# Amelogenesis

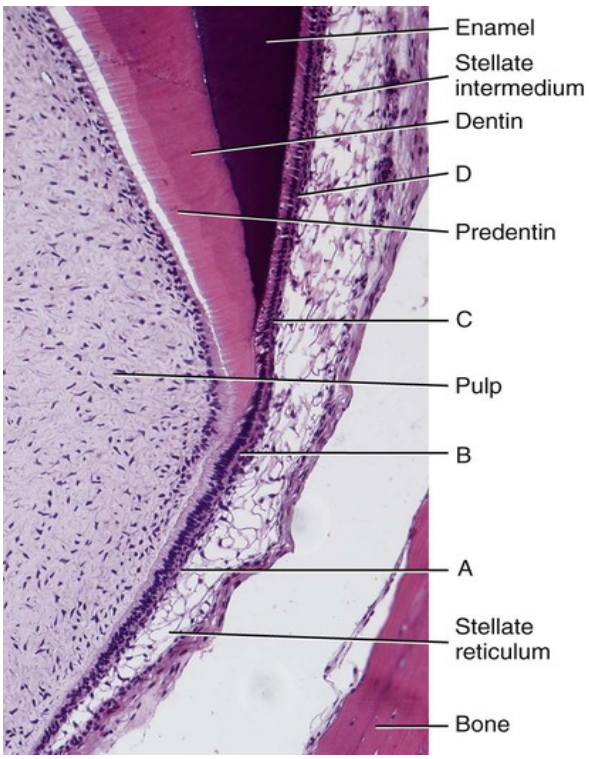
**Amelogenesis :** the process of enamel formation and maturation by ameloblasts ( that undergo various functional stages during their life cycle )

Amelogenesis is a 2 step process :

1. production of **immature enamel** (partially mineralized enamel) -**70% minerals**
2. Production of **mature enamel** ( which contains 96% minerals )

**Ameloblasts :**

- are responsible for both processes ( production of immature and mature enamel )
- have a unique life cycle which reflects its function in E formation and maturation




**The major functional stages of Ameloblasts :**

1. presecretory stage
2. secretory stage
3. maturation stage

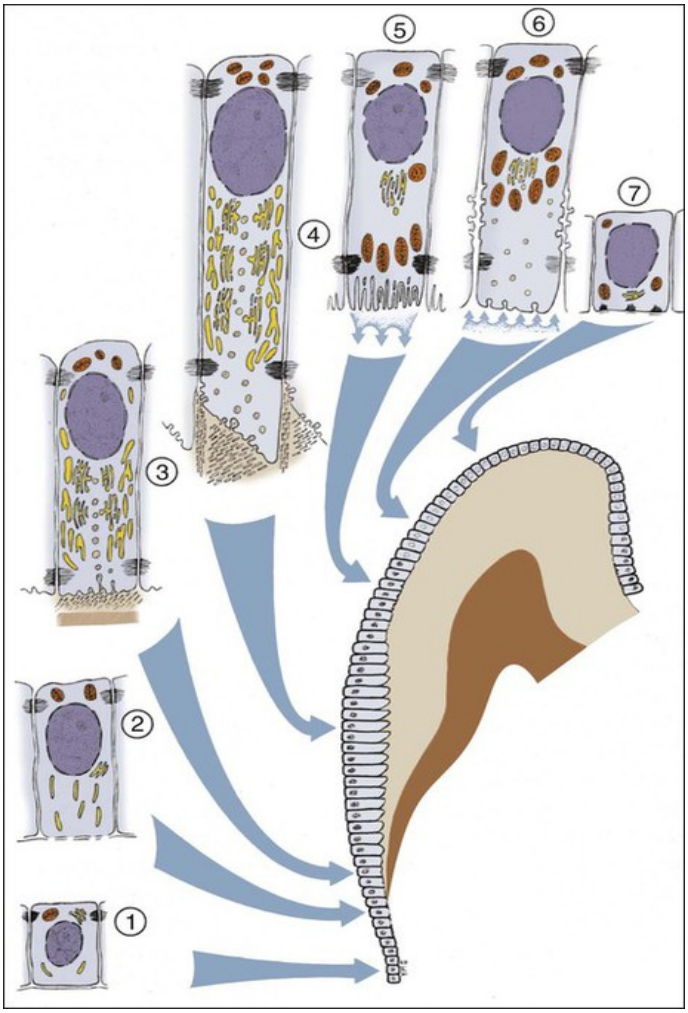
Q: name the following + mention the stage according to the picture :

- D= Ameloblasts in secretory
- C= presecretory Ameloblasts
- A= IEE
- Stage : crown stage or late bell stage
- cells in the pulp region – fibroblasts

➔ The DEJ was a basement membrane but when ameloblasts started secreting enamel it will cause the Basement membrane to break down .

First layer of Enamel secreted is called **= initial enamel which is rod less enamel**

## The life cycle of Ameloblasts :




1. **Inner Enamel Epithelium :**
   - Cell is not polarized
   - Resting on BM
   - Not secreting
2. **Pre-secretory ameloblasts :**
   Elongation of the IEE and the cell is now polarized ( since the nucleus moves away from the center )
3. **Initial secretory Ameloblast**
4. **Secretory stage :** from the junctional complex an extension of the cytoplasm forms as toms process .
   **Toms process** → forms rods
   Toms process will disappear later on to form cells similar to the initial secretory ameloblasts which will secret the Rodless final enamel .
5. **Ruffle ended ameloblast of maturative stage**
6. **Smooth ended ameloblasts of maturative stage**
7. **Reduced enamel epithelium ( protective stage ) –** made from the **whole enamel organ**
   Provide protection to the enamel until the time of eruption .

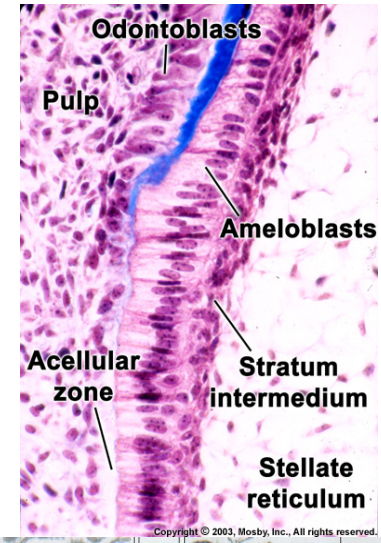
Maturation stage takes around 2 years ( it takes time to remove organic material and water – they remove 26% )

During eruption the REE will fuse with the oral epithelium and form the junctional epithelium .

## Ameloblasts differentiation :

- **Morphogenetic ( IEE ) :** cuboidal
- **Differentiating :** elongate and the nuclei shifts proximally
- **Secretory :** basal lamina disintegrates + matrix secreted and mineralized
- **Maturation :** enamel proteins removed and rapid crystal growth
- **Protective :** basal lamina secreted and hemi desmosomes formed

**The transfer from morphogenesis to differentiation happens by induction ( the dental papilla cells induce the IEE to differentiate into preameloblasts etc … )**



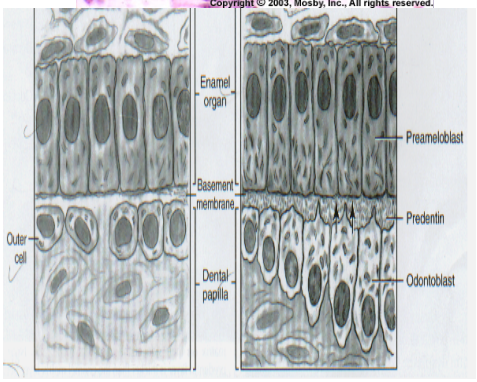

1) **Inner Enamel Epithelium : morphogeneic ameloblast**
   1. Cuboidal or low columnar
   2. Large centrally located nuclei
   3. **Golgi located adjacent to stratum intermedium**
   4. **Mitochondria and other organelles are scattered thought out the cytoplasm**

   **Reciprocal induction : b/w ectomesenchymal and epithelial cells**

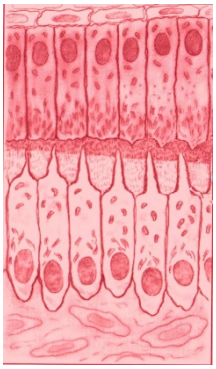
   IEE induce the dental papilla cells to differentiate into odontoblasts and secrete dentin

   Dentine secretion will induce IEE to differentiate to pre secretory ameloblasts

   - **Differentiating cells can't divide but IEE can divide because it is not differentiating .**
   - **Pre odontoblasts will secrete predentin .**



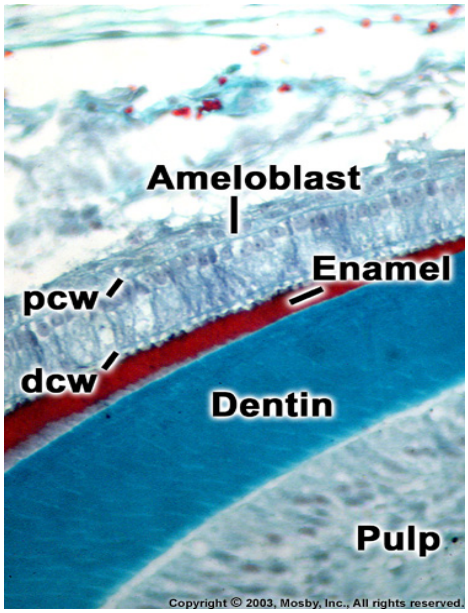

2) **Pre ameloblast ( differentiating ameloblast )**
   1. Differentiation of IEE into pre ameloblasts
   2. Elongation of cells
   3. Nucleus will shift proximally ( towards the Stratum intermedium )
   4. Golgi complex which moves centrally
   5. RER increases
   6. Cell becomes highly polarized



3) **Secretory ameloblast – no tomes processes :**
   1. Columnar ( contains many secretory granules – that contain enamel protein )
   2. Synthetic activity on the rER → Golgi body → membrane bound secretory granules
   3. Granules migrate to the **distal end of the cell** and are released against newly formed dentine to form initial enamel ( irroded )
   4. Ameloblasts move away from the DEJ.

**4) Secretory ameloblasts :**

1. Tall columnar
2. Develop tome's processes ( from the distal junctional complex )- since the nutrition source is the dentine .
3. Secrete roded enamel ( inner and outer enamel )
4. 0.023 mm secreted per day - Partially mineralized matrix **by alkaline phosphatase enzyme** – a marker for minerlaization to know whether this cell is active in mineralization .
   ( to check for alkaline phsophatase we use stain )




**Roded enamel is much stronger than irroded enamel .**

**The junctional complex is made of :**

1. tight junctions
2. adherent junctions
3. desmosomes

- basement membrane degenerates is doesn't calcify .
- The most common protein during secretory stage is amelogenin , after secretory stage it will be removed and only enamilin remains .
- IEE is dividing , but pre secretory ameloblasts is not dividing ( because it is already differentiated ) .

The first formed enamel is immature : ( 70% water and inorganic matrix – 30% organic matrix / proteins ) **amelogenin >>> enamilin**

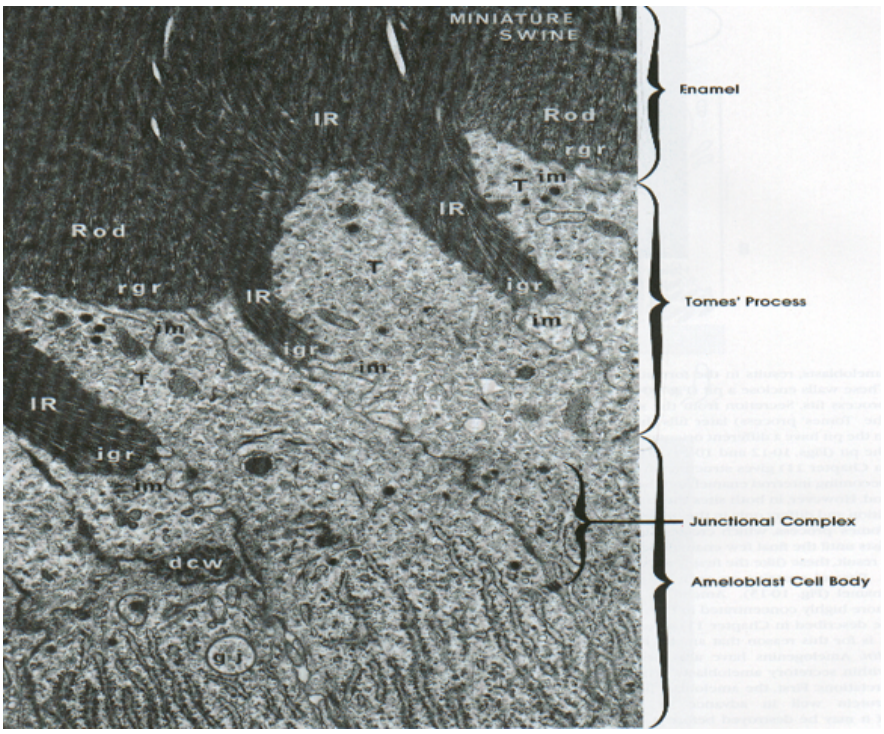
- ➔ Differentiating ameloblasts move away from dentine surface and form a conical projection
- ➔ Cells form elongated tomes processes – give a rod Shaped enamel
- ➔ At the end of secretory stage , ameloblasts shorten and lose tome's processs and secrete the final layer of enamel which is irroded ( aprismatic enamel )
- ➔ Aprismatic enamel is rodless enamel that is the most superficial layer of enamel .
- ➔ When the nucleus is located near the basement membrane this is called reversed polarity .

**Q:What is the orign of the odontoblats ? ectomesenchymal cells of the dental papilla**

**explain what you see :**

ameloblast body with the proximal and distal sides

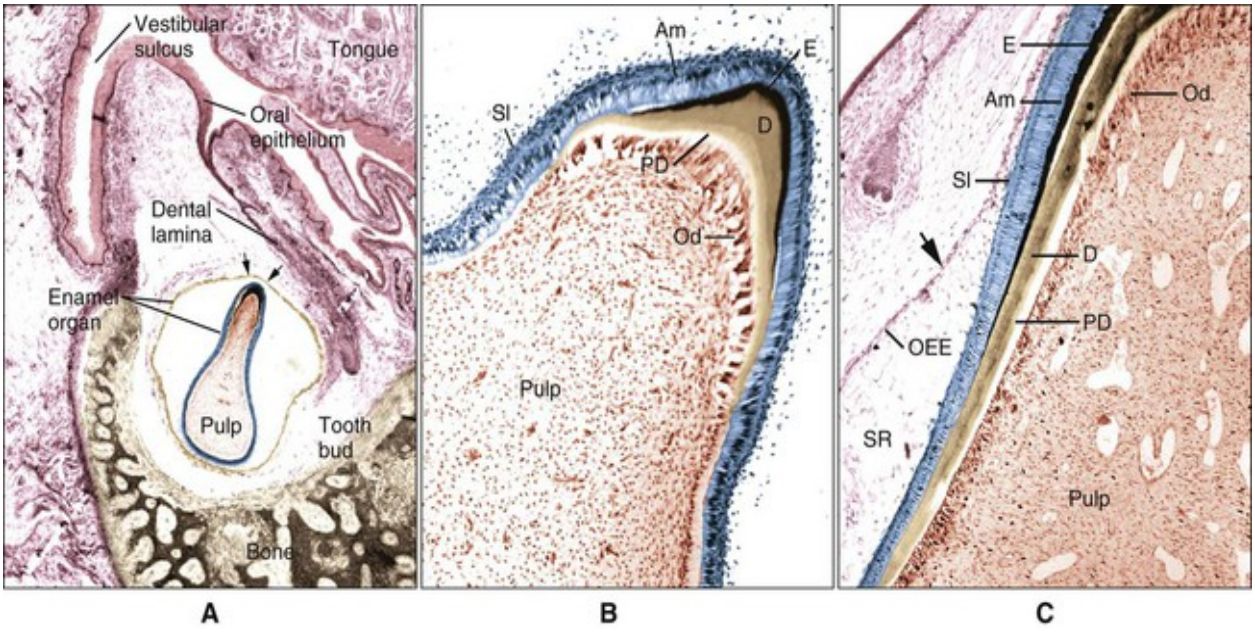
( which contains the tome's process – extension of the cytoplasm from the distal junctional complex ) – they secreted rods and interrod.




**This is a calcified section ( since you see enamel ) and seen under a TEM .**

**The rods and interrods are formed by different parts of the same tome's process .**

**When the ameloblast lose tome's process they will secrte rodless enamel and become shaoter .**




**A= enamel organ , the calcified tissue started forming at the tip of the incisal edge . ( tooth is not erupting , since the crown is not yet fully formed )**

**B /C = ameloblasts differentiated and started secreting enamel and odontoblasts are secreting dentin**

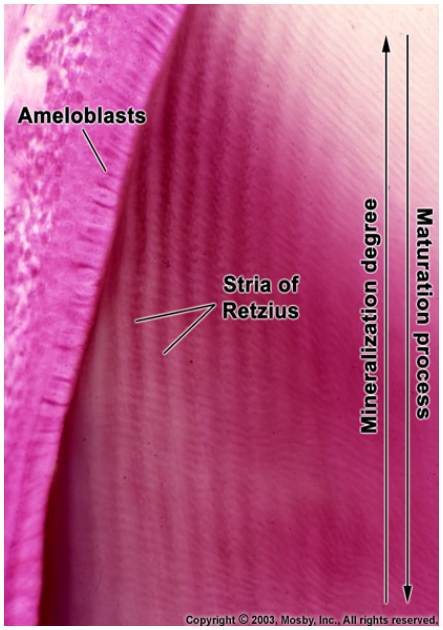
**Enamel maturation :**

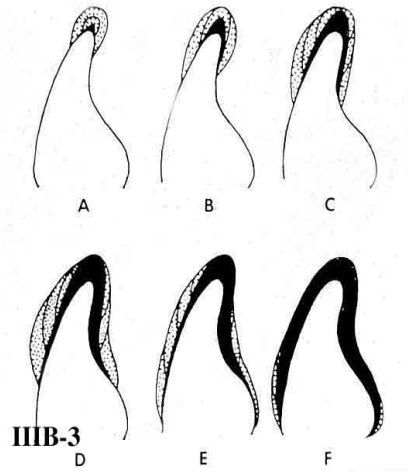
**Characterized by :**

1- Removal of water
2- Reduction of protein content from 30% to 1%
3- Increasing mineral content

**Location : begins at the DEJ of cusp tips ( growth centers )**

- Further mineralization + growth of HA crystals
- Loss of water + protein
- Most amelogenins are removed leaving high molecular weight enamilin dominant




**The sequence of enamel formation and maturation :**

The enamel that formed the earliest matures the earliest.

Maturation begins near the growth centers and proceeds towards :

- The enamel surface
- Cervically along the DEJ

- Transition stage : a stage between secretory ameloblasts and maturation ameloblasts( between secretion and maturation )
- Q: Do all ameloblasts reach maturation stage ? no only 75% of the ameloblasts

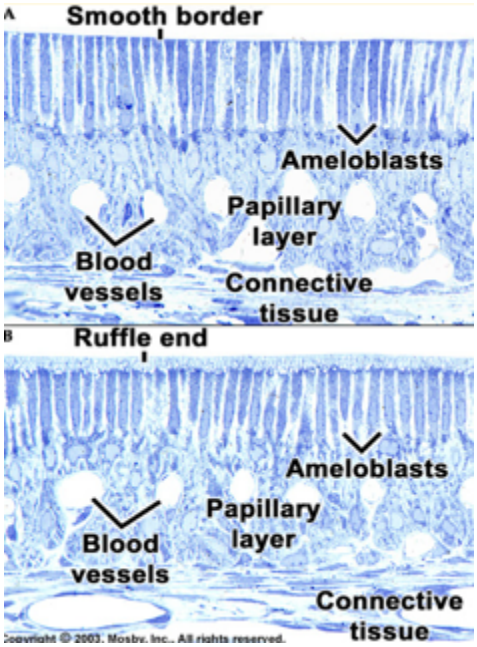
Do all maturation stage ameloblasts have the same shape ? no , some are ruffled and some are smooth ( 80% ruffle , 20% smooth )

Maturation also happens incrementally ( layer by layer ) and the first area is the cusp tip and the last area to mature is the cervix

- **In maturation stage :** the SI above the rough ended ameloblasts is called papillary layer
- The papillary layer = the collapsed enamel organ + they contact the blood vessels in the dental sac

**Maturation stage ameloblasts :**

Once the full thickness of enamel is laid down around 25% of post secretory ameloblasts die . ( undergo apoptosis – programmed cell death by the division of the cytoplasm into many vesicles ingested by phagocytes )



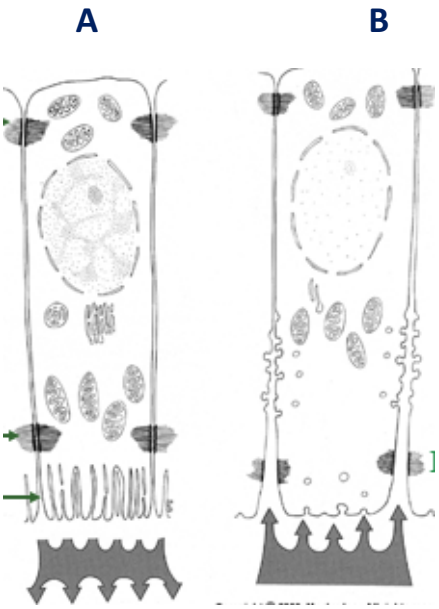

Further ultrastructure changes occur :

1- The cell becomes shorter
2- the volume and organelles decrease
3- the cell becomes involved in enamel maturation
4- Reformation of **atypical basal lamina** at the distal border of maturation ameloblasts .

**Modulation :** the process by which the apexes of maturation ameloblasts alternate between smooth ended and ruffle ended border .

Importance of modulation: if the enamel is kept under ruffle ended ameloblasts , the area will become too acidic and instead of growing of the crystals , it will dissolve the crystals .

The 3 other layers of enamel organ → amalgamated together to form the papillary layer .




| **A- Ruffle ended ameloblasts** | **B- Smooth ended ameloblasts** |
|---|---|
| Proximal junctional complex = Leaky | Proximal junctional complex = Tight |
| Distal junctional complex = tight | Distal junctional complex = leaky |

- Maturation stage ameloblasts : rich with rER , Golgi , Mitochondria
- Enamel can't be seen under the microscope in decalcified sections so the area is called enamel space .

hypotheses that have been proposed to explain the progressive removal of enamel protein during maturation :

- Mature enamel possesses alternating acidic and neutral zones.
- The acidic zone were correlated with ruffled border ameloblasts ( RA ) whereas neutral were correlated with smooth border ameloblasts (SA)
- Enamel under RA is acidified by proton pump from the ruffled border of ruffle – ended ameloblast. In addition to acidity created as a result of enamel crystal formation.
- Acidity of enamel is kept by the tight Junctional complexes between RAs.

Q:How did they discover those alternating areas ?

A:Using indicator dyes to detect regional variations in PH along maturing enamel which corresponds the modulation cycle of ameloblasts .

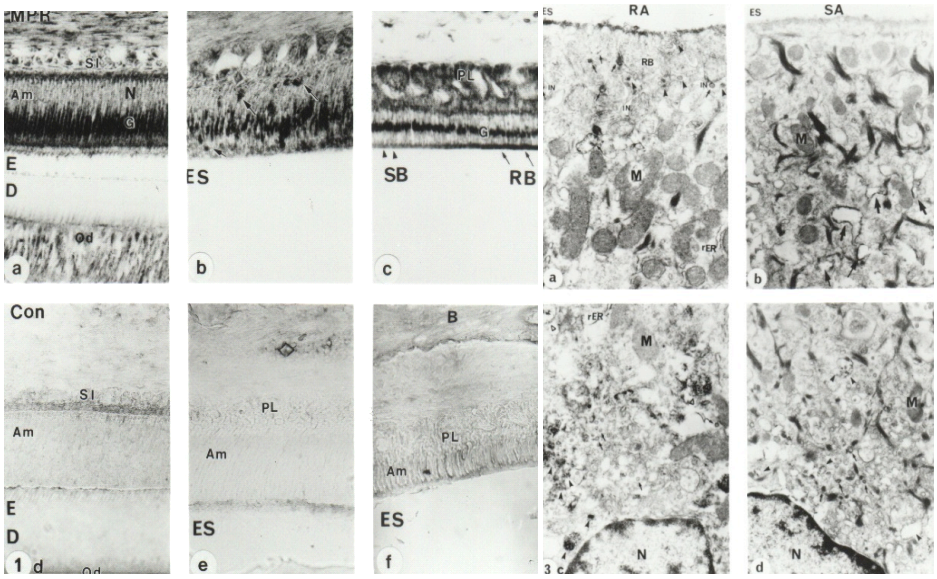
**Hypothesis :**

- Maturation ameloblasts secret lysosomal enzymes extracellularly into enamel.
- These secreted enzymes are targeted to the ruffled border by **mannose-6-phosphate receptors (MPR) – is a marker for lysosomal enzymes**
- It is postulated that acidification of enamel adjacent to RA is necessary for activation of the hydrolytic enzymes.
- When acidity reaches a critical level, ruffle-ended ameloblasts modulate into smooth-ended with leaky junctional complexes allowing the interchange between enamel and cellular fluid causing the neutralizing of enamel under SAs.

**Reduced Enamel Epithelium : ( protective ameloblasts )**

After completion of maturation of enamel processes, 25% of ameloblasts will die and only 50% 0f the ameloblasts become part of the reduced enamel epithelium (REE) .

- cells of the OEE, SR & SI lose their appearance → form a stratified layer of cells adjacent to ameloblasts ( ameloblasts are no longer distinguishable )
- REE later disintegrates during tooth eruption , it fuses with the Oral epithelium and forms the junctional epithelium .




There is no staining of Golgi apparatus in the smooth ended ameloblasts – meaning that there is no secretion of mannose 6 phosphate.

SUMMARY :

1. Enamel is ectodermally derived tissue produced by the enamel organ
2. Cells of the IEE differentiated into ameloblasts
3. Various phases
4. Partially mineralized enamel produced initially that eventually matures
5. Completed enamel is protected by the REE.

**Defects of Amelogenesis :**

- Common : Enamel pearls
- Genetic – inherited , spontaneous mutations: Amelogenesis imperfecta- Metabolic disorder
- Systemic diseases
- Febrile disease ( childhood viruses that cause fever )- band like appearance on the teeth , which indicates that the ameloblasts stopped forming enamel at the same time .
- Drugs ( tetracycline ) – greyish teeth , tetracycline attaches to calcium so it affects all calcified tissues .
- Excess fluoride ( fluorosis / trauma )


Febrile diseases


Fluorosis

The regular amount of fluoride is 0.01 ppm .

**Amelogenesis imperfecta :**

Caused by the malfunction of proteins in the enamel :

1. Ameloblastin
2. Enamilin
3. Tuftelin
4. Amelogenin

Teeth with abnormal color : yellow , brown , grey.

Can affect any number of teeth in both dentitions.


The teeth with Amelogenesis imperfecta:

1. have a higher risk for dental cavities
2. hypersensitive to
   a- temperature changes
   b- rapid attrition
   c- excessive calculus deposition
   d- gingival hyperplasia.