNA repair, distaste, antibodies

Structures at the plasma membrane



Cortical granule also has digestive enzymes, as well as sugars and proteins needed to block polyspermy and support cleavage blastomeres.

DEVELOPMENTAL BIOLOGY, 9e, Figure 4.6

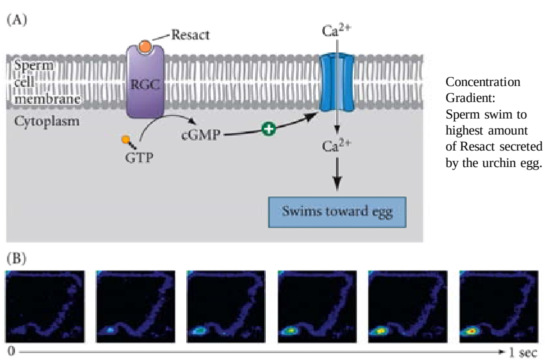
C. External Fertilization is Better Understood

- Even today much of what we truly understand about fertilization has come from the study or organisms who fertilize their eggs outside of the female's body
- Understanding internal fertilization is big-money big-science

The 8 Big Ideas of Sea Urchin Fertilization

1. Chemoattraction
2. The Acrosome reaction
3. Sperm Recognition of Egg ECM
4. Membrane Fusion
5. Block to Polyspermy
6. Cortical Granule Reaction
7. Activation of Egg Metabolism
8. Fusion of Genetic Material

Chemotactic Migration: Flagellar swimming to source of signal.

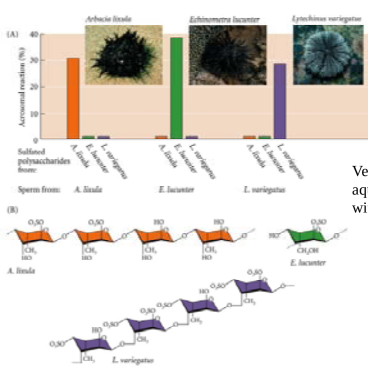


Concentration Gradient: Sperm swim to highest amount of Resact secreted by the urchin egg.

[Calcium] in sperm cytosol

DEVELOPMENTAL BIOLOGY, 9e, Figure 4.10

Secretion of the contents of the acrosome is species-specific

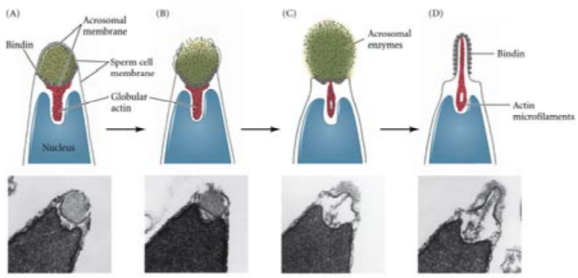


Very important in an aqueous environment with mixed sperm.

DEVELOPMENTAL BIOLOGY, 9e, Figure 4.11

Figure 4.12 Acrosome reaction in sea urchin sperm

The acrosome reaction release digestive enymes to get through the jelly layer and inverts Bindin proteins on the acrosomal membrane onto surface of process.



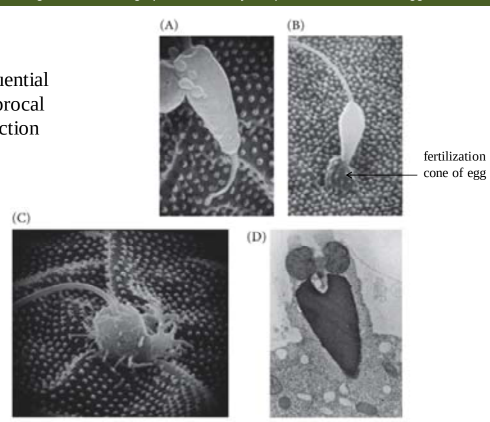
Bindin proteins on the sperm bind to receptors on egg plasma membrane and facilitate membrane fusion and in turn stimulate egg fertilization cone.

DEVELOPMENTAL BIOLOGY, 9e, Figure 4.12

- Bindin proteins and bindin receptors are mutational "hotspots"
  - They are rapidly changing DNA sequences
  - Very close species have different proteins

Figure 4.16 Scanning electron micrographs of the entry of sperm into sea urchin eggs

Sequential reciprocal induction

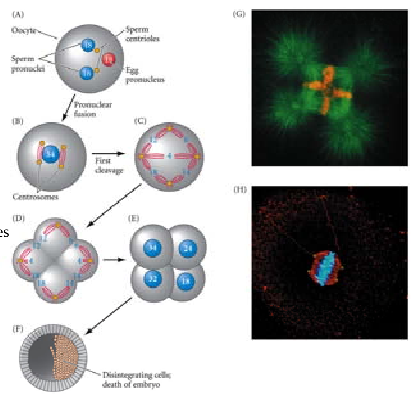


DEVELOPMENTAL BIOLOGY, 9e, Figure 4.16

- Sperm entry provides:
  - The male haploid DNA contribution
  - A single centriole
- The male centriole divides to form both pieces of the first mitotic spindle
  - The female centriole is degraded

Polyspermy kills the embryo

There is no mechanism to ensure each cell gets same # chromosomes

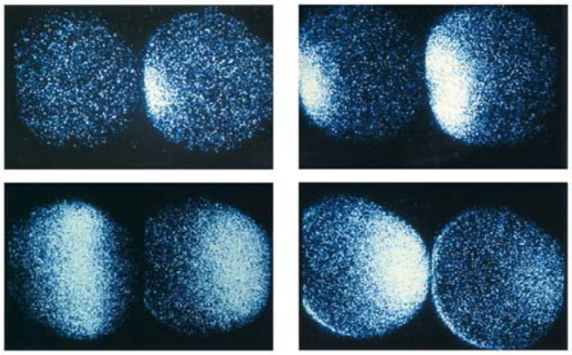


DEVELOPMENTAL BIOLOGY, 9e, Figure 4.17

Block to Polyspermy

- There are two mechanisms in sea urchins
  - The Fast Block
    - Rapid change in membrane potential: Na+ channels open on membrane fusion, blocks bindin
    - Occurs in inverts and frogs, not mammals
  - The Slow Block
    - A wave of Ca2+ channel openings sweeps from sperm entry site around egg to opposite side
    - Causes release of the cortical granules and formation of the fertilization envelope
    - Occurs in many animals, including mammals

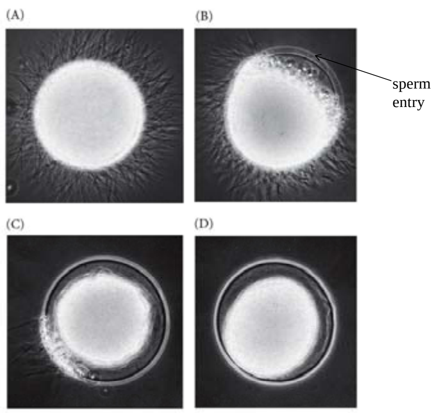
Figure 4.21 Wave of Ca^{2+} release across a sea urchin egg during fertilization



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.21

Figure 4.19 Formation of the fertilization envelope and removal of excess sperm

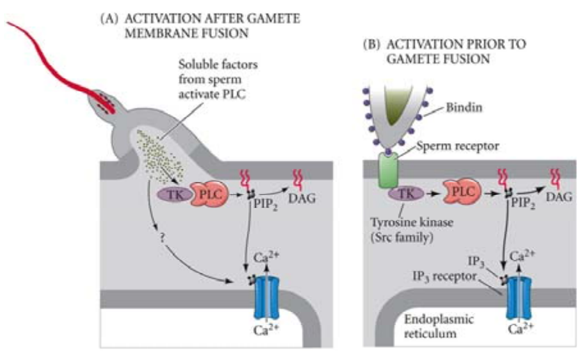
Takes about a minute



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.19

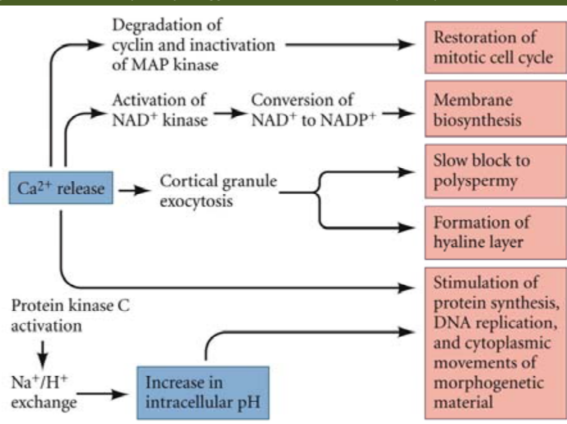
- Key changes:
  - Enzymes degrade bindin and bindin receptors
  - Sugars attract water and push vitelline envelope away from plasma membrane
  - Tough protein cross-linking and hyalin layer give support to cleavage blastomeres

Activation of egg metabolism occurs in the cytosol, independent of the pronuclei



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.23

Figure 4.26 Postulated pathway of egg activation in the sea urchin (Part 2)



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.26 (Part 2)

TABLE 4.1 Events of sea urchin fertilization *(Part 1)*

Event	Approximate time postinsemination[a]
EARLY RESPONSES	
Sperm-egg binding	0 seconds
Fertilization potential rise (fast block to polyspermy)	within 1 sec
Sperm-egg membrane fusion	within 1 sec
Calcium increase first detected	10 sec
Cortical granule exocytosis (slow block to polyspermy)	15–60 sec

Main sources: Whitaker and Steinhardt 1985; Mohri et al. 1995.

[a]Approximate times based on data from *S. purpuratus* (15–17°C), *L. pictus* (16–18°C), *A. punctulata* (18–20°C), and *L. variegatus* (22–24°C). The timing of events within the first minute is best known for *Lytechinus variegatus*, so times are listed for that species.

DEVELOPMENTAL BIOLOGY, 9e, Table 4.1 (Part 1)

TABLE 4.1 Events of sea urchin fertilization *(Part 2)*

Event	Approximate time postinsemination[a]
LATE RESPONSES	
Activation of NAD kinase	starts at 1 min
Increase in $NADP^+$ and NADPH	starts at 1 min
Increase in O_2 consumption	starts at 1 min
Sperm entry	1–2 min
Acid efflux	1–5 min
Increase in pH (remains high)	1–5 min
Sperm chromatin decondensation	2–12 min
Sperm nucleus migration to egg center	2–12 min
Egg nucleus migration to sperm nucleus	5–10 min
Activation of protein synthesis	starts at 5–10 min
Activation of amino acid transport	starts at 5–10 min
Initiation of DNA synthesis	20–40 min
Mitosis	60–80 min
First cleavage	85–95 min

Main sources: Whitaker and Steinhardt 1985; Mohri et al. 1995.

[a]Approximate times based on data from *S. purpuratus* (15–17°C), *L. pictus* (16–18°C), *A. punctulata* (18–20°C), and *L. variegatus* (22–24°C). The timing of events within the first minute is best known for *Lytechinus variegatus*, so times are listed for that species.

***DEVELOPMENTAL BIOLOGY*, 9e, Table 4.1 (Part 2)**

Fusion of the Genetic Material in Sea Urchins

- Sperm carries its nucleus, centriole, mitochondria and flagellum into egg
  – Mito's and flagellum disintegrate in cytosol
  – Interestingly, in mice, centriole also degrades
- DNA decondenses to form pronucleus
  – First the membrane breaks up, exposing DNA
  – Then sperm's histones are replaced by egg's
  – This loosens up nucleosomes for replication

Fusion of the Genetic Material in Sea Urchins

- Male pronucleus then aligns with its centriole between it and female pronucleus
- The centriole sends out microtubes that integrate with egg's to form an aster between the two pronuclei
- The pronuclei are then pulled together to allow fusion and formation of the diploid zygote nucleus

D. What We Know of Internal Fertilization

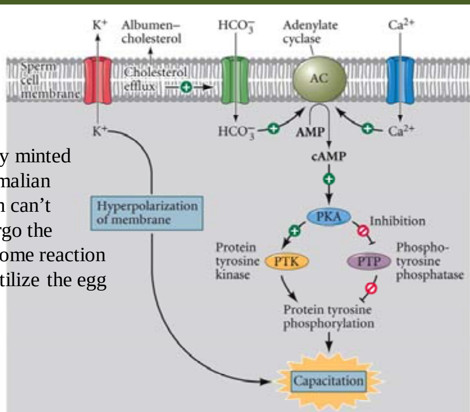
- Tough to study
  - Fertilization occurs in the female oviducts
    - We don't yet know all conditions sperm encounter
  - Sperm is ejaculated at nearly every developmental stage
    - Of 280 million, 200 reach the egg
    - We don't yet know why the winners are the winners

The Female Reproductive Tract Aids Transport and Maturity of Gametes

- The ampulla is the region of oviduct where fertilization takes place
  - Uterine contractions help get sperm there
  - There is a holding region just before ampulla
  - Sperm flagella are stimulated near egg
  - Directional cues coming from egg or cummulus
  - Along the migration route sperm are stimulated to mature "Capacitation"

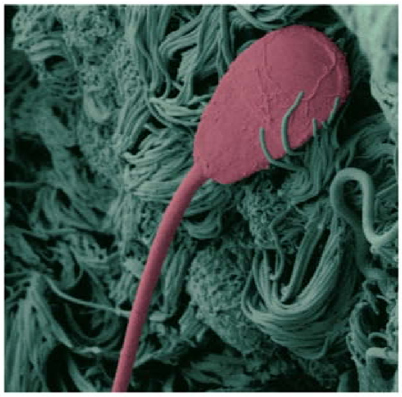
Figure 4.29 Hypothetical model for mammalian sperm capacitation

Newly minted mammalian sperm can't undergo the acrosome reaction or fertilize the egg



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.29

Figure 4.30 SEM (artificially colored) showing bull sperm as it adheres to the membranes of epithelial cells in the oviduct of a cow prior to entering the ampulla



This takes time. The really speedy sperm get there too soon.

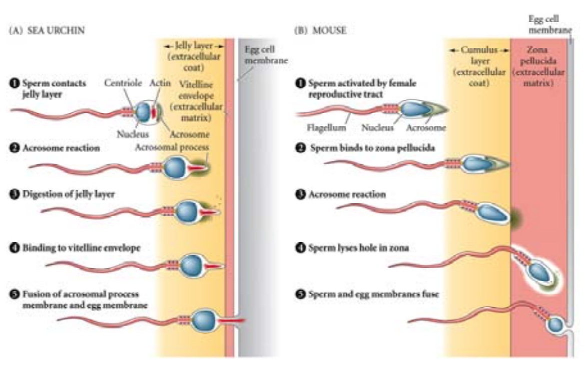
DEVELOPMENTAL BIOLOGY, 9e, Figure 4.30

Key Molecular Events of Capacitation

1. Sperm cell membrane loses cholesterol which localizes proteins for binding egg
2. Protein and carbohydrate "caps" on binding proteins are removed
3. Leakage of K+ cause shift in membrane potential activating Ca+ channels
4. Intracellular proteins are activated by phosphorylation following signal from oviduct
5. The acrosomal membrane is changed to prepare for fusion

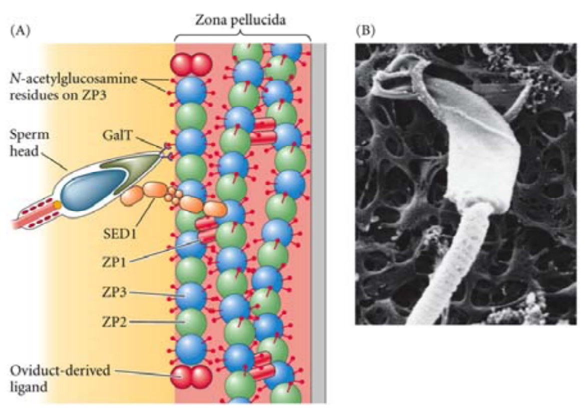
- Capacitation is accompanied by directional cues....
  - Thermotaxis: sperm can sense 2ºC gradient
  - Chemotaxis: sperm follow progesterone

Figure 4.8 Summary of events leading to the fusion of egg and sperm cell membranes in the sea urchin and the mouse



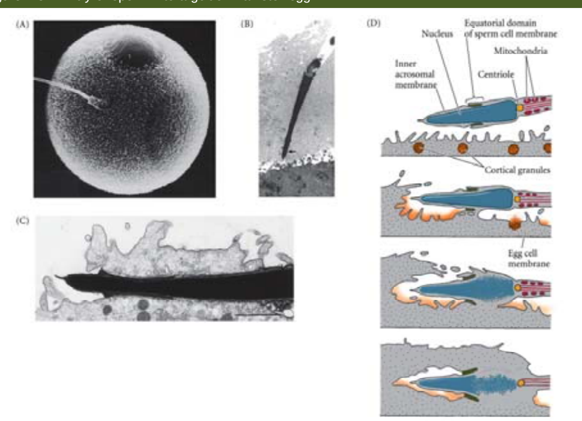
DEVELOPMENTAL BIOLOGY, 9e, Figure 4.8

Figure 4.31 Sperm-zona binding



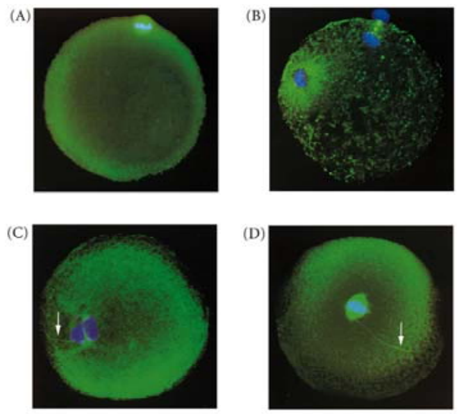
DEVELOPMENTAL BIOLOGY, 9e, Figure 4.31

Figure 4.34 Entry of sperm into a golden hamster egg



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.34

Figure 4.35 Pronuclear movements during human fertilization



DEVELOPMENTAL BIOLOGY, 9e, Figure 4.35

II. Cleavage

A. Background Information

B. Invertebrates
1. Sea Urchins
2. Snails
3. Tunicates
4. C. Elegans
5. Drosophila melanogaster

C. Vertebrates
1. The Frog
2. Zebrafish
3. The Chick Embryo
4. Mammals

A. Background Information

1. The Model Organisms
2. Structure-Process-Structure
3. Rapid Mitotic Cell Divisions
4. Cleavage Patterns
5. Mid-Blastula Transition

1. The Model Organisms

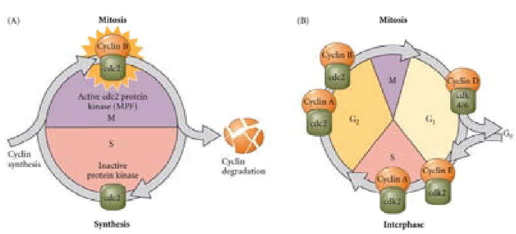
a. Echinoderms (sea urchins)
b. Gastropod molluscs (snails)
c. Tunicates (ascidians)
d. Nematode worms (*C. elegans*)
e. Insects (*D. melanogaster*)
f. Amphibians (*Xenopus laevis*)
g. Fish (*Danio rerio*)
h. Avians (Gallus gallus)
i. Mammals (human and mouse)

Cleavage is a developmental process that takes the organism from fertilization through the blastula stage

Structure → Process → Structure

Cleavage is rapid mitotic cell division

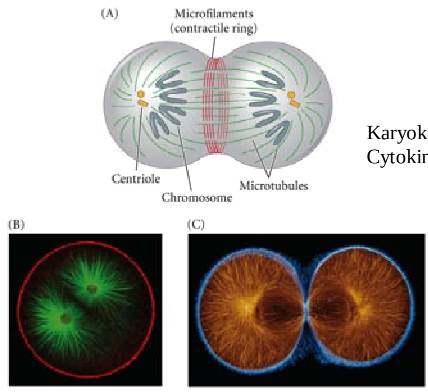
The G-phases of somatic mitosis allow for cell growth so that the daughter cells are equal in size to the parent cell. In blastomeres we are trying to reduce the volume of the egg to somatic levels.



Frogs can make 37,000 cells in 43 hours.
Fruit flies can make 50,000 in 12 hours (10 min!)

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.1

Figure 5.2 Role of microtubules and microfilaments in cell division



Karyokinesis = tubulin
Cytokinesis = actin

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.2

Mid-Blastula Transition

G-phases added back as cleavage goes on.

- Xenopus adds G1 and G2 back after 12 round
- Drosophila adds G1 at round 14 and G2 at 17

Synchronicity is lost as cells "go own way"

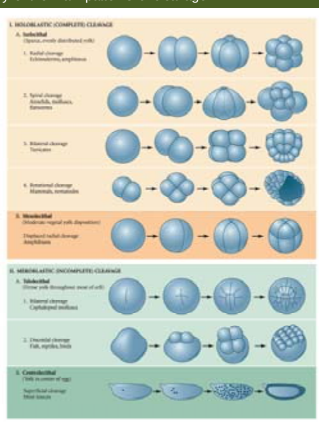
- Different regions of egg produce different cycle controls

New mRNAs are transcribed

- The beginnings of true differentiation

Figure 5.3 Summary of the main patterns of cleavage

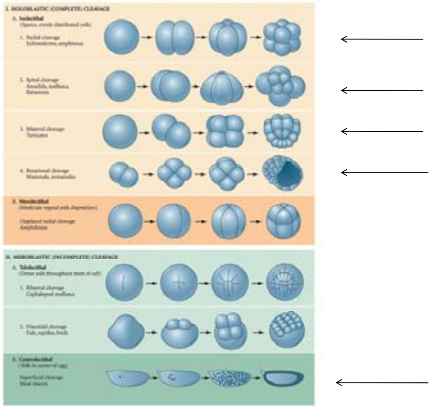
Cleavage pattern reflects combination of how cell aligns its centrioles and how much yolk the egg has.



Yolk inhibits cleavage

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.3

We'll start with cleavage

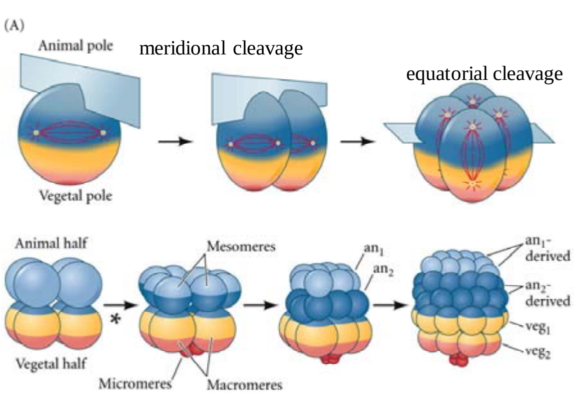


DEVELOPMENTAL BIOLOGY, 9e, Figure 5.3

B. Invertebrates

1. We'll start with the sea urchin....
   - Holoblastic: all of the egg forms into cells
   - Isolecithal: sparse yolk throughout cytosol
   - Animal-Vegetal: a little more yolk in vegetal end

Figure 5.6 Cleavage in the sea urchin (Part 1)

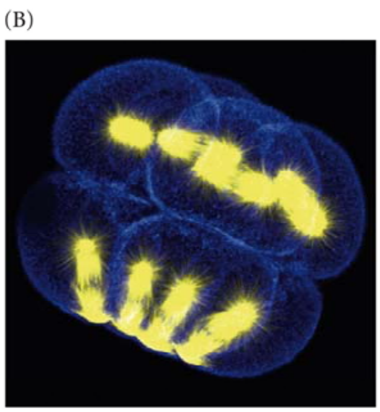


DEVELOPMENTAL BIOLOGY, 9e, Figure 5.6 (Part 1)

- C1: meridional and C2: meridional
- C3: equatorial
- C4: animal pole 4 divide meridionally
- vegetal 4 divide unequally equatorial
- C5: animal 8 divide equatorially
- vegetal 4 macros divide meridionally
- vegetal 4 micros divide unequally
- C6: animal 16 divide meridionally
- vegetal divide equatorially
- C7: animal 16 divide equatorially
- vegetal divide meridionally

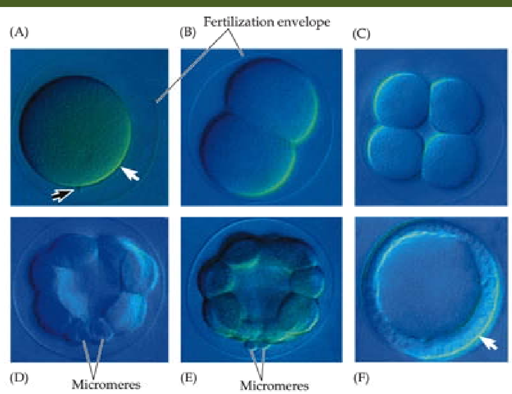
Figure 5.6 Cleavage in the sea urchin (Part 2)

C4



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.6 (Part 2)

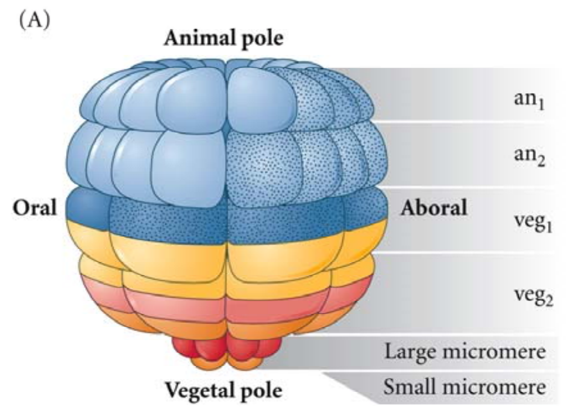
Figure 5.7 Micrographs of cleavage in live embryos of the sea urchin *Lytechinus variegatus*, seen from the side



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.7

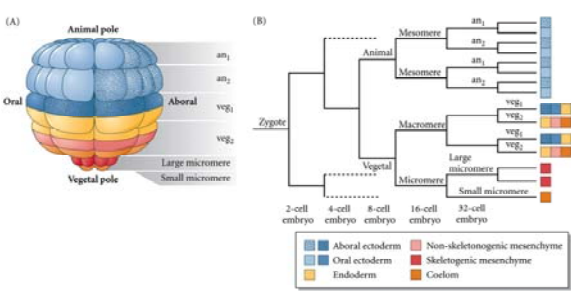
- Proteins secreted from the inner surface of cells draw water from outside
- Results in hollow blastula

Figure 5.8 Fate map and cell lineage of the sea urchin *Strongylocentrotus purpuratus* (Part 1)



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.8 (Part 1)

Figure 5.8 Fate map and cell lineage of the sea urchin *Strongylocentrotus purpuratus*



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.8

Micromeres induce presumptive ectodermal cells to acquire other fates

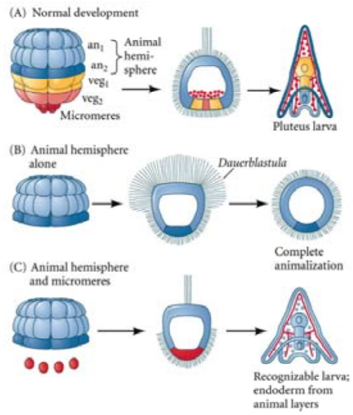
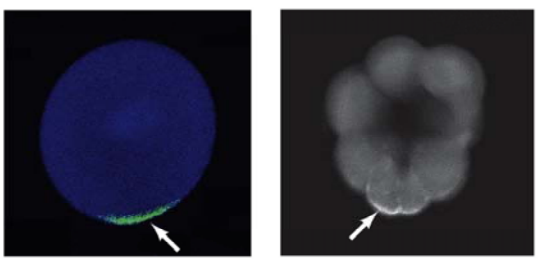


Figure 5.11 Role of Disheveled and β-catenin proteins in specifying the vegetal cells of the sea urchin embryo (Part 1)

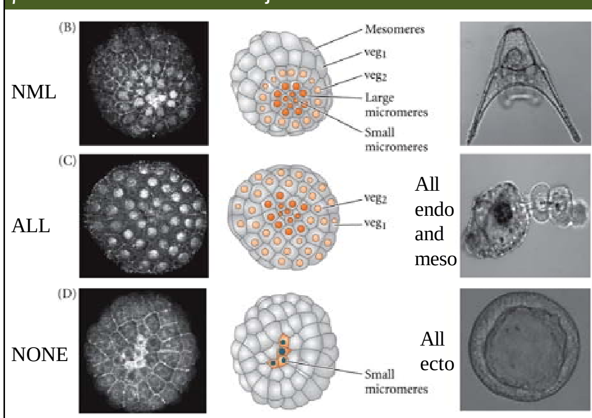
Vegetal end of egg | Vegetal micromere cells

(A)



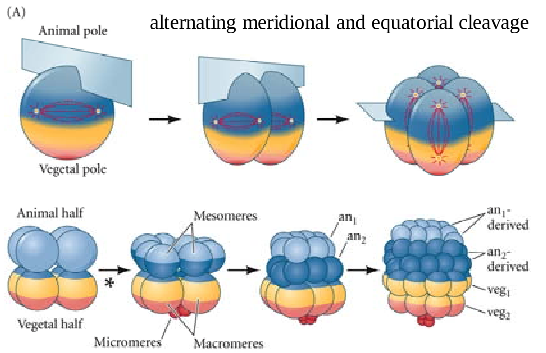
Wnt receptor disheveled

β-catenin does the Wnt job



Radial, Holoblastic Cleavage in the Sea Urchin

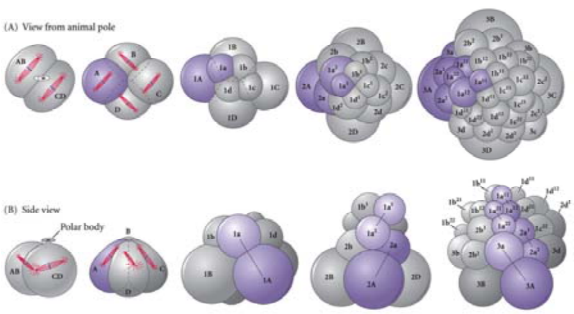
alternating meridional and equatorial cleavage



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.6 (Part 1)

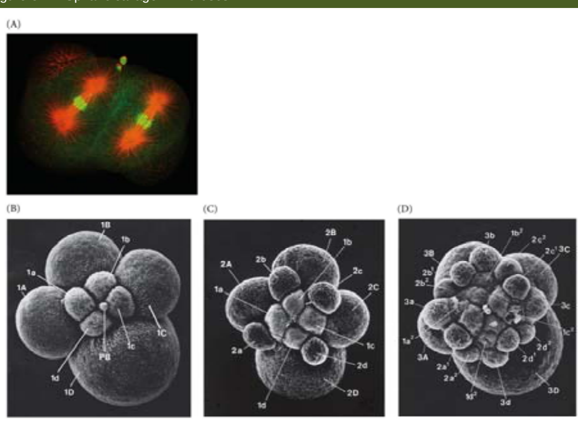
The adult organism has radial symmetry

2. Spiral, Holoblastic Cleavage of the Snail



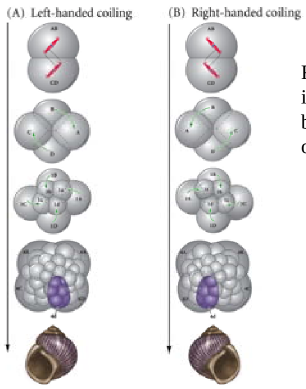
DEVELOPMENTAL BIOLOGY, 9e, Figure 5.23

Figure 5.24 Spiral cleavage in molluscs



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.24

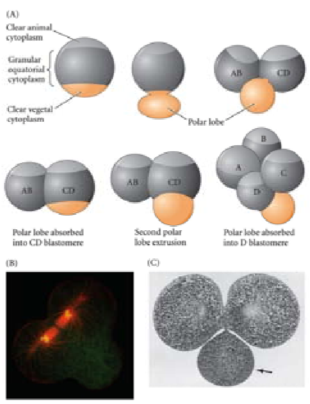
Figure 5.25 Looking down on the animal pole of left-coiling (A) and right-coiling (B) snails



Right-hand coiling is genetically dominant but it really depends on mom's genetics

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.25

Polar lobe formation in certain mollusc embryos

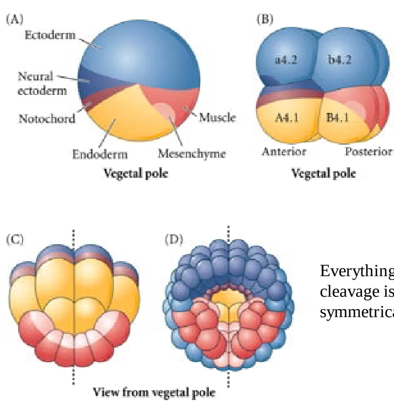


Function much like micromeres in sea urchins

Common Theme
The Organizer

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.28

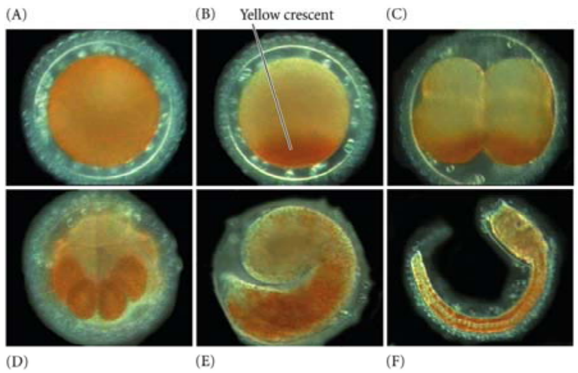
3. Bilateral, Holoblastic Cleavage in Tunicates



Everything from the first cleavage is focused on the symmetrical midline

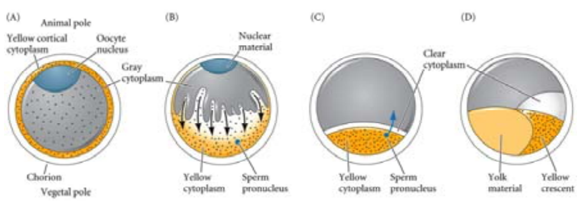
DEVELOPMENTAL BIOLOGY, 9e, Figure 5.34

Figure 5.36 Cytoplasmic segregation in the egg of *Boltenia villosa*



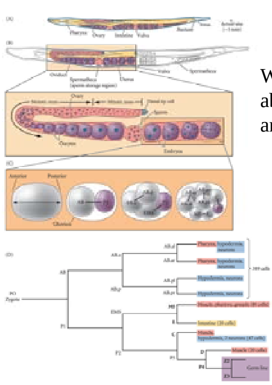
DEVELOPMENTAL BIOLOGY, 9e, Figure 5.36

Figure 5.35 Cytoplasmic rearrangement in the fertilized egg of *Styela partita*



DEVELOPMENTAL BIOLOGY, 9e, Figure 5.35

4. The nematode *Caenorhabditis elegans*

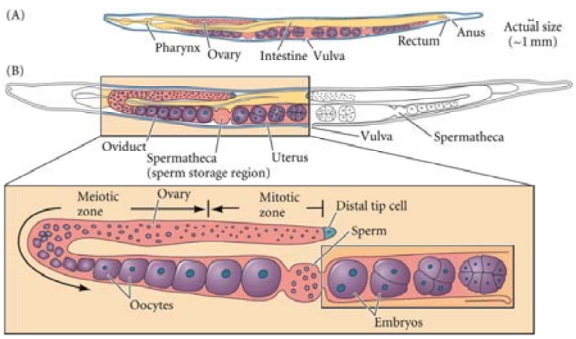


We may know more about *C. elegans* than any other organism:

- every cell division
- every cell death
- every differentiation
- every migration
- full genome

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.42

Rotational, Holoblastic Cleavage in the nematode *Caenorhabditis elegans*

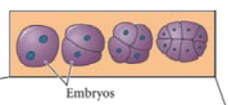


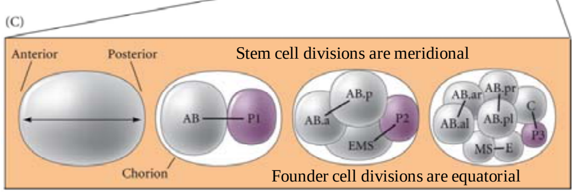
hermaphrodite

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.42 (Part 1)

Figure 5.42 The nematode *Caenorhabditis elegans* (Part 2)

Cleavage divisions drive rotation: Some are asymmetrical, producing a stem cell (P-lineage) and a “founder” cell. The descendents of founder cells give all of the larval cells (558 cells).





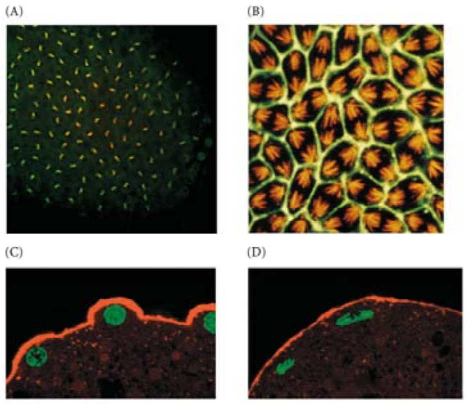
An adult hermaphrodite has 959 cells, males have 1031

DEVELOPMENTAL BIOLOGY, 9e, Figure 5.42 (Part 2)

5. Superficial cleavage in a *Drosophila* embryo

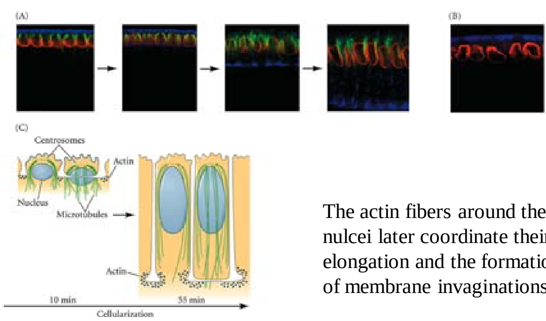
DEVELOPMENTAL BIOLOGY, 9e, Figure 6.1

Figure 6.2 Nuclear and cell division in *Drosophila*



DEVELOPMENTAL BIOLOGY, 9e, Figure 6.2

Figure 6.3 Formation of the cellular blastoderm in *Drosophila*



The actin fibers around the nulcei later coordinate their elongation and the formation of membrane invaginations.

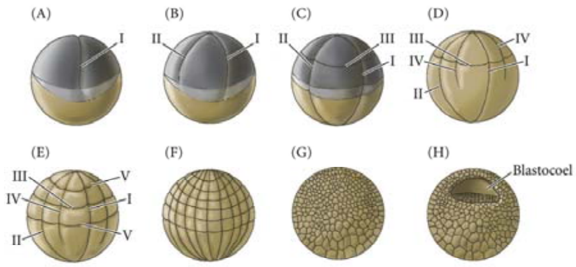
DEVELOPMENTAL BIOLOGY, 9e, Figure 6.3

C. Vertebrates

1. The Frog
2. Zebrafish
3. The Chick
4. Human

Radial, Holoblastic Cleavage of a frog egg

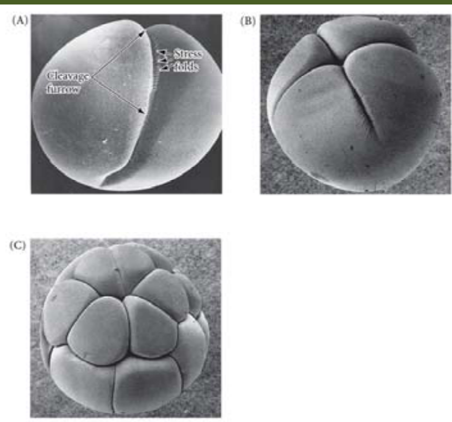
Same basic design as the sea urchin



It is unequal because of the large amount of yolk in the vegetal end

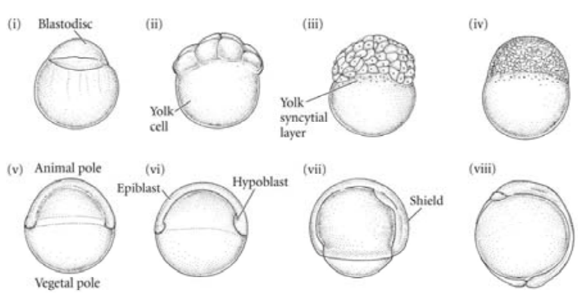
DEVELOPMENTAL BIOLOGY, 9e, Figure 7.2

Figure 7.3 Scanning electron micrographs of frog egg cleavage



DEVELOPMENTAL BIOLOGY, 9e, Figure 7.3

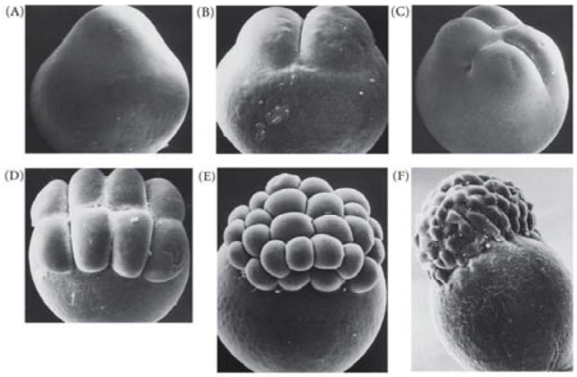
2. Discoidal, Meroblastic Cleavage in Zebrafish



Very high yolk content

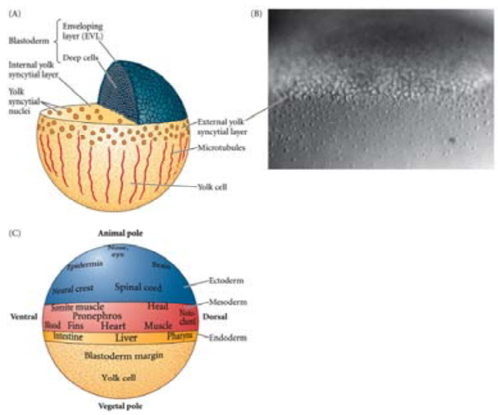
DEVELOPMENTAL BIOLOGY, 9e, Figure 7.37 (Part 1)

Figure 7.40 Discoidal meroblastic cleavage in a zebrafish egg



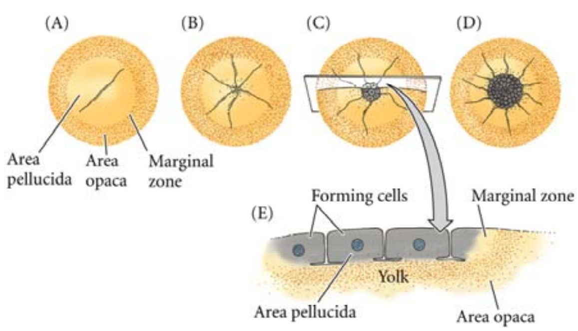
DEVELOPMENTAL BIOLOGY, 9e, Figure 7.40

Figure 7.41 Fish blastula



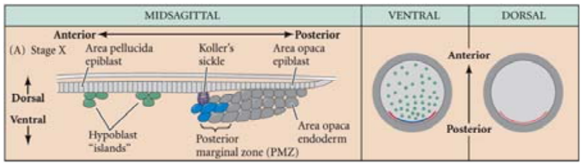
DEVELOPMENTAL BIOLOGY, 9e, Figure 7.41

3. Discoidal, Meroblastic Cleavage in a chick egg



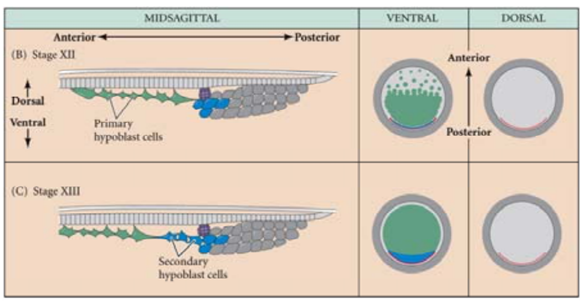
DEVELOPMENTAL BIOLOGY, 9e, Figure 8.1

Figure 8.2 Formation of the chick blastoderm (Part 1)



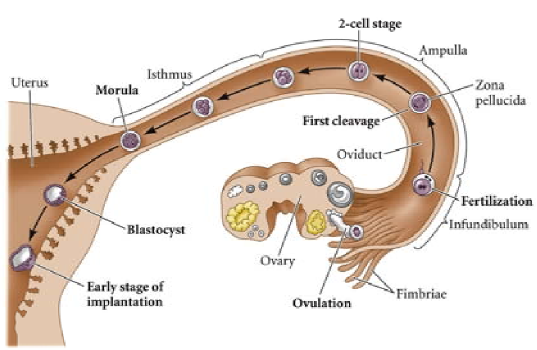
DEVELOPMENTAL BIOLOGY, 9e, Figure 8.2 (Part 1)

Figure 8.2 Formation of the chick blastoderm (Part 2)



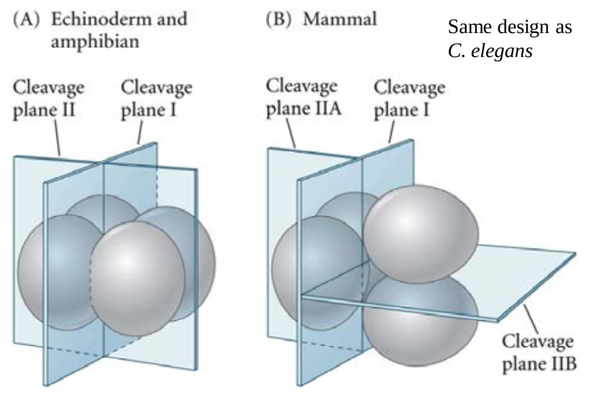
DEVELOPMENTAL BIOLOGY, 9e, Figure 8.2 (Part 2)

4. Mammals: Development of a human embryo from fertilization to implantation



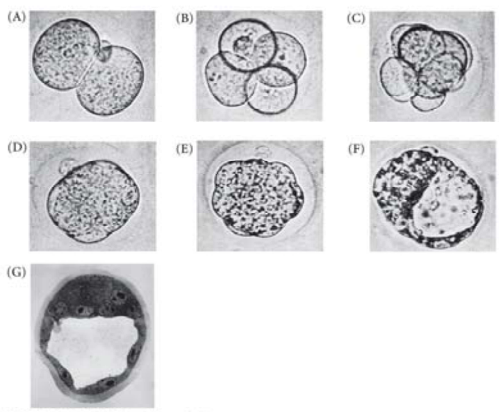
DEVELOPMENTAL BIOLOGY, 9e, Figure 8.15

Rotational, Holoblastic Cleavage in Mammals



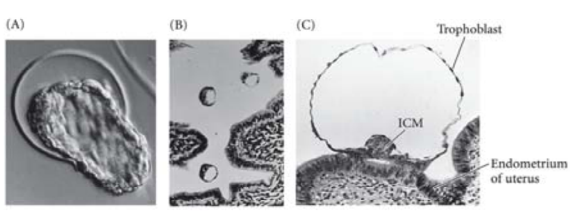
DEVELOPMENTAL BIOLOGY, 9e, Figure 8.16

Figure 8.17 Cleavage of a single mouse embryo in vitro



DEVELOPMENTAL BIOLOGY, 9e, Figure 8.17

Figure 8.20 Hatching from the zona and implantation of the mammalian blastocyst in the uterus



DEVELOPMENTAL BIOLOGY, 9e, Figure 8.20