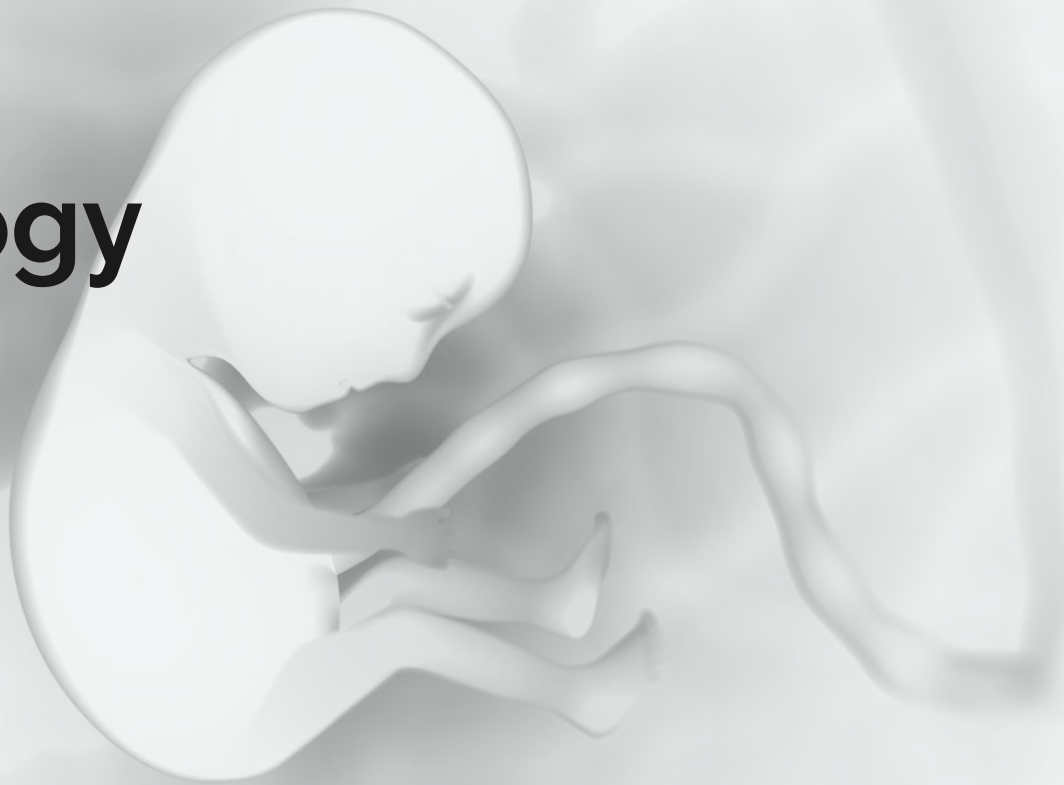
Embryology



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Development of The Face (Week 1-12)

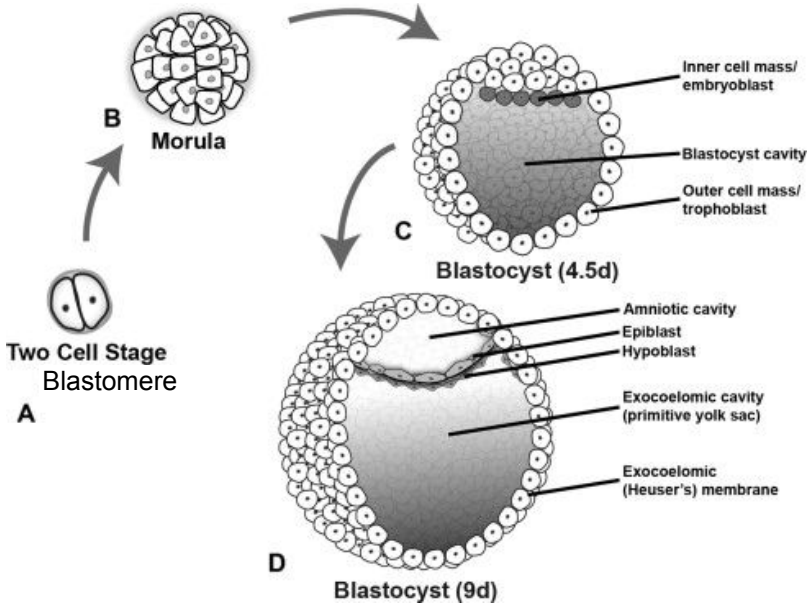
Week 1 - Germ Cell Formation and Fertilisation

Ovum and sperm contact in distal part of the uterine tube -> zygote, undergoes mitosis and cleavage to form -> cell mass (morula) -> increases in size to form a (blastocyst)

2 types of cells in a blastocyst:

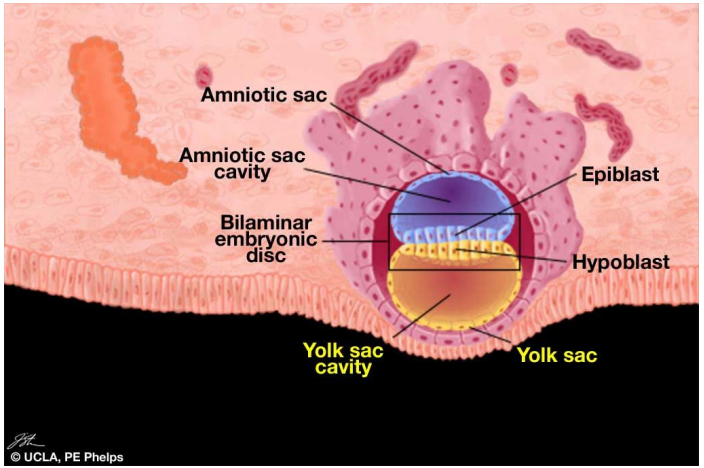
- Trophoblast lines the cavity (primary yolk sac) -> helps with implantation of the embryo
- Embryoblast -> forms the embryo proper

On the 7th day - implantation occurs on the inner lining of the uterus on the back wall

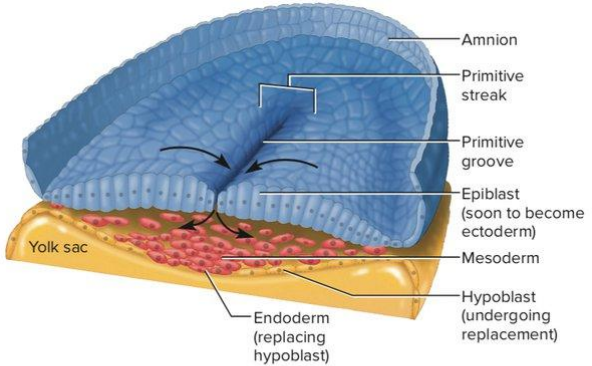


Week 2 - Formation of a Bilaminar Embryonic Disc

- Continued proliferation of the embryonic cells within the blastocyst eventually forms the bilaminar embryonic disc
- Disc is suspended between the:
  - Amniotic cavity -> formed by epiblast, primary ectoderm, (dorsal) columnar cells
  - Yolk Sac -> formed by hypoblast, primary endoderm, (ventral) cuboidal cells
  - Prochordal plate -> region were the ectoderm and endoderm meet



Week 3 - Formation of the Trilaminar Disc



- Cells within the amniotic cavity of the bilaminar disc will begin to differentiate and burrow to form a **'primitive streak'.**
    - Rostral end of the streak ends in a pit (depression) = primitive node (notochord) -> supporting the primitive embryo (early cartilage and vertebrae formation)
- Cells from the epiblast (soon to be ectoderm) moves towards the hypoblast (soon to be endoderm) forming the mesoderm
- **Ectoderm, mesoderm and endoderm = trilaminar embryonic disc**
    - This event from a bilaminar to a trilaminar disc is called **gastrulation**

Derivatives of the Germ Layers

Ectoderm

- Epidermis of skin and its derivatives (including sweat glands, hair follicles)
- Epithelial lining of mouth and anus
- Cornea and lens of eye
- Nervous system
- Sensory receptors in epidermis
- Adrenal medulla
- Tooth enamel
- Epithelium of pineal and pituitary glands

Mesoderm

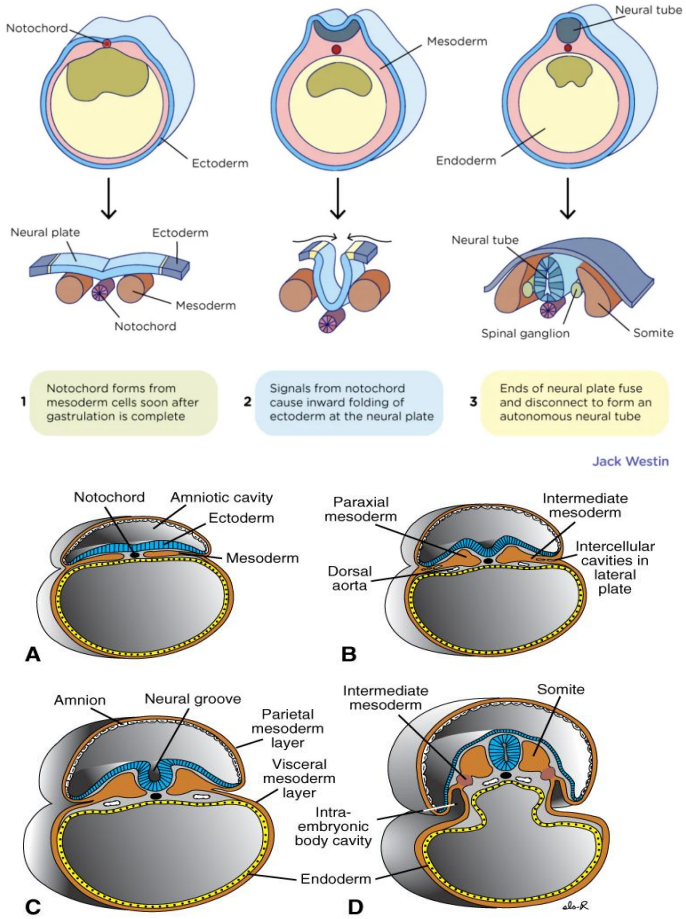
- Notochord
- Skeletal system
- Muscular layer of stomach and intestine
- Excretory system
- Circulatory and lymphatic systems
- Reproductive system (except germ cells)
- Dermis of skin
- Lining of body cavity
- Adrenal cortex

Endoderm

- Epithelial lining of digestive tract
- Epithelial lining of respiratory system
- Lining of urethra, urinary bladder, and reproductive system
- Liver
- Pancreas
- Thymus
- Thyroid and parathyroid glands

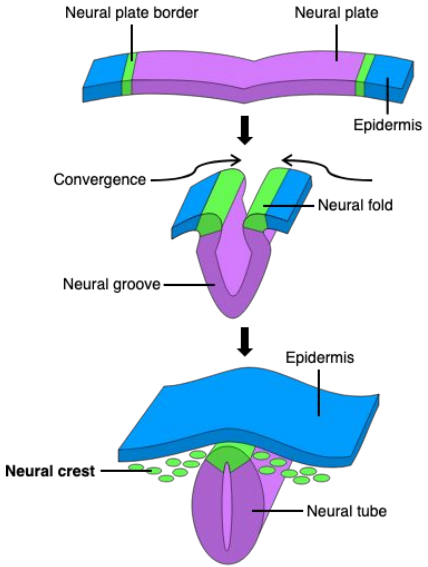
NEURULATION
(Neural plate to Neural tube)

- Cells of the **ectoderm** differentiate and thickens to form the the **neural plate** (central band of cells that extends from the rostral to the caudal end)
- Plate grows and thickenings invaginate inwards to form the **neural groove surrounded by neural folds**
- **Neural folds** will undergo **fusion and forms the neural tube** which eventually separates from the ectoderm
    - Neural tube eventually becomes your CNS and breaks off from the ectoderm



Neural Crest Cells (NCCs)

- NCCs develop from neuroectoderm which undergo an epithelial - mesenchymal transformation and migrate from the crests of the neural folds to join the mesoderm and form mesenchyme
    - Will go on to develop ectomesenchyme **-> forms pulp, dentine, cementum, alveolar process and periodontal ligament BUT NOT enamel and certain types of cementum**



How do NCC's know what to do?

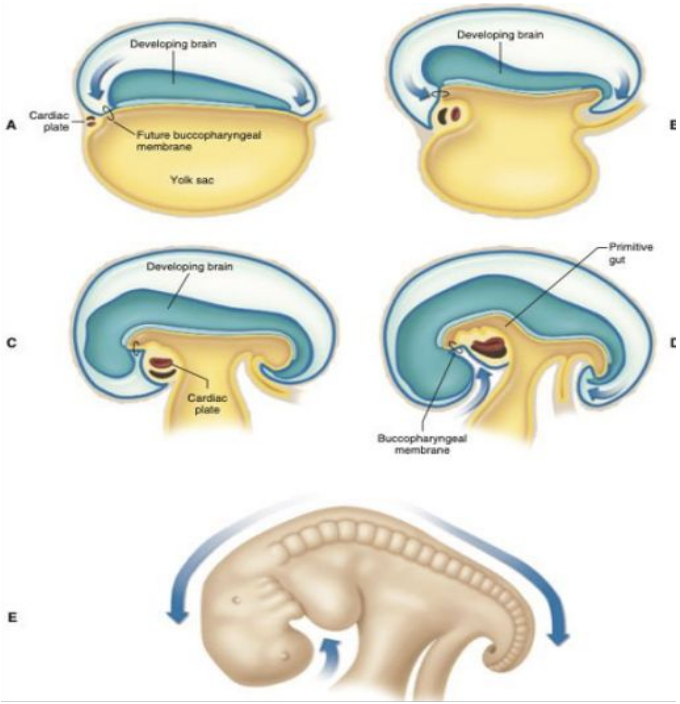
- Signalling events
- Common signalling pathways = sonic hedgehog (Shh), fibroblast growth factor (Fgf)

By the end of the 3rd week:

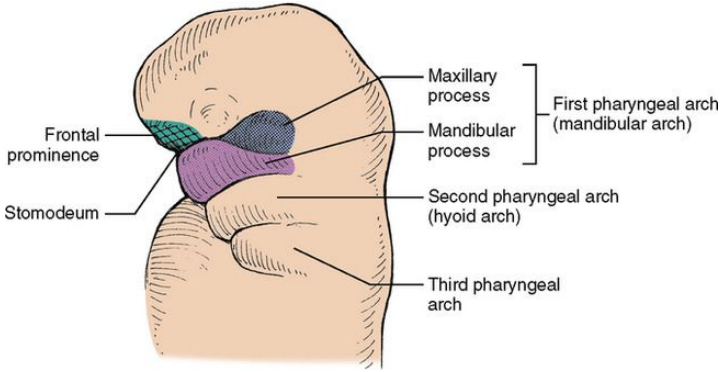
- Mesoderm also differentiates and divides into somites

Week 4 - Formation of the Stomodeum

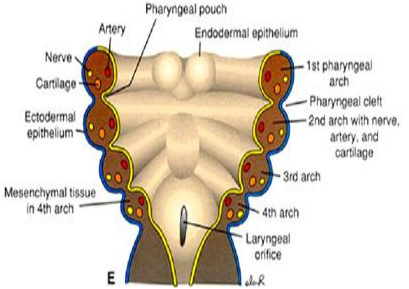
- Folding of embryo along the rostrocaudal axis and the lateral axis
  - **Primitive stomatodeum (the oral cavity) formed by the rostrocaudal axis**; separates from the gut by the buccopharyngeal membrane (where the ectoderm and endoderm meet)
  - Lateral fold: ectoderm lines the embryo on all external surfaces (surface epithelium)
- Rostral end of the embryo -> neural tube expands and forms **primitive forebrain -> produces the frontal prominence**
- Stomodeum:
  - Sits between the frontal prominence and cardiac bulge and bounded laterally by the 1st branchial arch



Branchial Arches Formation



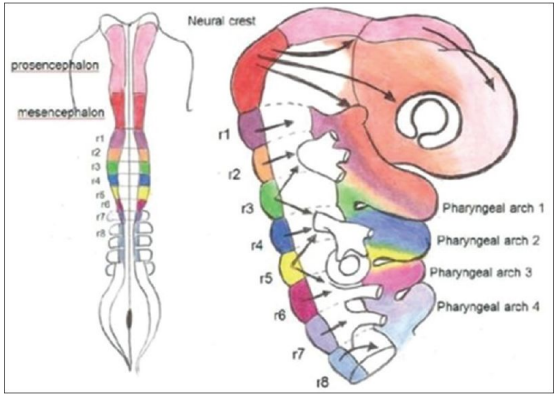
- Five paired swellings (thickenings of the mesoderm) that surround the embryonic foregut
- Structure:
  - Lined by ectoderm externally forming depressions = pharyngeal grooves
  - Lined by endoderm internally forming depressions = pharyngeal depressions
  - Own artery, nerve, cartilage rod “skeleton”, and a group of muscle cells
- Two bulges of tissue appear inferior to the stomodeum
  - Consists of a core of mesenchyme formed partly by NCCs from the midbrain
  - Fuse to become the mandibular arch (first branchial arch). This first branchial arch will go to split into:
    - Upper - maxillary process
    - Lower - mandibular process
  - Hindbrain NCCs will contribute to the formation of 2, 3, 4, 6 branchial arches
  - Note: NCCs from the forebrain does not contribute to the pharyngeal arches



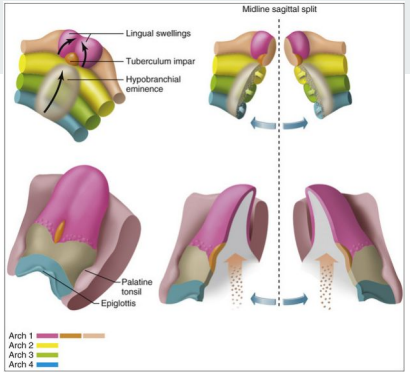
Derivatives of Branchial Arches!

Derivatives of the Pharyngeal (Branchial) Arches in Humans

Pharyngeal Arch	Nerve	Artery	Muscles	Skeletal and Ligamentous Elements
First (Mandibular arch)	CN V (Trigeminal nerve)	Maxillary artery	Masticatory muscles - Temporalis, Masseter - Pterygoids - Mylohyoid Anterior belly of Digastric Tensor tympani, Tensor palati	Malleus and incus Meckel's cartilage Tympanic ring Mandible bone +maxilla +maxillary process Sphenomandibular ligament, Anterior ligament of the malleus
Second (Hyoid arch)	CN VII (Facial n.)	Hyoid, stapedial a.	Muscles of facial expression Stapedius m. Stylohyoid m. Digastric (posterior)	Stapes Styloid process Lesser Cornu of hyoid Upper part of hyoid body stylohyoid ligament Reichert's cartilage
Third	CN IX (Glossopharyngeal n.)	Internal carotid a.	Stylopharyngeus m.	Greater Cornu of hyoid Lower part of hyoid body
Fourth	CN X Superior laryngeal	Right subclavian a. and aorta	Pharyngeal and laryngeal muscles	Laryngeal skeleton (thyroid, cricoid, arytenoid, corniculate, and cuneiform cartilages)
sixth	CN X Recurrent laryngeal			



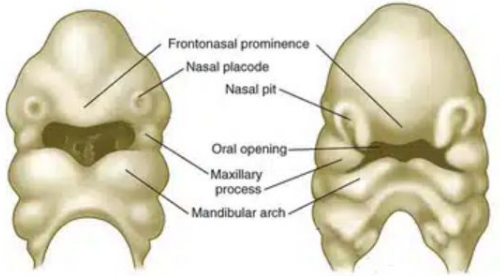
Week 4 - Development of tongue

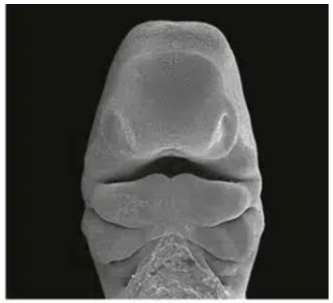


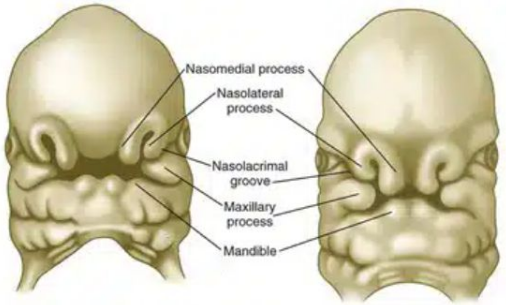
Part of the tongue	Innervation	Derivatives
Mucous membrane of Anterior ⅔ of tongue	Mandibular branch of trigeminal nerve	1st branchial arch ● Tuberculum impar ● 2 lingual swellings
Mucous membrane of Posterior ⅓ of tongue	Glossopharyngeal nerve	3rd branchial arch ● Hypobrachial eminence
Muscles	Hypoglossal nerve	Occipital somites

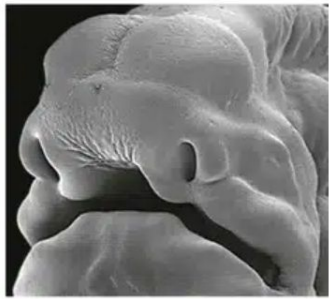
Week 5: Olfactory placode & nasal process development

- Olfactory placode formation: localised thickenings develop within the ectoderm of the frontal prominence
- Rapid proliferation of mesenchyme around placodes crease horse-shoe shaped ridge → forms lateral and medial nasal processes and the nasal pit
    - Lateral arm of horseshoe = lateral nasal process.
    - Mesial arm of horseshoe = medial nasal process



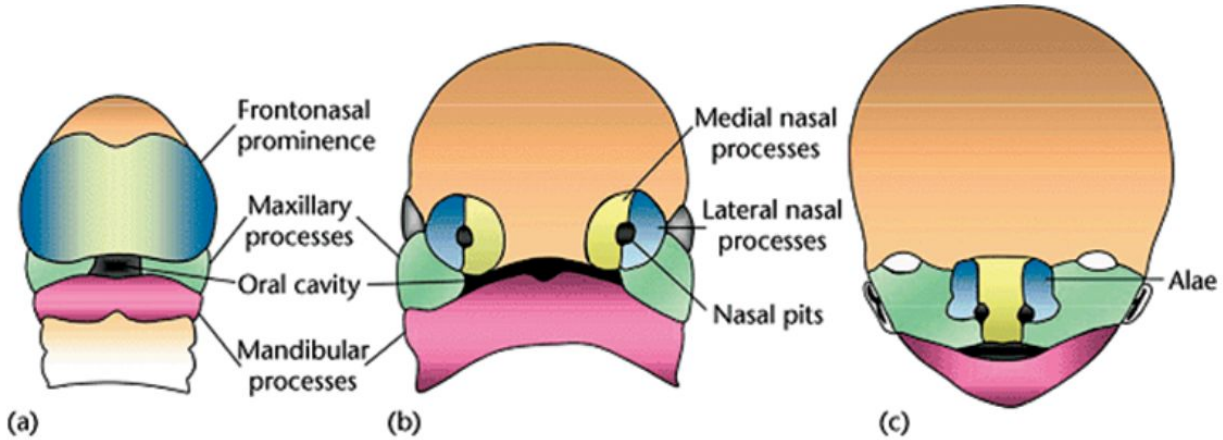






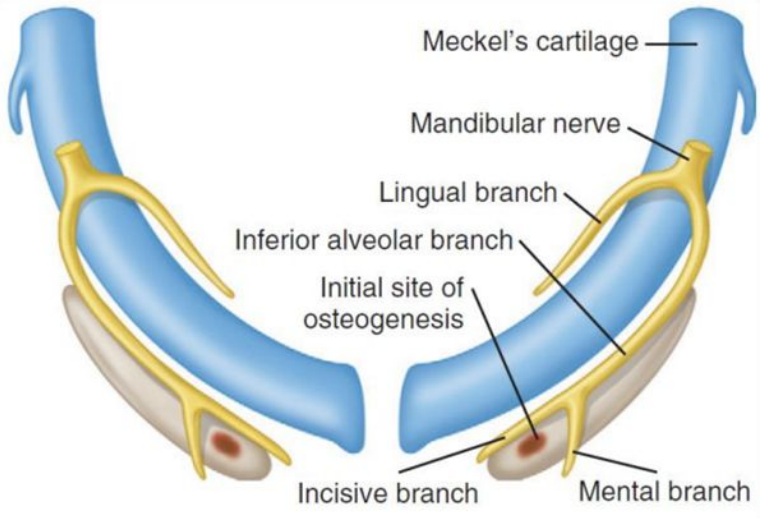
Week 6: Formation of the lip, philtrum, primary palate & anterior maxilla

- **Upper lip, philtrum, primary palate (median palatine process), and anterior maxilla** = merging of the medial nasal processes + frontonasal processes



- **Lateral part of the upper lip** = fusion of the anterior portion of the Mx. Process and the lateral aspect of the medial nasal process (overcomes the bucconasal groove to form the lateral aspect of the upper lip)
- **Lower lip** = merging of mandibular processes at midline.
- NOTE: The hard and soft palate consists of two parts:
  - The primary palate: develops first as an anterior triangular section
  - The secondary palate: develops later and makes up most of the palate

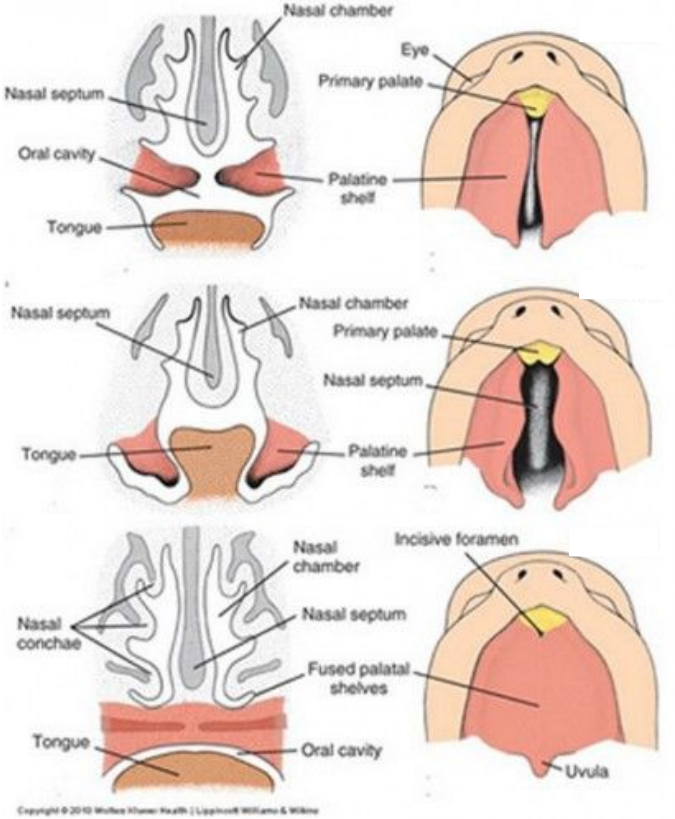
Week 6: Formation of the rudimentary mandible



- Meckel's cartilage (hyaline) present; separated in midline by mesenchyme
- Cartilage extends to developing ear
- Mandibular nerve begins ⅔ along Meckel's cartilage + divides into IAN and lingual nerves (run along medial and lateral aspects of cartilage)
- **Intramembranous ossification**: begins at week 7 where the inferior alveolar nerve divides into the mental and incisive branches
- **Bone formation** occurs rapidly → spreads anteriorly + posteriorly from the division of the inferior alveolar nerve
- Two areas of ossification remain separated until mandibular symphysis occurs after birth
- NOTE: ossification occurs outside of Meckel's cartilage and Meckel's cartilage is resorbed (persists as malleus of the inner ear)

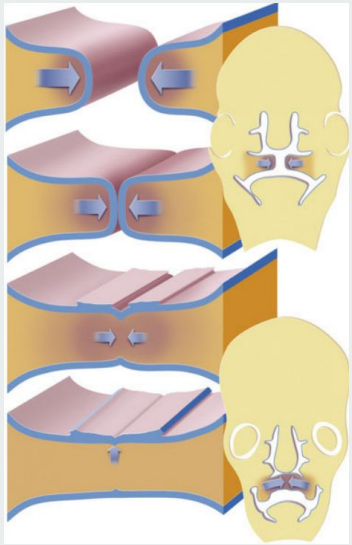
Week 7-9: Formation of the secondary palate

- Nasal septum grows downwards (from frontonasal process) & the palatine shelves/processes grow from the maxillary processes.
- Shelves initially grow downwards until the tongue descends and moves away from shelves.
- This allows the shelves ascend and merge with the primary palate→ palatine shelves move towards each other and fuse with the nasal septum to fuse along the midline.
- Nasal and oral cavities are now separated.
- NOTE: Fusion of the palatine shelves are true fusion (week 9)
    - Epithelium eliminated between two shelves meet as epithelial cells differentiate into mesenchymal cells



The word fusion is used quite loosely to describe 2 structure coming together BUT this can mean 2 different things.

True fusion

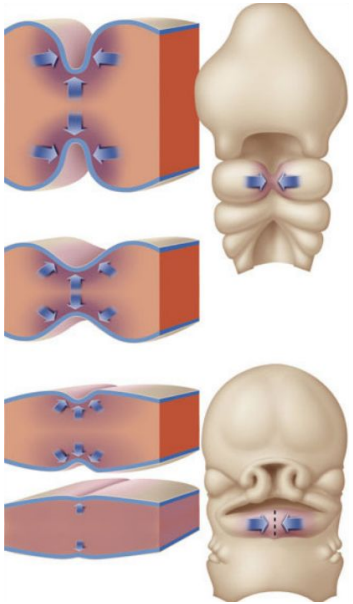


- Involves breakdown of surface epithelium
- For example: true fusion of palatal process in the formation of the secondary palate

VS

Apparent fusion

- More an elimination of an existing furrow
- For example: the branchial arches do NOT truely fuse, fusion of facial processes is not true fusion.



Cleft lip and palate

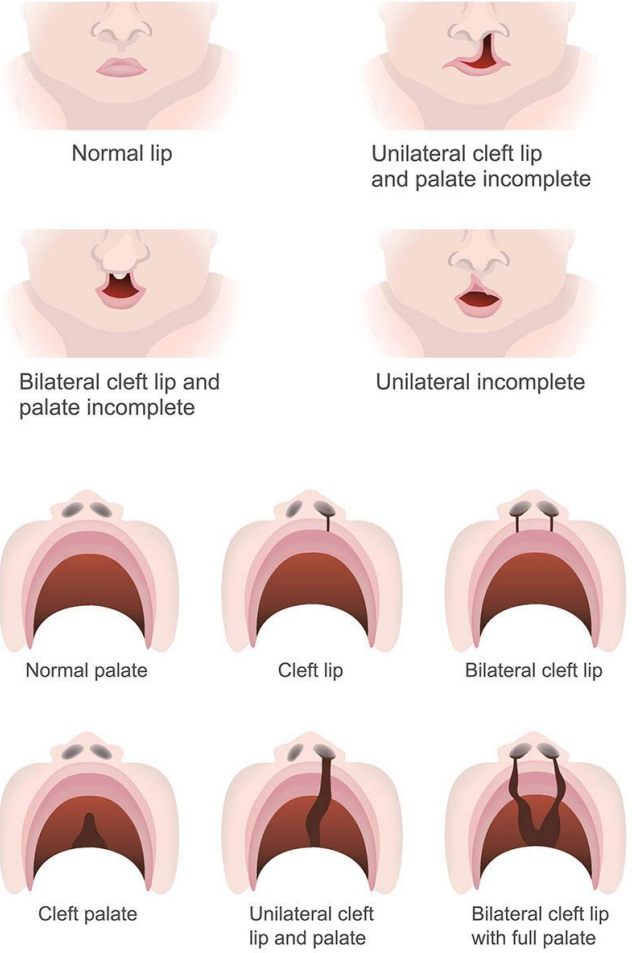
Cleft lip & Palate (CLP)

Contributing factors	Examples
Genetics (Brief knowledge of 1 or 2)	Mutation of genes that encode for development of orofacial complex ● Transforming growth factor β (TGFβ) - involved in lip & craniofacial development ● Fibroblast growth factor (FGF) - involved cell proliferation & palatine shelves formation ● Homeobox genes
Environment	Teratogens such as: ● Maternal smoking, malnutrition, maternal alcohol consumption, folic acid deficiency, etc...

Note: Embryo most susceptible to developing CLP at week 4-8 lip & palate development

Types of Cleft lip & Palate

Type	What went wrong?
Unilateral/Bilateral cleft lip	Fusion failure between medial nasal process & Mx processes
Oblique cleft lip	Fusion failure between lateral nasal process & Mx processes
Median lip	Fusion failure between both medial nasal processes
Cleft Palate (Extent of the cleft depends on the duration of disturbance)	Multiple causal factors ● Failure of shelves to contact ● Failure of epithelium breakdown ● Post-fusion rupture ● Physical obstruction of shelf elevation



Genetics & Linkage

Genetics and linkage

- Genetic risk factors related to CLP
- Synapsis and recombination
- Linkage mapping using the test cross, recombinant frequency
- Hardy Weinberg mathematics
- Content from BDS1:
  - Punnett squares
  - Interpreting pedigrees

Linkage refers to genes on the same chromosome being “linked” and therefore more likely to co-segregate. We can test which genes are linked & how close different genes on a chromosome are through linkage mapping.

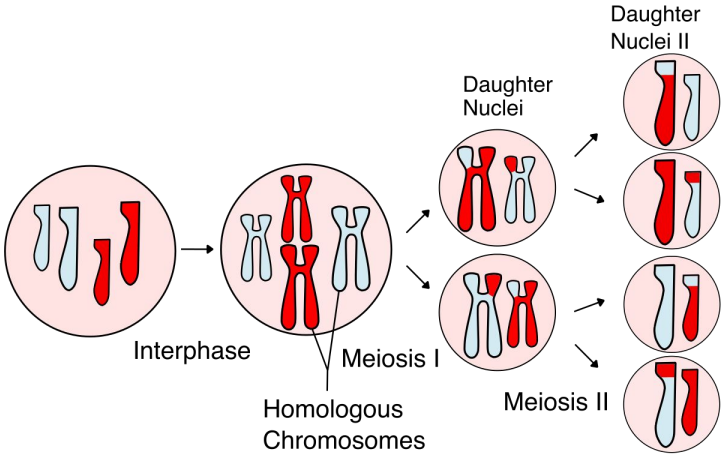
But first we need to understand why synapsis and recombination happens in the first place.

Mendel's laws

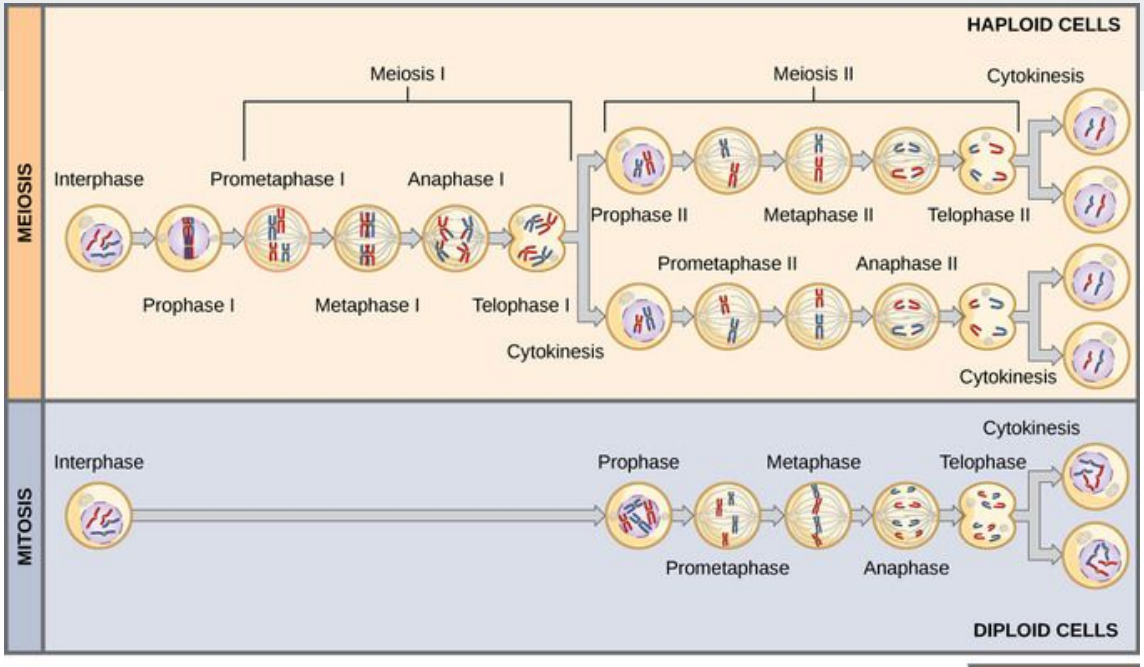
- Mendel's laws apply to meiosis, a type of cell division with for gamete cells.
  - Meiosis 1 separates homologous pairs of chromosomes
  - Meiosis 2 separates sister chromatids

Mendel's laws (both occur in meiosis 2)

1. Law of Segregation: 2 alleles segregate during gamete formation and end up in different gametes
2. Law of independent assortment: each pair of alleles segregates independently of each other pair of alleles during gamete formation



Meiosis



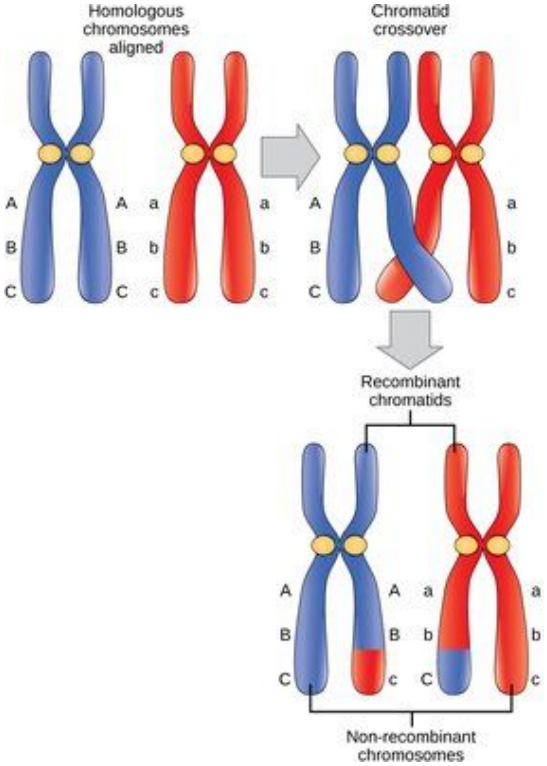
						OUTCOME
PROCESS	DNA synthesis	Synapsis of homologous chromosomes	Crossover	Homologous chromosomes line up at metaphase plate	Sister chromatids line up at metaphase plate	Number and genetic composition of daughter cells
MEIOSIS	Occurs in S phase of interphase	During prophase I	During prophase I	During metaphase I	During metaphase II	Four haploid cells at the end of meiosis II
MITOSIS	Occurs in S phase of interphase	Does not occur in mitosis	Does not occur in mitosis	Does not occur in mitosis	During metaphase	Two diploid cells at the end of mitosis

Recombination

To ensure new genetic combinations in the offspring, there are 2 sources of genetic recombination during meiosis:

1. **Crossing-over/synapsis** (during prophase 1) ->recombines genetic material within chromosomes
   - Non-sister chromatids of a chromosome pair exchange their genetic material
2. **Independent assortment-**> recombines genetic material between chromosomes
   - Each pair of alleles segregates independently of each other pair of alleles during gamete formation (i.e. during metaphase there are different ways for the homologous chromosomes to line up & be separated)

Fertilization between haploid gametes results in a third source of genetic recombination because there is the combining of chromosomes from different individuals (parents).



Test cross and linkage mapping

Test cross = crossing the F1 gene back to the recessive parents

- By using the test cross we can find out the recombinant frequency and therefore how far apart 2 genes are in linkage map units (linkage mapping)

Considerations:

1. Alleles from homozygous recessive parents don’t contribute to the phenotype meaningful
2. Recombinant combinations are at most 50%
3. Parental phenotypes should be in similar proportion (as should recombinant phenotypes)

- Recombinant frequency = (number of recombinants)/total
- Linkage map units= multiply recombinant frequency by 100 (units in centimorgans, RELATIVE distance of how far apart the two genes are)

Example linkage question

(Question from Dr Hughes' linkage lecture)

Pea example: Let's say we counted the phenotypes plants produced from 1000 test crosses.

- Biallelic locci, 2 traits
- Trait 1: Pea colour- Yellow (YY, Yy);Green (yy)
- Trait 2. Pea appearance - Smooth (SS, Ss), Wrinkled (ss)

Purple/Smooth	Purple/Wrinkled	Yellow/Smooth	Yellow/Wrinkled
375	140	160	325

Find the recombinant frequency

- Parental phenotypes should be in similar proportion (as should recombinant phenotypes)
- Recombinant combinations are at most 50%. This means that the blue group is the recombinant group as 140+160 = 300 which is less than 50% of 1000
- Now calculate the recombinant frequency: (140+160)/1000 = 0.3

This means that the two genes (pea colour and appearance) are 0.3x 100= 30 centimorgans apart.

The lower the recombinant frequency, the closer the relative distance between 2 genes. This makes sense as the closer together two genes are, the less likely they are to be recombined due to a crossover.

Hardy Weinberg Equilibrium

- Hardy Weinberg Equilibrium allows us to measure allele frequency in a population (as it is hard to just by phenotype).
- Hardy Weinberg Equilibrium law = Allele and genotype frequencies remain constant from generation to generation when the population meets certain assumptions:

1. No new mutations
2. No migrations in or out of a population (gene flow)
3. No selection (equal fitness of all genotypes)
4. Random mating
5. Very large population

We can find out allele frequencies using mathematics:

Hardy-Weinberg mathematics

For a dimorphic gene (two alleles, A and a):

1. Allele frequency
   - p = frequency of A
   - q = frequency of a
   - **$p + q = 1$**

2. Genotype frequency
   - p^2 = frequency of AA homozygotes
   - 2pq = frequency of Aa heterozygotes
   - q^2 = frequency of aa homozygotes
   - **$p^2 + 2pq + q^2 = 1$**

   (H-W equation, binomial distribution)

Q&A

Are Hox genes a subtype of homeobox genes?

Yes!

There are 39 genes in the subgroup of homeobox genes that constitute the human HOX gene family.

Homeobox genes are a large group of genes that encode transcription factors that help regulate developmental processes

Exam Style Questions

Question 1

John is a 8 months old baby with a unilateral left cleft lip & palate. What are the key issues for John in regards to feeding? (5 marks)

- Insufficient anterior seal due to cleft lip
- Insufficient posterior seal due to cleft palate (if cleft palate involves soft palate)
  - Communication between nasal and oral cavities
- Suckling issues in breastfeeding (associated with cleft palate rather than cleft lip). This is because:
  - Air leaks from the nose to the mouth → reducing negative pressure (pressure gradient) → reducing suction
  - Expression (compression of breast nipple by tongue against hard palate to squeeze milk) can also be compromise
  - Nasal regurgitation - milk may leak out of the baby's nose

Question 2

Tim Jones is a 24 year old patient who presents to your practice with unilateral left cleft lip. What could have gone wrong for his cleft lip to occur? (6 marks)

- Organised response:
  - Molecular levels - cell signalling
  - Cellular Levels - NCCs
  - Tissue Levels - what kind of cleft lip/palate is it?

PCC