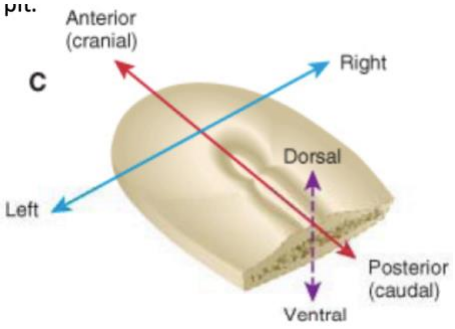
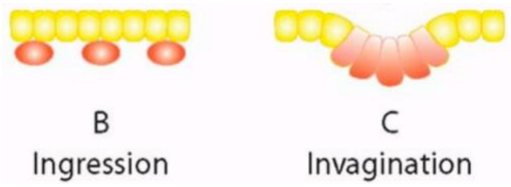
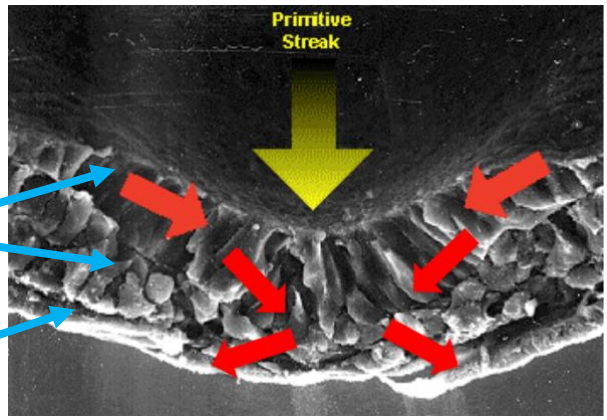
Gastrulation (day 14-16)

- **Gastrulation** is the formation of the 3 germ layers; ectoderm, endoderm and mesoderm
    - **Ectoderm** gives rise to the epithelial layer (epidermis), lens retina, internal ear, neural crest, neural tube, middle and posterior pituitary and pineal gland
    - **Mesoderm** gives rise to muscles, connective tissue, dermis, circulatory system, kidney, reproductive tract, ureters, gonads, endothelium and skeleton
    - **Endoderm** gives rise to the midgut, bladder, lungs, liver, pancreas, pharynx, thyroid, thymus and anterior pituitary (digestive tract)
- Occurs after implantation in humans
- As invasion of the endometrial tissue occurs, the inner cell mass divides into 2 layers to form the bilaminar embryo
- The hypoblast spreads out and covers the blastocoel to form the **yolk sac** (extraembryonic tissue that produces blood cells, and will eventually form the placenta)
- The epiblast divides into 2 layers
    - The amnion forms the protective layer around the embryo
    - The embryonic epiblast undergoes gastrulation (**trilaminar**)

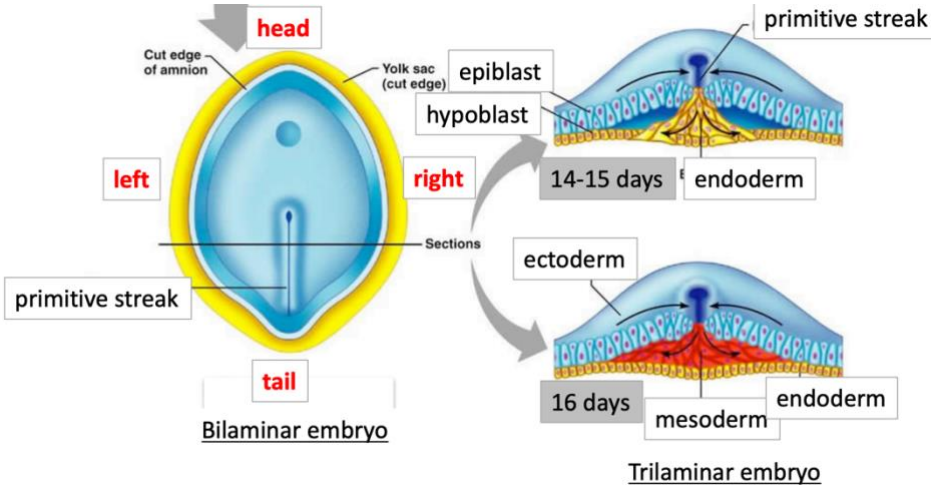


- First sign of gastrulation is the primitive streak on the surface of the epiblast
- The streak in a 15- to 16-day embryo is clearly visible as a narrow groove with slightly bulging regions on either side
- The primitive node at the anterior/cranial end of the primitive streak consists of a slightly elevated area surrounding the small primitive pit
- Anterior to the primitive node is the notochord





- Cells of the epiblast migrate toward the primitive streak
- They become flask-shaped, detach from the epiblast, and slip beneath it (**invagination**)
- Cells remaining in the epiblast form the ectoderm
- Cells between the epiblast and endoderm form the mesoderm
- Once the cells have invaginated, some displace the hypoblast, creating the embryonic endoderm
- Position of cells in the mesoderm determines what type of mesoderm (notochord, paraxial or lateral)



- **Caudal dysgenesis (Sirenomelia)** is caused by insufficient mesoderm formed in the caudal-most region of the embryo
- Causes hypoplasia, fusion of the lower limbs, vertebral abnormalities, renal agenesis, imperforate anus and anomalies of the genital organs
- This mesoderm contributes to formation of the lower limbs, urogenital system (intermediate mesoderm), and lumbosacral vertebrae

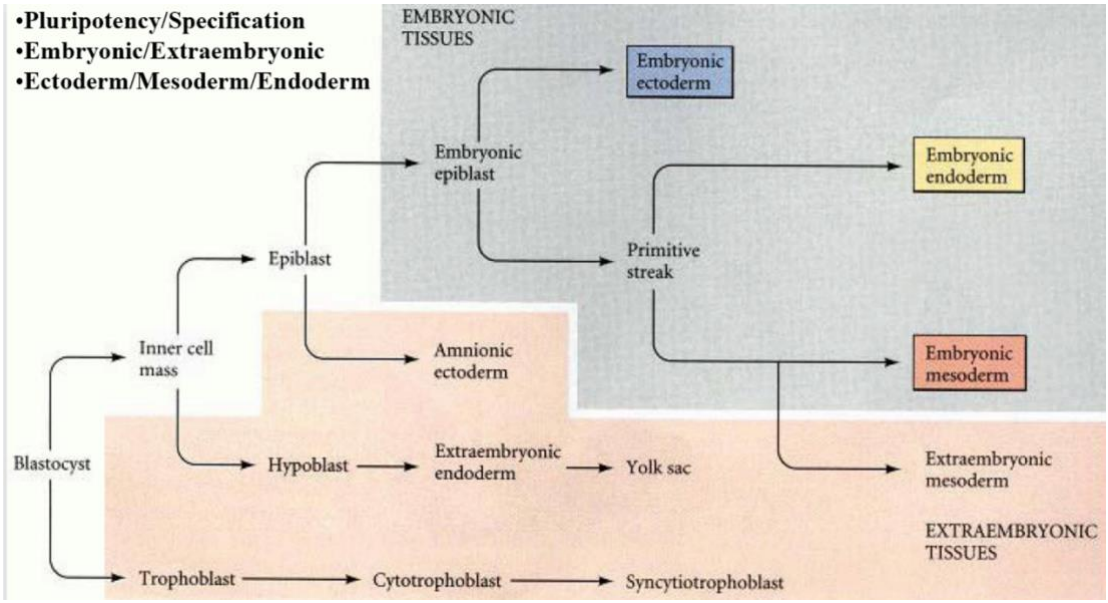
Model Organisms to Study Gastrulation

Sea Urchins

- Transparent = easy to visualise
- External development (outside the mother)

Chicken

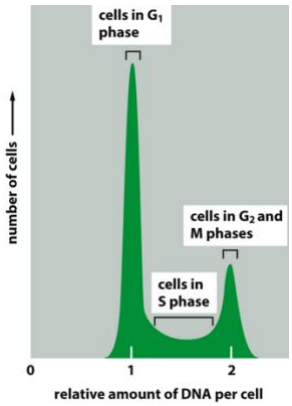
- Human and chicken gastrulas have similar morphology
- Chicken embryos can be visualised and manipulated in vivo
- Gastrulation occurs within 5 hours of laying
- Anatomical, experimental and genetic studies are possible



Regulation of Cell Cycle

G_1/S checkpoint

- Checks for damaged DNA and if it is environmentally favourable
- Growth factor stimulates cell to leave G1
- Cyclin D is produced, and binds to CDK4 which results in the transcription of genes including cyclin E
- Cyclin E-CDK2 binds and activates, preparing the cell for S phase
- Levels of the transcription factors that promote the expression of the S-phase cyclins and DNA synthesis enzymes are increased
- Levels of the molecules involved in inhibition of entry into S phase are decreased (controlled degradation)
- In the S phase, active S cyclin-CDK complexes phosphorylate proteins that form pre-replication complexes on DNA replication origins
    - Ensures each pre-replication complex is activated to initiate chromosomal replication, new complexes are prevented from forming and the genome is only replicated once



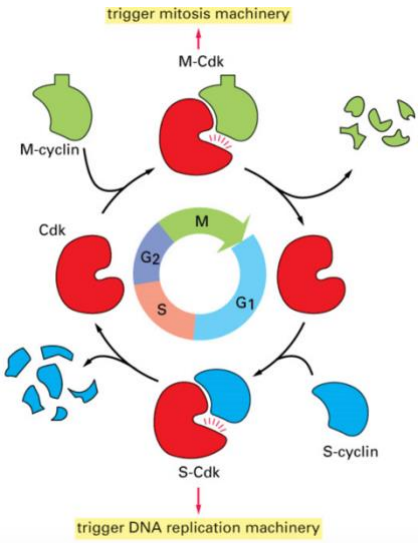
G_2/M checkpoint

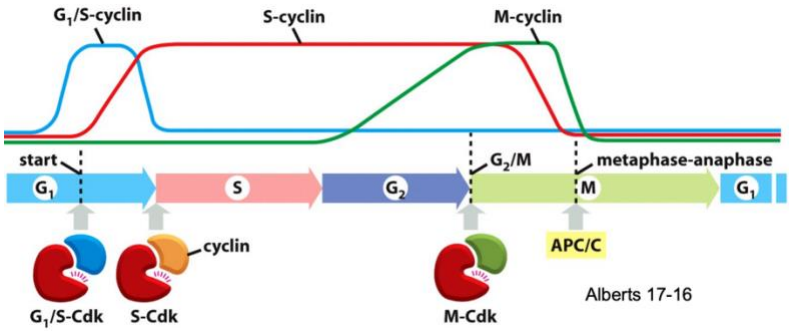
- Checks if all DNA is correctly replicated and not damaged
- Checks if it is environmentally favourable (enough energy?)

M checkpoint (metaphase-anaphase)

- Checks if all chromosomes are attached to spindles
- In the M phase, mitotic cyclin-CDK complexes are activated
    - Activates proteins involved in chromatin condensation, mitotic spindle formation, degradation of chromosomal structural proteins and progression of mitosis

- Checkpoints prevent entry into the next phase of the cell cycle until everything is normal
- Checkpoints ensure that incomplete/damaged/incorrect replication of DNA, chromosomes and organelles are not passed on to daughter cells
- Timing of events in the cell cycle is controlled by proteins called **Cyclins** (control transition from one phase of the cell cycle to the next)
- Cyclins form complexes with protein kinases called **Cyclin-dependent kinases** (Cdks)
- Cdks phosphorylate proteins that regulate each phase
- Cdks are only active when cyclin is bound
- S-Cdk phosphorylates proteins involved in DNA replication
- M-Cdk regulates proteins involved in mitosis
- Complexes are regulated by cdk activation and cyclin degradation/destruction





Rb Checkpoint

- Inherited homozygous mutations in Rb result in retinoblastoma
- When Rb is inactivated by phosphorylation, genes can be transcribed to allow the cell to enter S-phase
- Activated Rb stops the cell cycle (negative regulator)

p53 Checkpoint (damaged DNA)

- Genotoxic stress (damaged DNA) inhibits the cell cycle via Cdks
- Damaged DNA triggers a checkpoint arrest and activates the protein kinase transducers' ATM and ATR
- Downstream protein kinases Chk1/Chk2 proteins phosphorylated by active ATM and ATR
- p53 maintained in very low levels by association with Mdm2 - a ubiquitin ligase
- Mdm2 adds Ubiquitin to p53 targeting it to the proteasome
- p53 is phosphorylated by activated Chk1/Chk2
- p53 is a transcription factor
- Activated p53 dissociates from Mdm2 and enters nucleus, it initiates transcription of DNA repair enzymes
- p21 is transcribed, it is a CKI (Cdk inhibitor protein) that binds to active G1/S-Cdk and S-Cdk (with bound cyclins) and inhibits/inactivates the cyclin-dependant kinase complex, stopping the cell cycle from G1 to S
- After damage is repaired, p53 and p21 levels fall (they are ubiquitinated), and the cell cycle continues
- Activated p53 stops the cell cycle
- 50 % of all cancers carry mutations in the p53 gene

Paracrine Factors/Morphogens

- Paracrine factors regulate development of organs
- Morphogens produced by a cell can control the development of nearby cells
- The nearby cells develop and release morphogens which control the development of their nearby cells
- Growth factors (morphogens) diffuse between cells and have different effects depending on the concentration of the morphogen
- Some signalling pathways can replace each other (redundancy)
- There is a lot of interaction between pathways and cross talk at the intracellular level

Transforming Growth Factor beta (TGFbeta) Pathway

- Bone Morphogenetic Protein 4 (BMP4) is required for development of primordial germ cells

Hedgehog (Hh) Pathway

- Sonic Hedgehog (Shh) is expressed in many tissues, including gut, limb bud, nervous system, face
- Loss of Shh leads to cyclopia, forebrain reduction, craniofacial and limb defects

Wingless (WNT) Pathway

- Mutations in WNT3A in humans can lead to tetra-amelia (lack of all 4 limbs, craniofacial and urogenital defects (autosomal recessive))

Fibroblast Growth Factor (FGF) Pathway

- FGF8 is expressed in the primitive streak and is important for migration of mesodermal cells
- FGF8 knockout causes failure of cell migration in the gastrulating mouse embryo