Overview: Early development consists of cleavage and gastrulation

cleavage - rapid cell divisions; division of fertilized egg into many cells

gastrulation - cells undergo displacement
  - cells move to different parts of embryo
  - cells acquire new neighbors; new inductive relationships

- body axes established
  - anterior-posterior (head-anus)
  - dorsal-ventral (back-belly)
  - left-right
- embryonic cells begin to acquire their respective fates

Cleavage
- cleavage-stage cells = blastomeres
- initial rate of cell division and placement of blastomeres with regard to one another is under control of proteins and mRNAs stored in the oocyte
NOTE - mammalian zygotes control undergo early transcription; exercise some self-control of cleavage process

- initial phase; cleavage controlled by maternal factors
  - cytoplasmic volume does not increase
  - division of cytoplasm without increasing volume - normal cell cycle modified
    - early cell cycles abolish gap (G1, G2) periods when growth would normally occur
  - nuclear division occurs at a rapid rate
    - faster than tumor cells
      - e.g. frog egg can divide into 37k cells in 43 h (what temp?)
      - e.g. Drosophila - division every 10 min for 2 h; 50k cells in 12 h

Cell cycle

mitosis-promoting factor (MPF) controls entry into the cell cycle
  - first discovered as the agent responsible for reentry of frog oocytes into meiotic cycle

- blastomeres generally progress through a biphasic cell cycle:
  - M phase (mitosis)
  - S phase (DNA synthesis)
- MPF activity of early blastomeres highest during M; undetectable during S
  - MPF content cycles between the phases
- MPF structure: two subunits
  - cyclin B (large subunit)
    - shows cyclical behavior; accumulates during S phase; degrades during M phase
    - when sufficient cyclin B accumulates, cells move into M
    - cyclin B encoded in maternal stored messages
    - cyclin B controls the activity of cdc kinase (below)
  - cyclin-dependent kinase (cd kinase; the small subunit of MPF)
    - cd kinase activates mitosis; phosphorylates several target proteins
      - e.g. histones, nuclear envelope lamins, regulatory subunit of cytoplasmic myosin
      - stimulates chromosome condensation, nuclear envelope depolymerization, organization of mitotic spindle
  - presence of cyclin B controlled by egg cytoplasm proteins
    - ensure periodic synthesis and degradation
    - presence in egg means that cyclin B (and cell cycle control) can be independent of the zygotic genome
      - cell divisions are rapid and synchronous
    - however, when materials (i.e. mRNAs, proteins) are used up, embryo must synthesize new ones

- mid-blastula transition - period when embryonic genome takes over control of development
  NOTE - MBT does not occur in all species; e.g. major exception - mammals; some zygote-driven transcription takes place in early cell cycles
  - MBT:
    1) cell cycle post-MBT: addition of two gap (G) phases for cell growth
      - G1 - post mitosis
      - G2 - post DNA replication
    2) cell division becomes asynchronous
    3) new mRNA transcription (i.e. derived from zygotic nucleus)

The cytoskeletal mechanisms of mitosis
Cleavage is the result of two coordinated process
  - karyokinesis: mitotic division of nucleus
    - mechanical agent = the mitotic spindle
      - microtubules composed of tubulin
  - cytokinesis: division of the cell
    - mechanical agent = microfilaments
      - microfilaments made of actin

- mitotic spindle and contractile ring are perpendicular to each other
- spindle is internal to the contractile ring
- contractile ring creates a **cleavage furrow**
  - cleavage furrow bisects the plane of mitosis; creates two genetically equivalent blastomeres

- actin microfilaments found in the **egg cortex**
  - forms a distinct **cortical band** 0.1 μm wide
  - this band exists only during cleavage
  - extends 8-10 μm into the center of the egg

- cortical band exerts the force that splits the zygote; acts like an intercellular purse-string

**Patterns of embryonic cleavage**
The pattern of embryonic cleavage peculiar to a species is determined by two major parameters
1. amount and distribution of yolk protein within the cytoplasm
2. factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation

- amount and distribution of yolk determines:
  - where cleavage can occur
  - relative size of blastomeres

- when one pole is relatively yolk-free, cell divisions occur there at a faster rate than at the opposite pole
  - yolk-rich: **vegetal pole**
  - yolk-poor: **animal pole**
  - the zygotic nucleus is often displaced toward the animal pole
  - in general, yolk inhibits cleavage

**Cleavage classifications**
- sea urchins, mammals, snails: sparse, equally spaced yolk = **isolecithal**
  - **holoblastic cleavage**
    - cleavage furrow extends through entire egg
    - NOTE - with little yolk, embryos must have some other way of obtaining food; most generate voracious larval form; mammals obtain nutrition from maternal placenta

- insect (**centrolecithal**), fish, reptiles, birds (**telolecithal**): most of the cell volume taken up by yolk
  - **meroblastic cleavage** (Gr. *meros*, “part”); only a portion of the cytoplasm is cleaved
    - cleavage furrow does not penetrate the yolky portion of the cytoplasm
    - insects -centrolecithal eggs - divisions of cytoplasm occur only in the rim of cytoplasm around the periphery of the cell (A.K.A. **superficial**
cleavage)
- birds, fishes - telolecithal eggs - one small area is yolk-free; cell divisions occur only in this small disc (A.K.A. discoidal cleavage)

- inherited cleavage patterns are species-specific
  NOTE - isolecithal eggs (without constraint of yolk) show most cleavage variation
  - four basic patterns:
    1. radial
    2. spiral
    3. bilateral
    4. rotational

Gastrulation
Gastrulation gives blastula cells new positions and new neighbors
- also, multilayered body plan
  - endoderm and mesoderm inside
  - skin and nervous system cell spread over outside surface (ectoderm)
  - new inductive relationships can take place between cells that have been re-positioned

Cell movements
- Invagination - infolding of a region of cells (like indenting a rubber ball when poked)
- Involution - inturning or inward movement of an expanding outer layer; spreads over the internal surface of the remaining external cells
- Ingression - migration of individual cells from the internal surface layer into the interior of the embryo; cells become mesenchymal and migrate independently
- Delamination - splitting of one cellular sheet into two more-or-less parallel sheets; resembles ingestion, but results in a new layer of cells
- Epiboly - movement of epithelial sheets (usually of ectodermal cells) that spread as a unit (rather than as individuals) to enclose deeper layers of the embryo; can occur by cells dividing, by cells changing their shape, or by several layers of cells intercalating into fewer layers (often, all three mechanisms used)

Cell Specification and Axis Formation
Cell fates specified by either
- cell-cell interactions, or
- asymmetric distribution of patterning molecules into particular cells
  - usually transcription factors that activate or repress transcription of specific genes in those cells that acquire them
  - asymmetric distributions of patterning molecules happen during cleavage by several mechanisms:
    1. molecules bound to egg cytoskeleton; passively acquired by cells that obtain this cytoplasm
    2. molecules are actively transported along the cytoskeleton to one particular cell
3. molecules become associated with a specific centrosome; then follow that centrosome into one of the two mitotic sister cells

- once asymmetry has been established, one cell can specify a neighboring cell by paracrine or juxtacrine interactions

- embryos establish three axes:
  - anterior-posterior (or anteroposterior)
    - line extending from head to tail (mouth to anus)
  - dorsal-ventral (dorsoventral)
    - lined extending from back (dorsum) to belly (ventrum)
  - right-left
    - line between the two lateral sides of the body

EARLY DEVELOPMENT IN SEA URCHINS

Sea Urchin Cleavage
Radial holoblastic cleavage; first seven cleavages “stereotypic” - same pattern followed in every individual of the species

Cleavage #
1st - meridional
2nd - meridional (1 & 2 are parallel to each other)
3rd - equatorial, perpendicular; separates animal and vegetal halves
4th - animal tier cells divide meridionally into eight blastomeres, each with the same volume = mesomeres
    - vegetal tier cells undergo unequal equatorial cleavage; produces:
      - 4 large cells = macromeres
      - 4 smaller cells = micromeres
5th - mesomeres (8, at animal hemisphere) divide equatorially; form two tiers, an1, an2
    - macromeres (4) divide meridionally; form tier of 8 cells below an2
    - micromeres (4) divide; produce small cluster beneath the larger tier
6th - animal hemisphere cells divide meridionally
    - vegetal hemisphere cells divide equatorially
7th - animal hemisphere cells divide equatorially
    - vegetal hemisphere cells divide meridionally
    = 128 cells (blastula); divisions become less regular

Blastula formation
Blastula stage begins at 128 cell stage
- cells form hollow sphere surrounding a central cavity - blastocoel
- all cell are the same size (micromeres have slowed down division rate)
- every cell is in contact with proteinaceous fluid of blastocoel and hyaline layer on the outside
- **tight junctions** connect cells into **epithelial sheet**
  - cells continue to divide
    - blastula remains one cell thick
    - thins as it expands
      - cells adhere to hyaline layer
      - water influx (due to osmotic pressure from high protein and glycoprotein content of blastocoel)
  - rapid and invariant divisions last through 9th or 10th (species-dependent)
  - fates have been specified
  - each cell gets **ciliated** on the region of membrane farthest from the blastocoel
  - ciliated blastula begins to rotate within the fertilization envelope

- differences seen between cells
  - vegetal pole cells begin to thicken to form the **vegetal plate**
  - animal hemisphere cells synthesize and secrete a hatching enzyme
    - enzyme digests fertilization envelope
    - blastula hatches

**Fate maps and the determination of sea urchin blastomeres**

By the 60-cell stage, most embryonic cell fates are specified, but cells are not irreversibly committed
  - i.e. particular blastomeres consistently produced the same cell types in each embryo, but these cells remain pluripotent; can give rise to other cell types if experimentally placed in a different part of the embryo

**Cell fate determination**

- 60-cell stage: animal half of embryo consistently gives rise to ectoderm
- veg1 layer produces cells that can enter into either ectodermal or endodermal organs
- veg2 layer gives rise to cells that can populate three different structures
  - endoderm
  - coelom (internal mesodermal body wall)
  - secondary mesenchyme (pigment cells, immunocytes, muscle cells)
- 1st tier micromeres - primary mesenchyme cells - form larval skeleton
- 2nd tier micromeres - contribute cells to coelom

- most fates achieved by conditional specification (fate depends on position relative to neighboring cells)
- skeletogenic micromeres (1st tier) are the only cells whose fates are determined autonomously
  - isolated cells still form spicules
  - transplanted to animal region: they will form spicules and alter fate of nearby cells by inducing a second site for gastrulation
**β-catenin micromere specification**
- **β-catenin** is responsible for specifying micromeres; it also has the ability to induce neighboring cells
- **β-catenin** is a transcription factor; often activated by Wnt pathway
- **β-catenin** accumulates in cells fated to become endoderm and mesoderm
  - responsible for specifying vegetal half of embryo
  - may help specify mesodermal and endodermal fates of vegetal cells
  - treating sea urchin embryos with LiCl causes accumulation of **β-catenin** in every cell; transforms presumptive ectoderm into endoderm
  - inhibition of **β-catenin** accumulation in vegetal cell nuclei prevent formation of mesoderm and endoderm
- **β-catenin** critical for specification of micromeres and empowering them with ability to induce veg₂ cells above them
  - specification mediated by Pmar1 gene product
  - Pmar1 is a homeodomain transcription factor
    - acts as a transcriptional repressor
    - represses as yet unknown gene - product is general repressor of genes that characterize primary mesenchyme cells

**Specification of the vegetal cells**
- before veg₂ cells are specified by molecular signals, moderate levels of **β-catenin** bias the cells to be “**endomesoderm**”
  - then an “early veg₂ signal” emanates from the micromeres (4ᵗʰ cleavage), amplifies mesendoderm specification established by **β-catenin**
  - **Δelta** protein on micromeres signals activation of **Notch** pathway in the adjacent veg₂ cells
  - **Notch** pathway causes cells to become secondary mesenchyme rather than endoderm
    - both signals are needed, in order
- **Wnt8** is made by the micromeres and endoderm cells (i.e. endomesoderm cells not receiving Delta signal)
  - **Wnt8** acts in autocrine manner to boost specification of both veg₂ endoderm cells and micromeres; facilitates separation into two distinct lineages

**Differentiation: Combinations of transcription factors**
- Once transcription factors are present in their specific regions, they can activate genes that characterize different cell types in the embryo
  - e.g. **Endo16** - endodermal protein
  - secreted product of endodermal cells
  - probably an adhesion protein that allows cell rearrangement in the archenteron
  - **endo16** upstream regulatory region contains 7 modular elements to which at least
13 different transcription factors bind
- some activate, some repress endo16 transcription

**Axis specification**
- cell fates line up along animal-vegetal axis established in the egg cytoplasm prior to fertilization
- animal-vegetal axis appear to structure future anterior-posterior axis
- vegetal region sequestering those maternal components necessary for posterior development

- dorsal-ventral and left-right axes specified after fertilization (not well understood)

- oral-aboral axis (approximates ventral-dorsal axis) usually delineated by the first cleavage plane
  - oral pole of future oral-aboral axis lies 45 degrees clockwise from the first cleavage plane (viewed from the animal pole)
  - oral-aboral axis mechanism may be similar to that used by vertebrates to establish right-left axes
  - oral ectoderm specified by expression of nodal gene (TGF-β family)
  - Nodal protein appears to act against a BMP

  - oral fates promoted by Nodal; aboral fates promoted by BMP2/4

- left-right axis of deuterostomes usually associated with an asymmetric expression of a Nodal gene
- sea urchins, L-R asymmetric expression of Nodal is observed in the larva long after oral-aboral axis has been established

**NOTE** - sea urchin Nodal expression is on the right side of the embryo; other deuterostomes asymmetric Nodal expression has been on the left side
- sea urchin - only lift side f larva develops a coelomic sac and the imaginal rudiment that will generate the adult
  - Nodal expression on the right side activates a pathway that prevents this sac from forming

**Sea Urchin Gastrulation**
Late blastula consists of a single layer of about 1000 cells; forms a hollow ball; somewhat flattened at the vegetal end
- blastomeres derived from different regions of the zygote
  - different sizes and properties

**Ingression of the primary mesenchyme**
after hatching, a group of cells derived from micromeres undergo epithelial-to-mesenchymal transformation
- **primary mesenchyme** cells
  - a.k.a. **skeletogenic mesenchyme** (form larval skeleton)
- cells change their cytoskeleton
- become bottle shaped
- lose adhesion to cells lateral to themselves
- break away from the apical layer to enter blastocoel

- primary mesenchyme cells extend and contract long, thin **filopodia** (250 nm long, 25 µm long)
- cells actively make and break filopodial connections to blastocoel wall
  - eventually become localized within prospective ventrolateral region of blastocoel
  - fuse into syncitial cable
  - form axis of **calcium carbonate spicules** of the larval skeletal rods

**Importance of extracellular lamina inside the blastocoel**
- ingression of micromeres results from cells losing affinity for neighboring cells and for hyaline membrane; gaining affinity for blastocoel proteins
  - originally blastula cells are connected on the outer surface to the hyaline layer, on inner surface of basal lamina secreted by the cells and on the lateral surface, another cell
  - prospective ectoderm and endoderm (descendants of mesomeres and macromeres, respectively) bind tightly to one another and to hyaline layer, but adhere only loosely to basal lamina
  - micromeres originally display similar pattern
    - however, at gastrulation primary mesenchyme precursors lose affinity for hyaline layer and neighbors
    - affinity for components of basal lamina and extracellular matrix; e.g. fibronectin) increase 100X
    - release attachments to external hyaline layer and neighboring cells
    - drawn in by basal lamina
    - migrate up into blastocoel
    - changes in affinity associated with changes in membrane surface molecules; e.g. fibronectin, integrin, laminin, L1, cadherins

- once inside the blastocoel, primary mesenchyme cells appear to migrate along the extracellular matrix of blastocoel wall
- extend filopodia in front of them
- proteins e.g. fibronectin, sulfated glycoprotein necessary to initiate and maintain migration
- migrating cells stop and forms spicules near equator of blastocoel
  - primary mesenchyme cells arrange themselves in a ring at a specific position along animal-vegetal axis

- at two sites near future ventral side of larva, primary mesenchyme cells cluster together,
fuse with one another, initiate spicule formation
- positional information may be provided by prospective ectodermal cells and their basal laminae
- only primary mesenchyme cells capable of responding to these patterning cues
- extremely fine filopodia (0.3 μm diameter) appear to explore and sense the blastocoel wall
- may be responsible for picking up dorsal-ventral and animal-vegetal patterning cues from ectoderm

The first stage of archenteron invagination
cells in vegetal region thicken and flatten; form **vegetal plate**
- remain bound to one another and to hyaline layer
- move to fill gaps caused by ingression of primary mesenchyme
- bend inward and invaginate up to ~ one-quarter to one-half the way into the blastocoel
- invagination ceases at that point

- cavity formed is termed the **archenteron**
- the vegetal plate cells surrounding the 2-8 cells at vegetal pole become bottle-shaped, constrict apical ends
- this causes cells to pucker inward
- also, the hyaline layer at vegetal plate buckles inward due to changes in composition
  - hyaline layer made of up of two layers:
    - outer lamina; made primarily of hyalin protein
    - inner lamina: composed of fibropellin proteins
      = fibropellins stored in secretory granules within oocyte; secreted after cortical granule exocytosis releases hyalin
- by blastula stage, fibropellins form mesh-like network over embryo surface
- at invagination, vegetal plate cell (only) secrete chondroitin sulfate proteoglycan into inner lamina
- chondroitin sulfate proteoglycan is hygroscopic
  - swells inner lamina, not outer lamina
  - causes vegetal region of hyaline layer to buckle

- second force arising from movements of epithelial cells adjacent to the vegetal plate; may facilitate invagination by drawing buckled layer inward

- fate of vegetal plate cells specified when skeletogenic mesenchyme cells ingress
- secondary mesenchyme is the first group of cell invaginating
  - forms tip of archenteron
  - pigment cells, musculature around gut, coelomic pouches
- endodermal cells adjacent to micromere-derived mesenchyme becomes foregut, migrating farthest distance into blastocoel
- next layer of endodermal cells become midgut
- last circumferential row to invaginate forms hindgut and anus

**Second and third stages of archenteron invagination**
- invagination of vegetal cells occurs in discrete stages; after brief pause following initial invagination, 2nd phase of archenteron formation begins
- archenteron extends; ~ 3X length
  - **convergent extension**
    - cells rearrange themselves by migrating over one another and flattening themselves
    - cell intercalate to narrow tissue; move forward

- 3rd stage of archenteron elongation
  - tension provided by secondary mesenchyme cells; (at tip of archenteron)
  - form filopodia through blastocoel fluid to contact inner surface of blastocoel wall
    - filopodia attach to wall at the junctions between the blastomeres
    - shorten, pulling up archenteron
  - specific target sites for filopodia on blastocoel wall
    - filopodia extend to random sites; retract
    - at particular region, remain attached
  - top of archenteron meets blastocoel wall in the target region
  - secondary mesenchyme disperses into blastocoel
    - proliferate to form mesodermal organs
  - where archenteron contacts wall, mouth eventually forms
  - mouth fuses with the archenteron to create a continuous digestive tube
    - blastopore turns into anus

as pluteus larva elongates, coelomic cavities form from secondary mesenchyme
- Nodal protein influences right coelomic sac to remain rudimentary
- right coelomic sac undergoes extensive development to form many of the structures of the adult sea urchin
  - left sac splits into three smaller sacs
  - invagination from the ectoderm fuses with the middle sac to form the imaginal rudiment

- imaginal rudiment develops a fivefold symmetry and skeletogenic mesenchyme cell enter the rudiment to synthesize first skeletal plates of the shell
- left side of pluteus becomes future oral surface of adult sea urchin

- during metamorphosis, imaginal rudiment separates form the larvae
- larva degenerates
- juvenile (imaginal rudiment) reforms digestive tract and settles on ocean floor
  - dependent on nutrition derived from larva