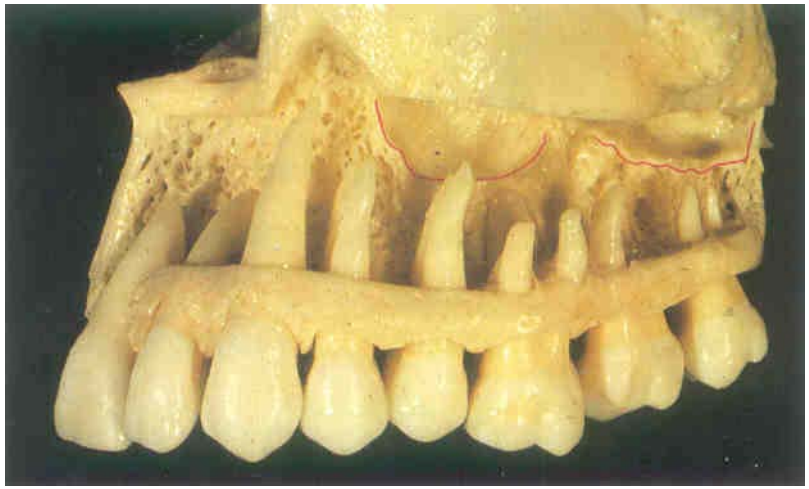
Tooth development

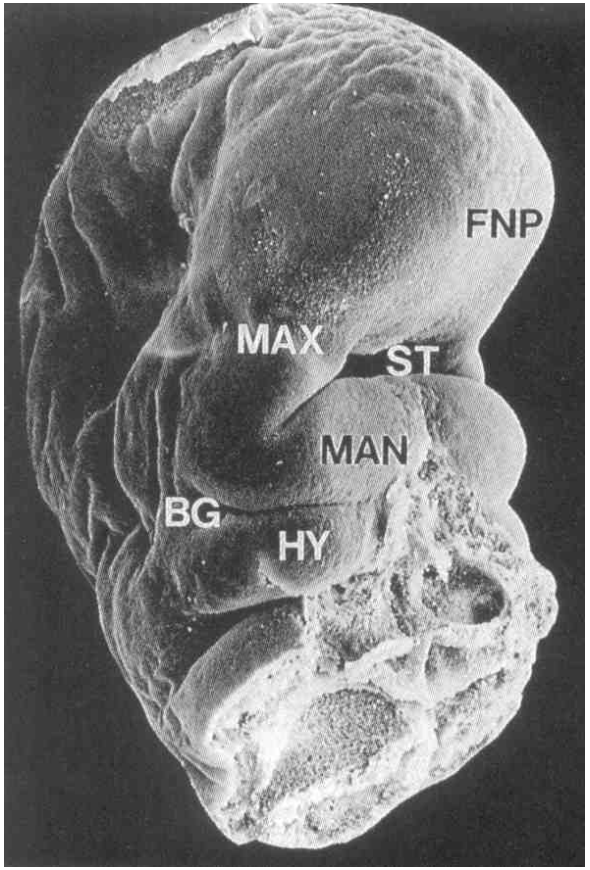
- Organised structures
- Regionally patterned
- Each tooth distinct by position and shape

- Clear example of developmental problem



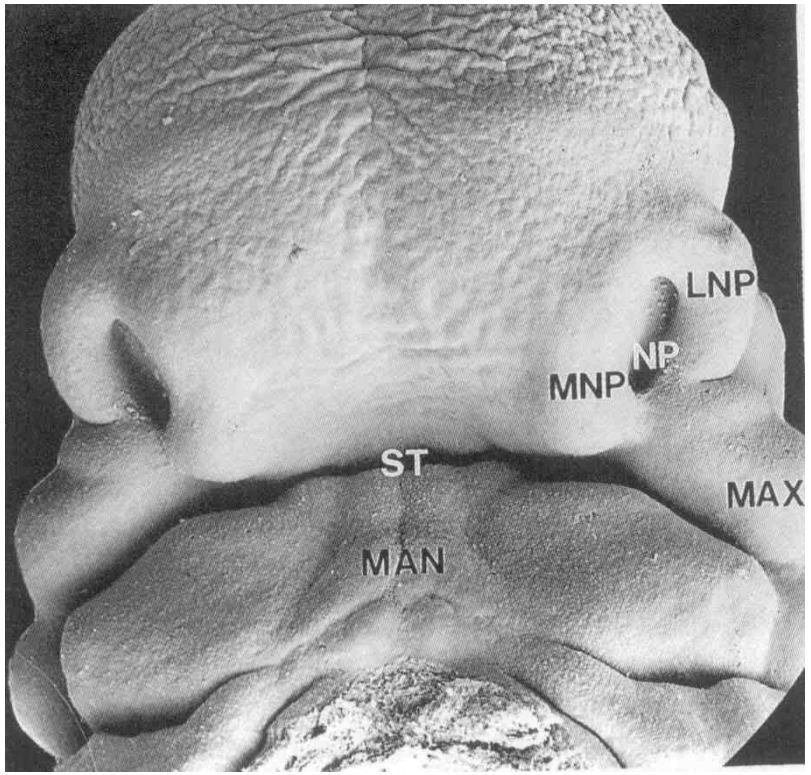
Tooth Origins

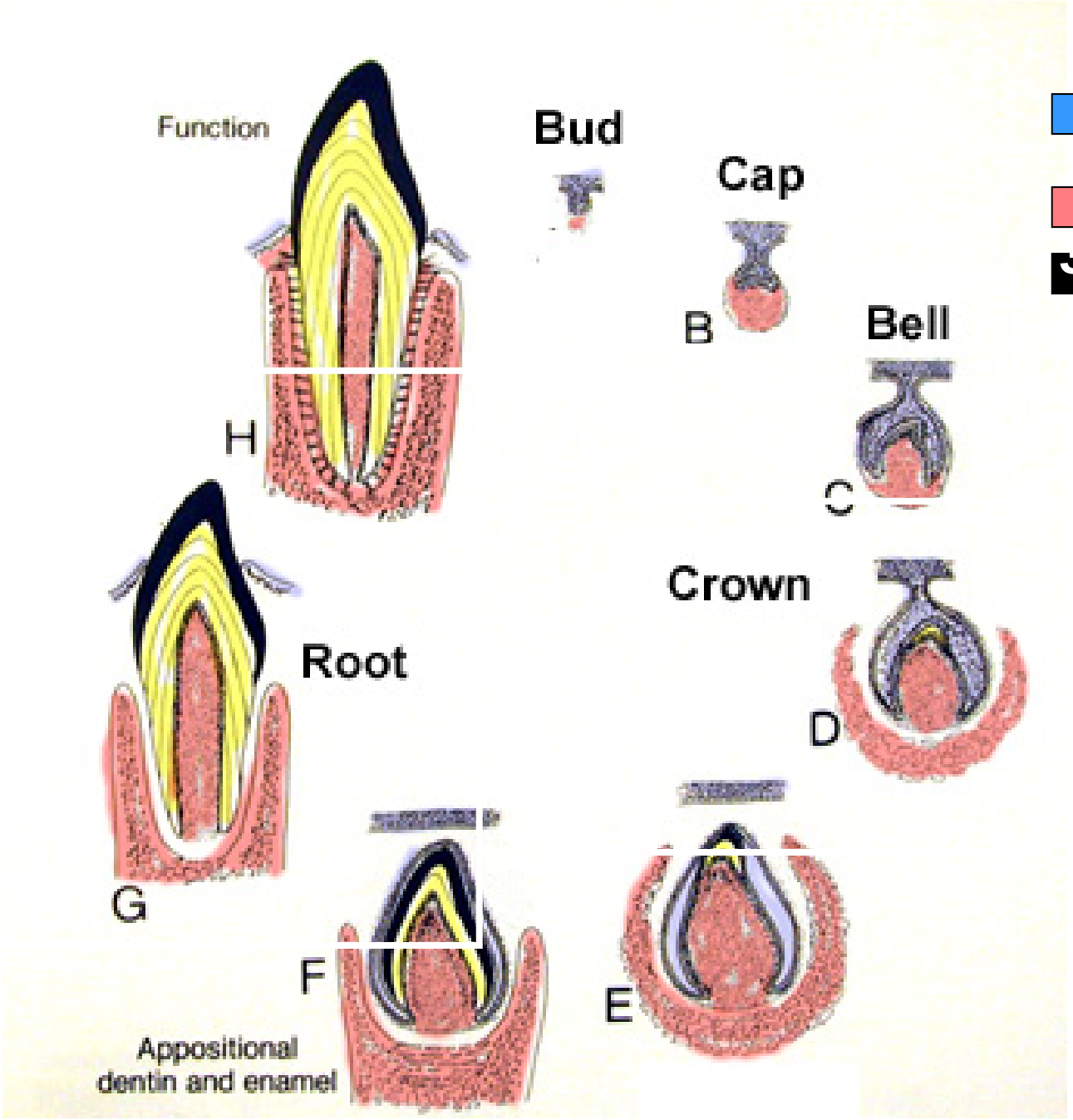
- First arch structures
- Combination of oral epithelium and underlying cranial neural crest
- Cranial crest distinct from trunk crest
- picture 26 day human embryo



Oral Tissues

- embryo 37 days old
- mandibular and maxillary processes evident
- tooth formation started



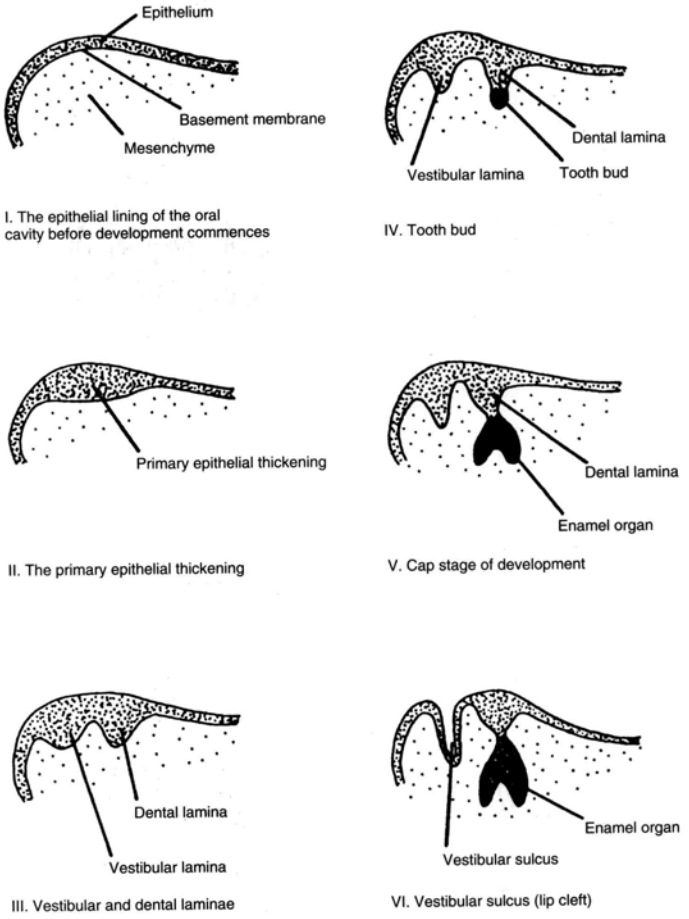

Function
Bud
Cap
B
Bell
C
H
Crown
D
Root
G
F
E
Appositional
dentin and enamel

Stages of tooth development

1. **Bud:** 1st condensation of mesenchyme around epithelium
2. **Cap:** Enamel organ forms “cap” above dental papilla
3. **Bell:** Shape of tooth crown begins to emerge in contours of enamel organ
4. **Crown:** mineralized crown tissue (enamel and dentine) deposition
5. **Root:** crown formation complete, roots grow and tooth erupts.

Formation of tooth buds

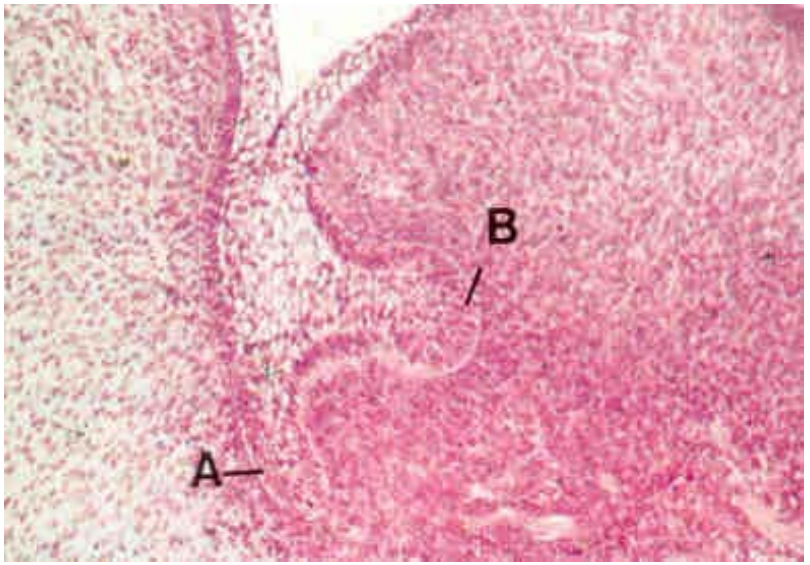
- 6-7 weeks - epithelial thickening
- 7 weeks division of lamina
- inner dental lamina forms
- tooth buds form locally in the lamina
- invaginates to form the enamel organ



Formation of dental lamina

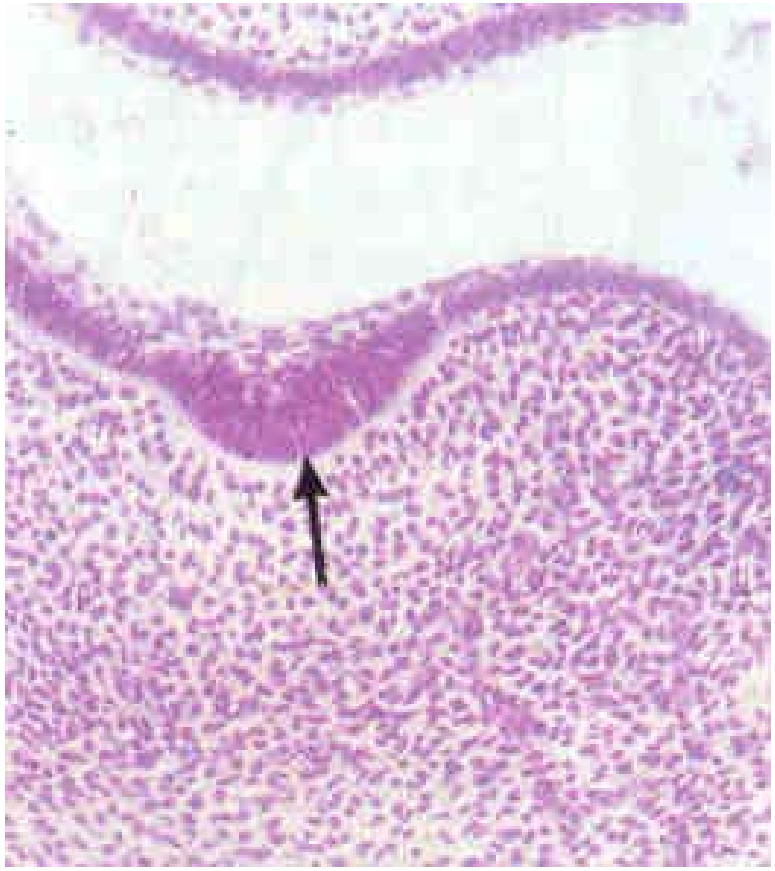
- 7 weeks separation of vestibular lamina from dental lamina

- How could separation occur at the cellular level?



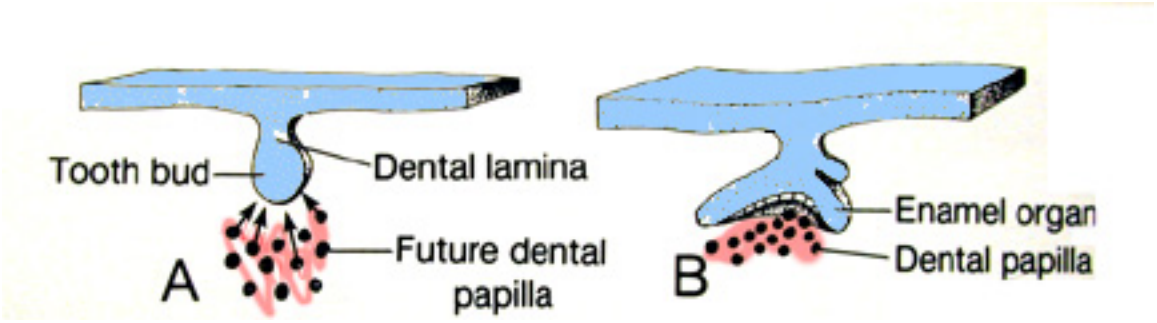
Primary epithelial band

- 6 weeks epithelial thickening in presumptive tooth forming region
- Consider possible developmental abnormalities



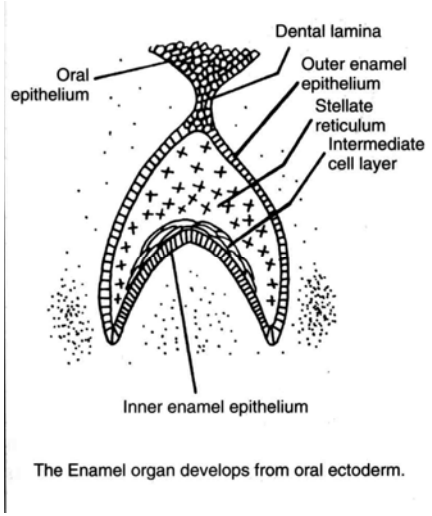
Bud Stage

1. Mesenchyme aggregates around ectodermal bud from dental epithelium
2. First bud stages reached by 7 weeks after fertilization

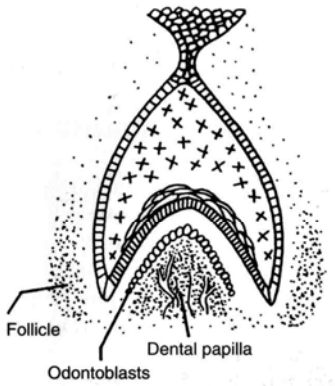


Structure of enamel organ

- At about 12 weeks enamel organ develops regional structure - the cap stage
- Separation of epithelium into inner and outer layers - future fates differ!
- Mesenchyme from jaw forms the dental papilla



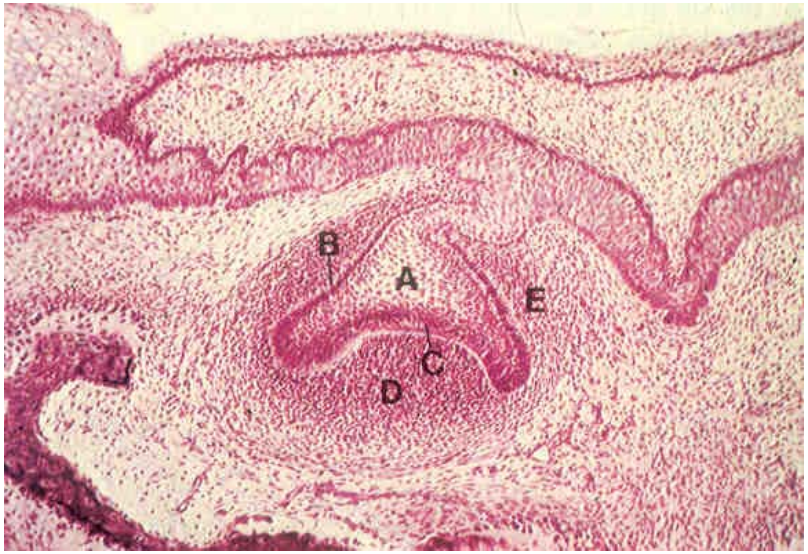
The Enamel organ develops from oral ectoderm.



The tooth germ consists of the enamel organ, the follicle and the dental papilla.

Histology of cap stage

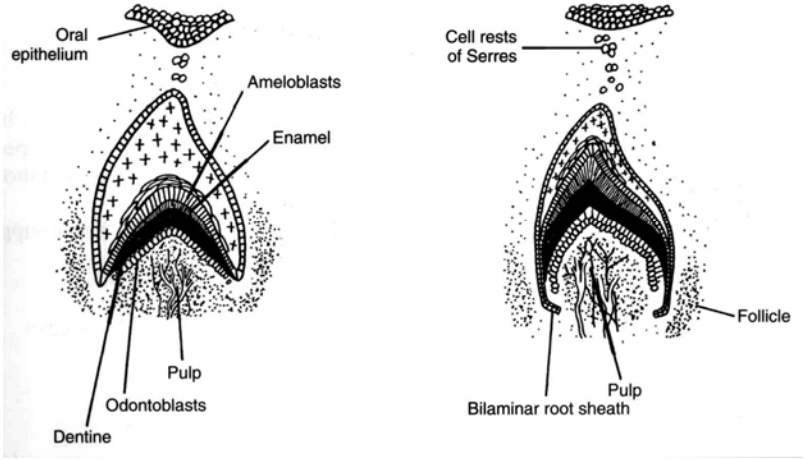
- Late cap stage 12 weeks
- A: Stellate reticulum
- B: Outer enamel epithelium - cuboidal
- C: Inner enamel epithelium - columnar
- D: Dental papilla
- E: Dental follicle



Cell differentiation

- Connection to oral epithelium severed

- inner enamel epithelium → ameloblasts
- dental papilla → odontoblasts + pulp
- deposition of enamel and dentine starts

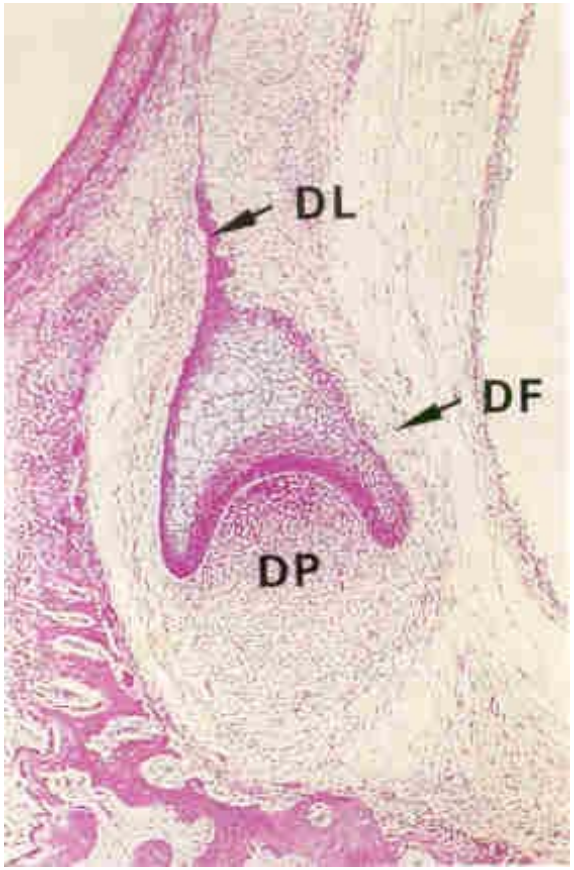


Bell stage

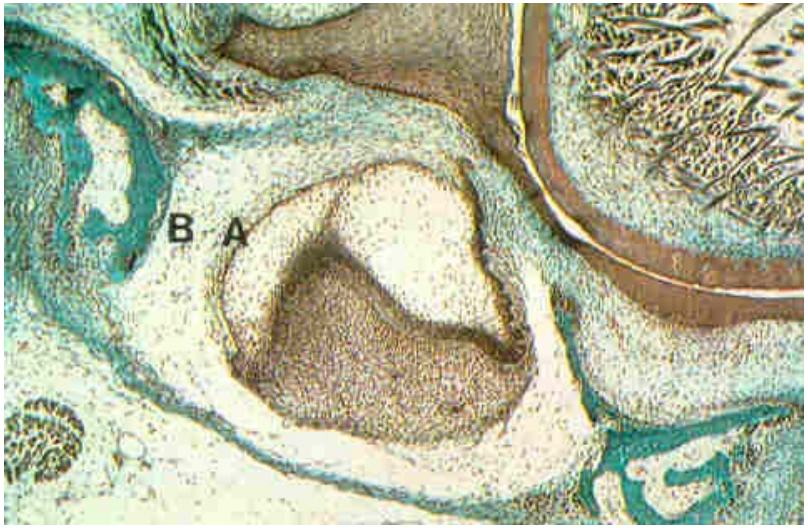
- Enamel organ tissues begin to differentiate.
- Enamel organ begins to fold into shape of finished crown.
- Dental papilla tissues begin to differentiate.
- First reached at 14 weeks.

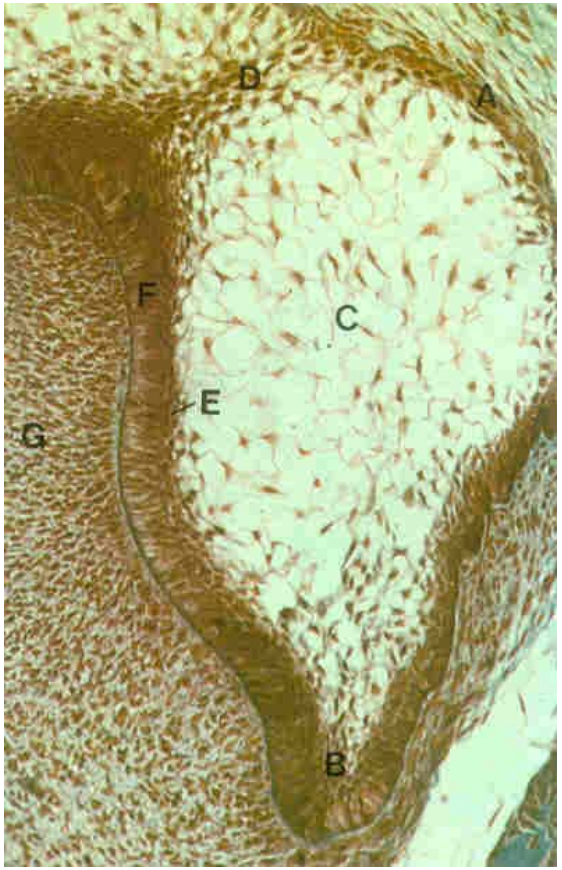
Early bell stage

- Breakdown of dental lamina
- Dental papilla becoming organised
- Condensation of mesenchyme form the dental follicle



Early bell stage



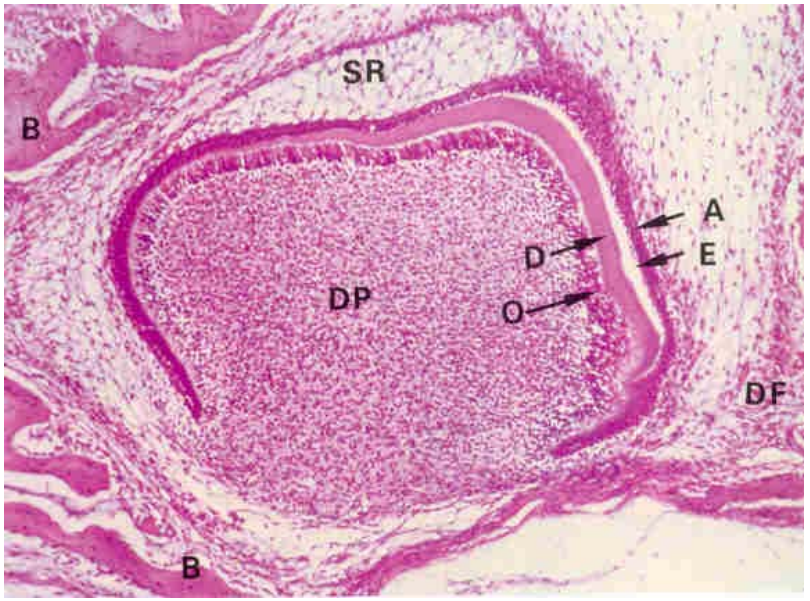


14 weeks - cusps defined
- organised patterns of cell differentiation

Bell stage

- Overt differentiation
- Dentine first to be laid down
- Organised layers of ameloblasts and odontoblasts evident

- Period of crown morphogenesis



Enamel knots

- Transitory structures in enamel organ
- Play a role in controlling cusp shape
- This knot marks future central fissure



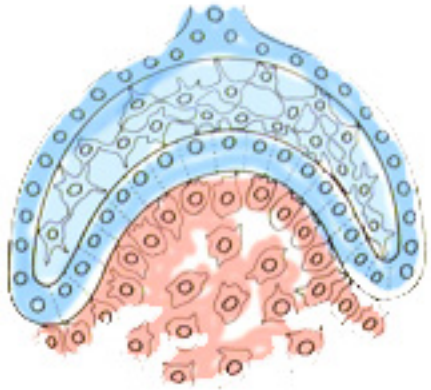
Crown Stage

1. **Defined by beginning of mineralization.**

2. **Differentiation continues away from cusp tips towards future cervical margin.**

3. **First reached at 18 weeks.**

4. **Crown shape now fully determined**



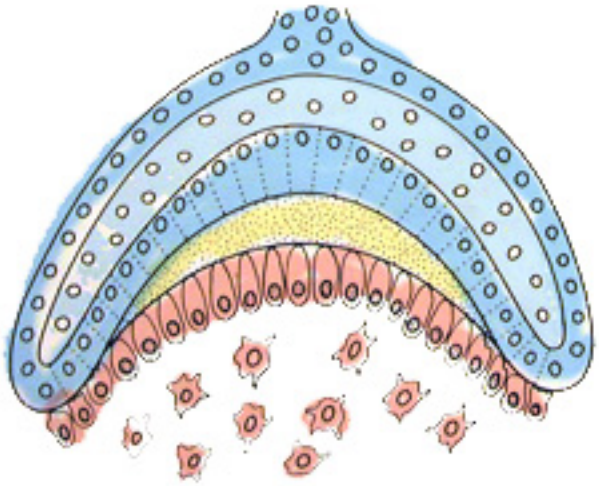
(a)

Epithlial derived

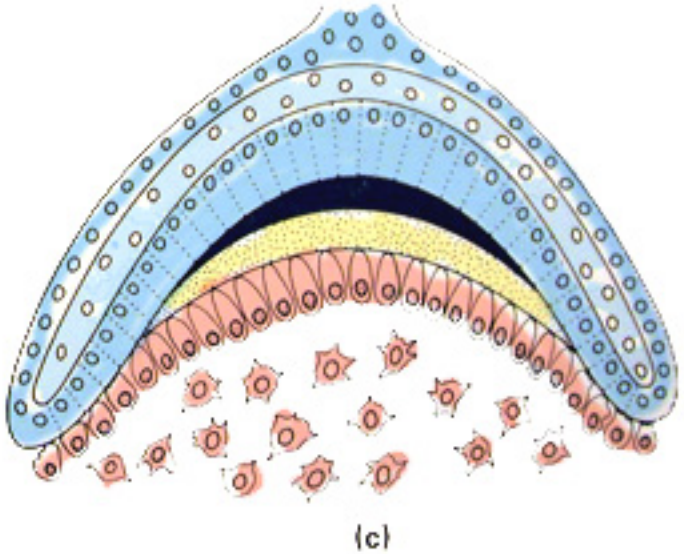
Mesenchymal derived

Dentine

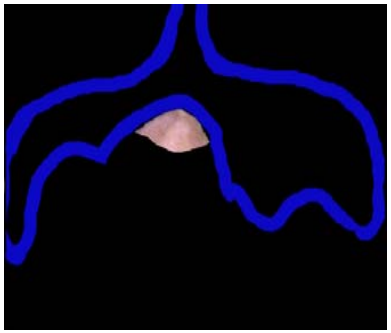
Enamel



(b)



(c)



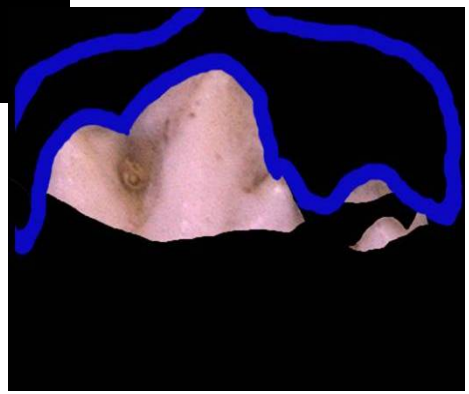
1.

Teeth mineralize cusp-tip to crown base



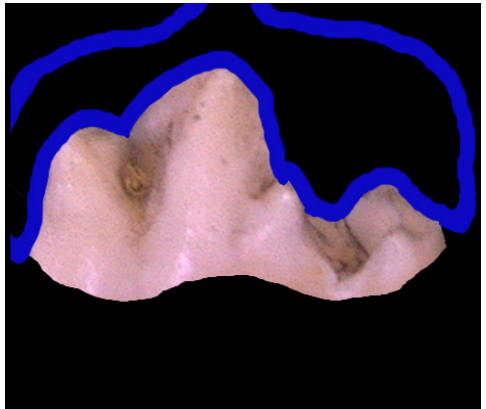
2.





3.

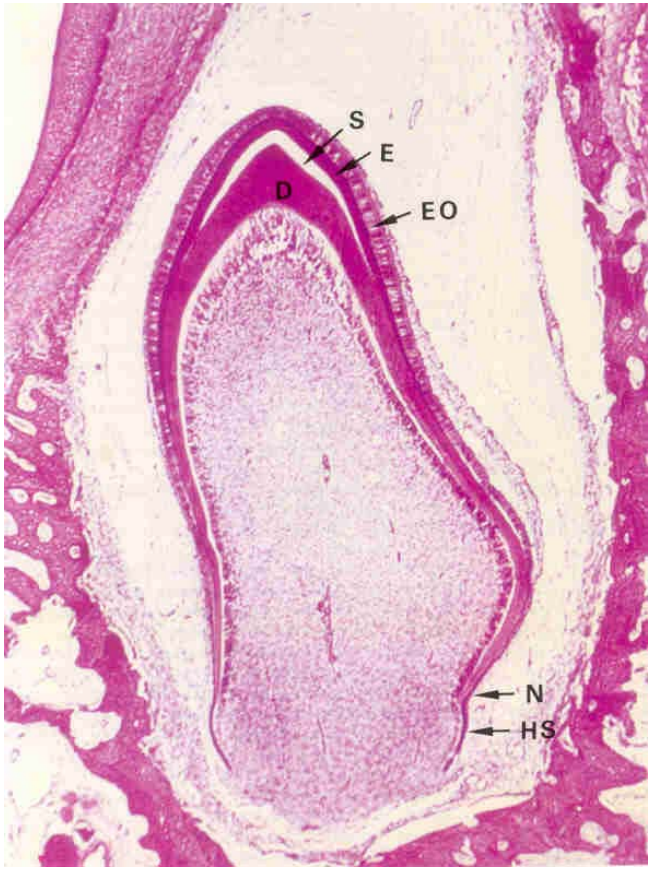
4.



Crown formation

- Incisor tooth - crown shape determined
- enamel deposited adjacent to dentine
- limit of enamel defined

- root formation started - root sheath evident

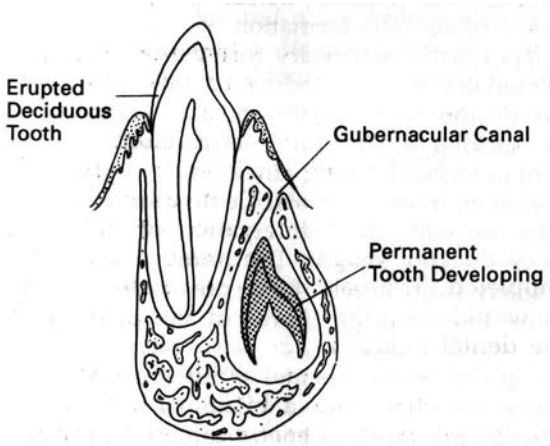


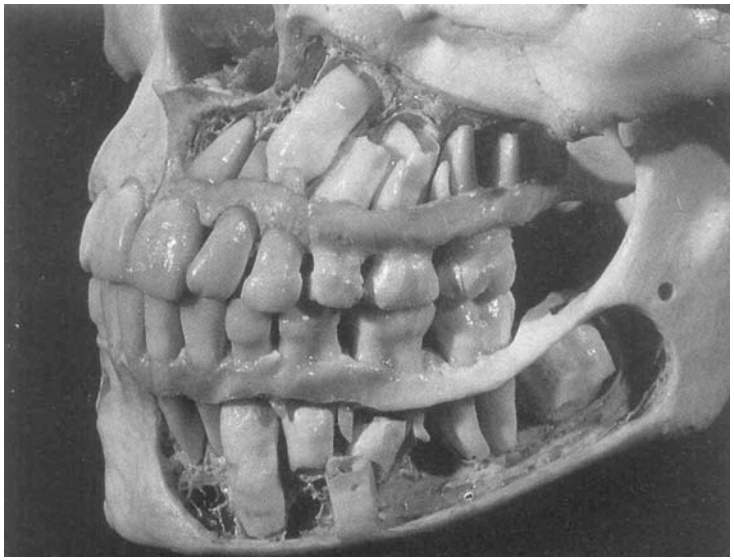
Crown formation II

- 18 weeks:
- downgrowth of lingual enamel epithelium from deciduous teeth develops into tooth germs of permanent teeth
- by 5 months tooth germs evident adjacent to incisors



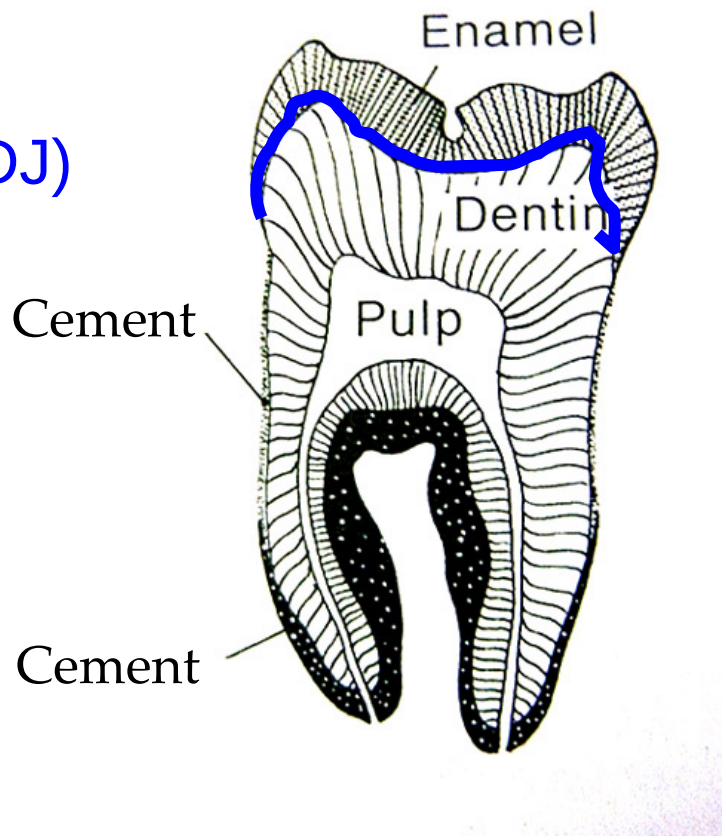
Deciduous and permanent teeth





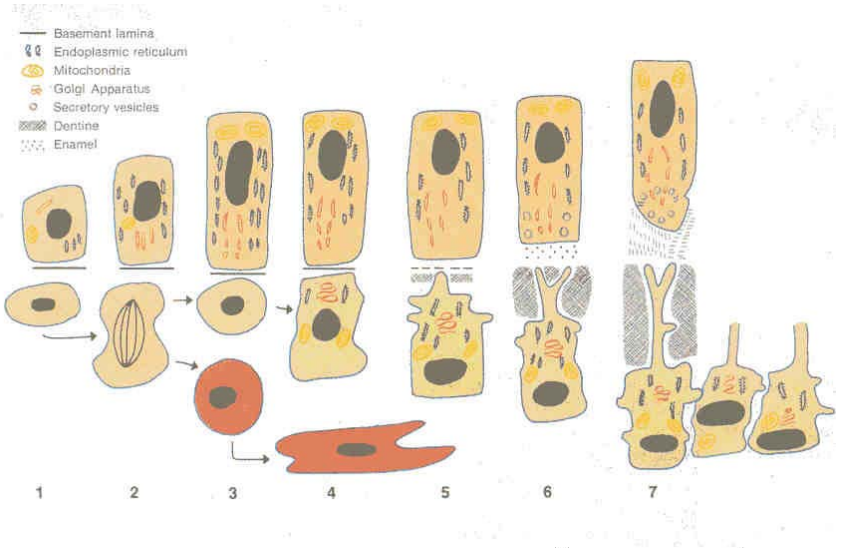
Specialised interface

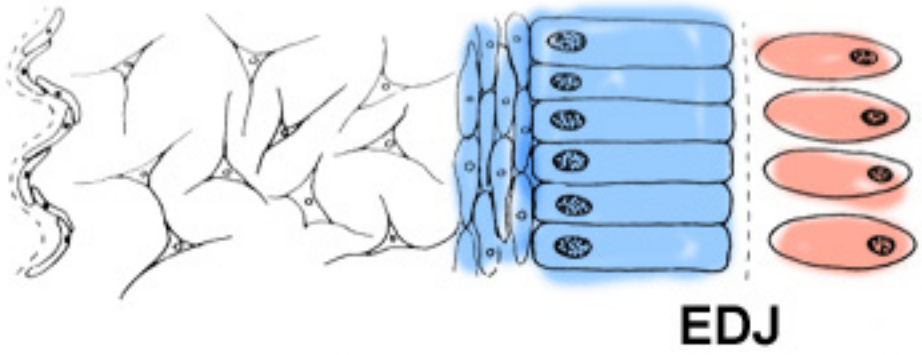
Enamel-
Dentine
Junction (EDJ)

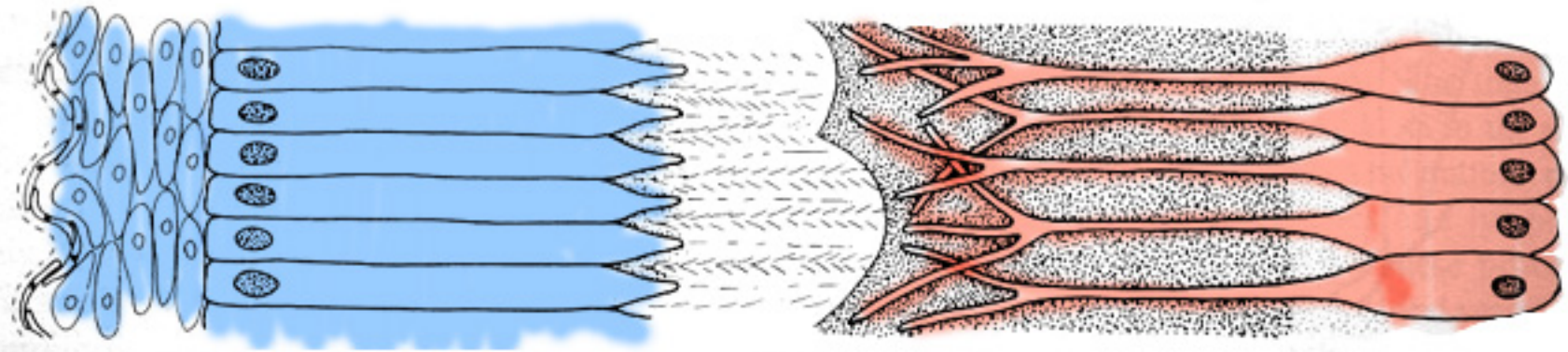


Formation of tooth structure

- differentiation of opposed odontoblasts and ameloblasts
- separated by basal lamina
- distinct process of mineralisation
- persistence of odontoblasts




EDJ

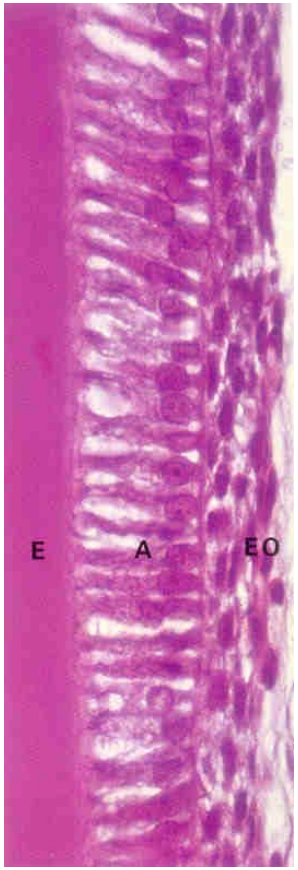

EEE
SI
Ameloblasts
EDJ
Odontoblasts

Dentinogenesis

1. Begins with induction of odontoblasts by ameloblasts.
2. Begins before amelogenesis.
3. Preodontoblasts secrete mantle dentine.
4. Odontoblasts grow long process, which lengths as dentine gets thicker.
5. Secretory phase: organic matrix laid down as predentine, which has lots of type 1 collagen.
6. Mineralization phase: happens after short period of time.

Formation of enamel

- Tall columnar cells with polarised secretory apparatus
- single layer of ameloblasts and other layers of the enamel organ
- degenerates at tooth eruption

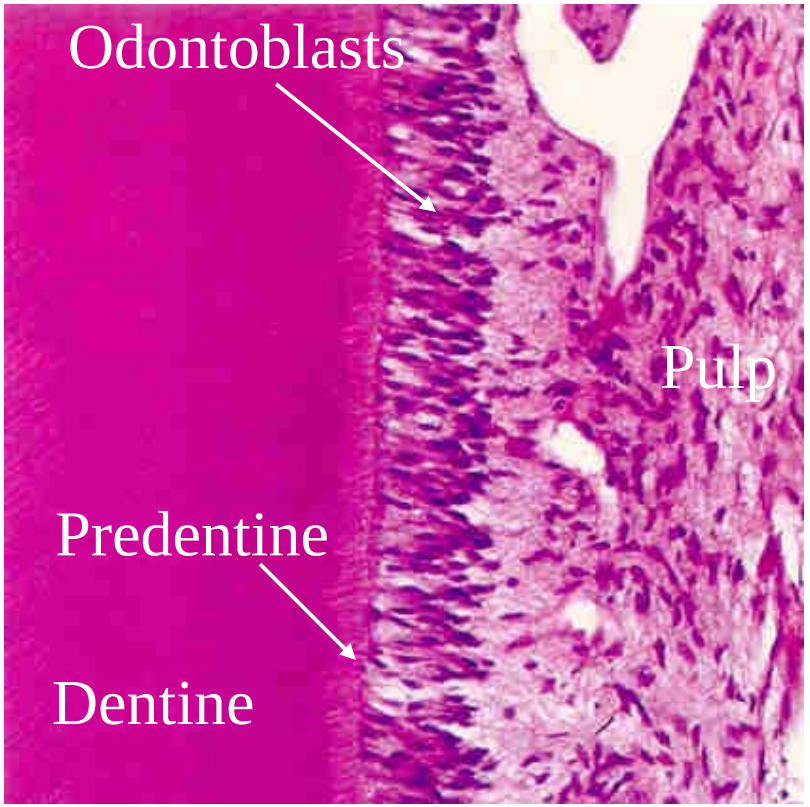


Amelogenesis (enamel formation)

1. Ameloblasts differentiate, become elongated (pulsing), and deposit enamel matrix.
2. Initial enamel is aprismatic, but shape of ameloblasts change resulting in prismatic enamel through most of crown.
3. Secretory phase: Organic matrix deposited and becomes mineralized almost instantly.
4. Maturation phase: hydroxyapatite crystals form and organic matrix largely removed.
5. Organic matrix largely made of albumin and amelogenin proteins.

Dentine formation

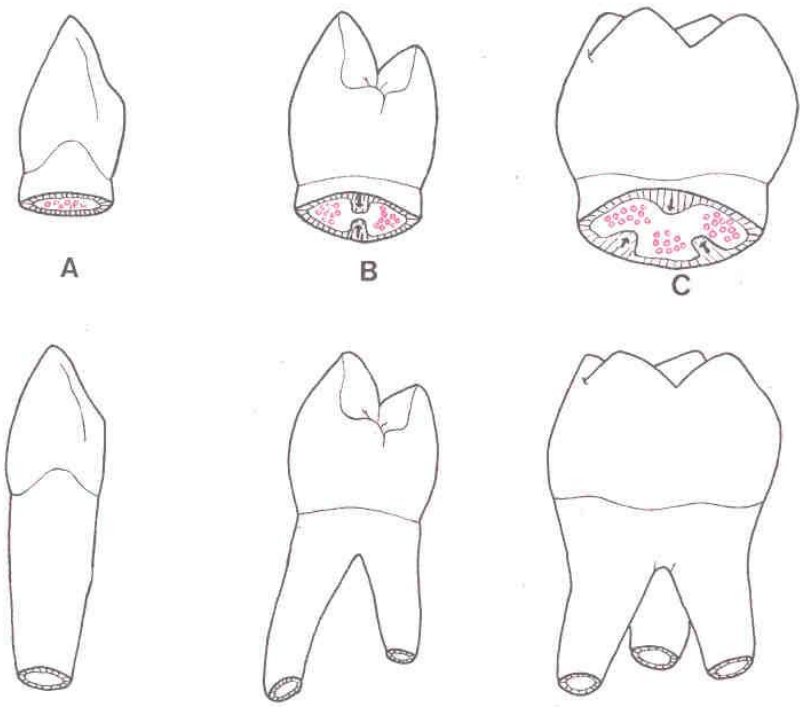
- dentine forms most of the tooth
- slender cellular processes remain
- odontoblasts persist in pulp cavity



Root formation and patterning

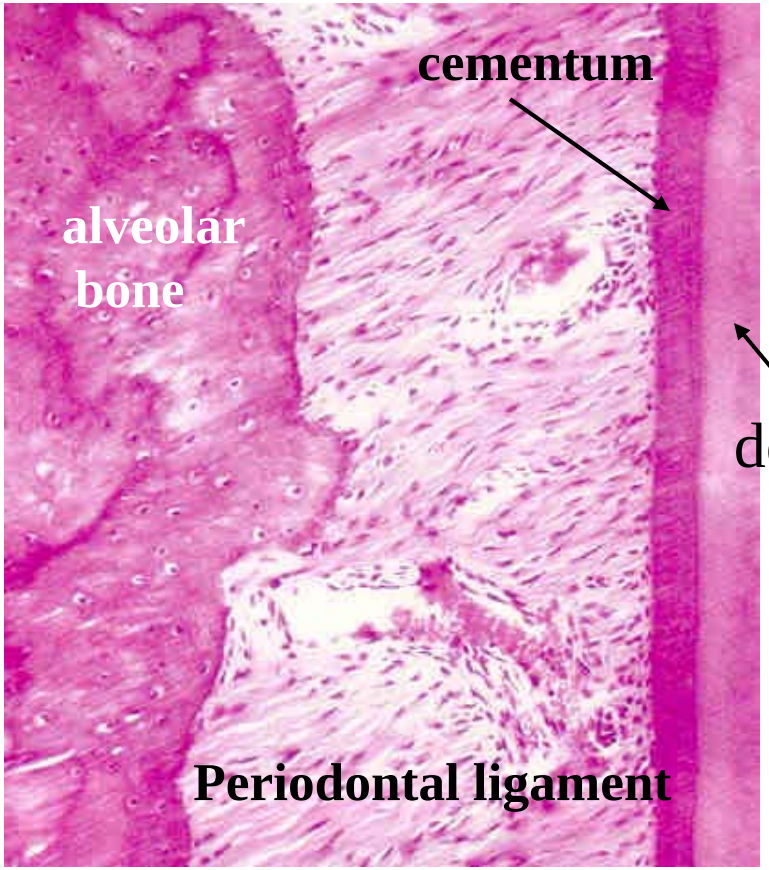
- during late stages of tooth formation and during eruption root formation continues

- characterised by an absence of enamel and development of periodontal attachment



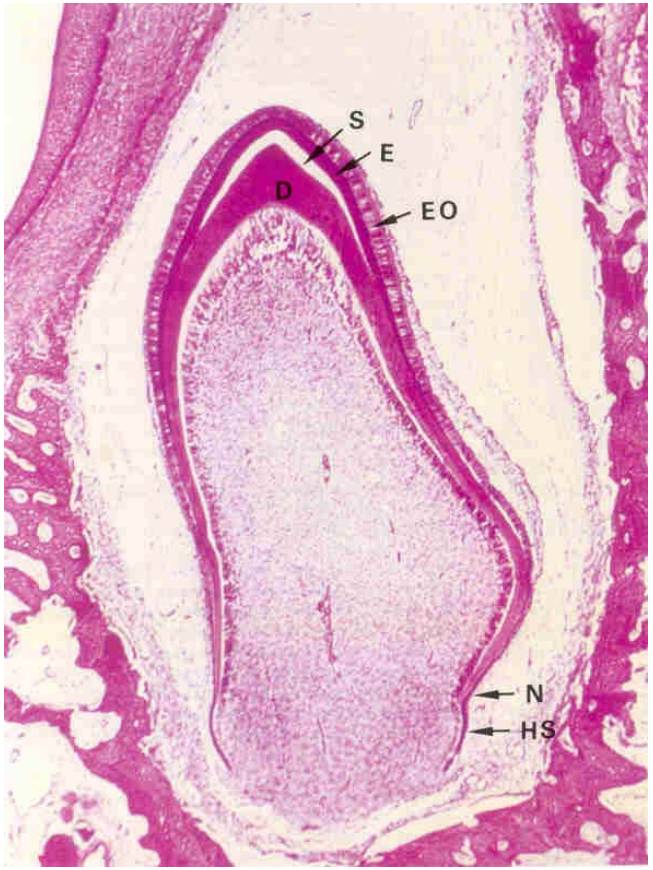
Root formation

- Root dentine covered in bone-like cementum
- originates from cementoblasts
- ligament cells attach tooth to alveolar bone



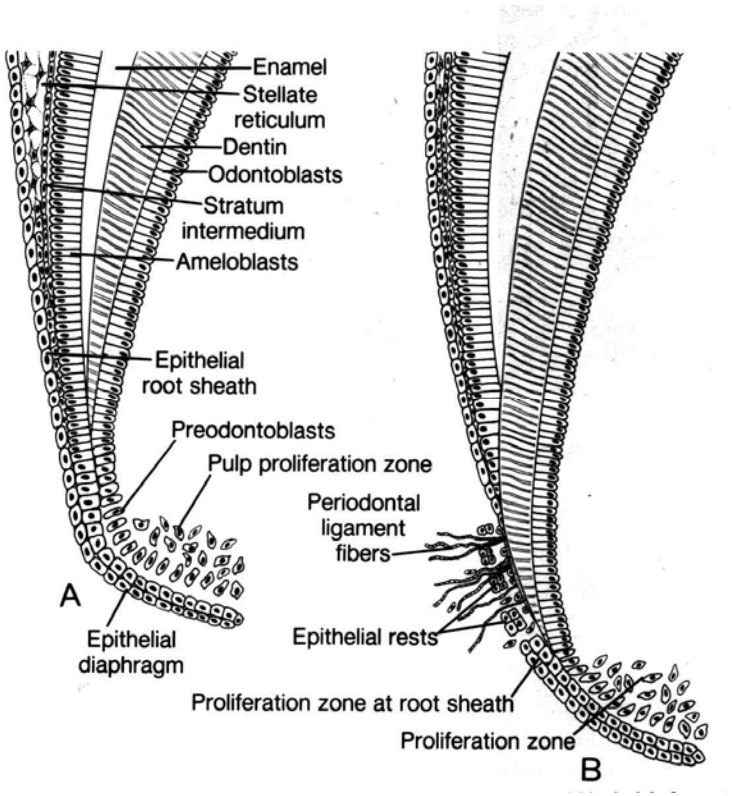
Root formation

- root formation started root sheath evident
- distinct organisation of cells forming cementum and ligament



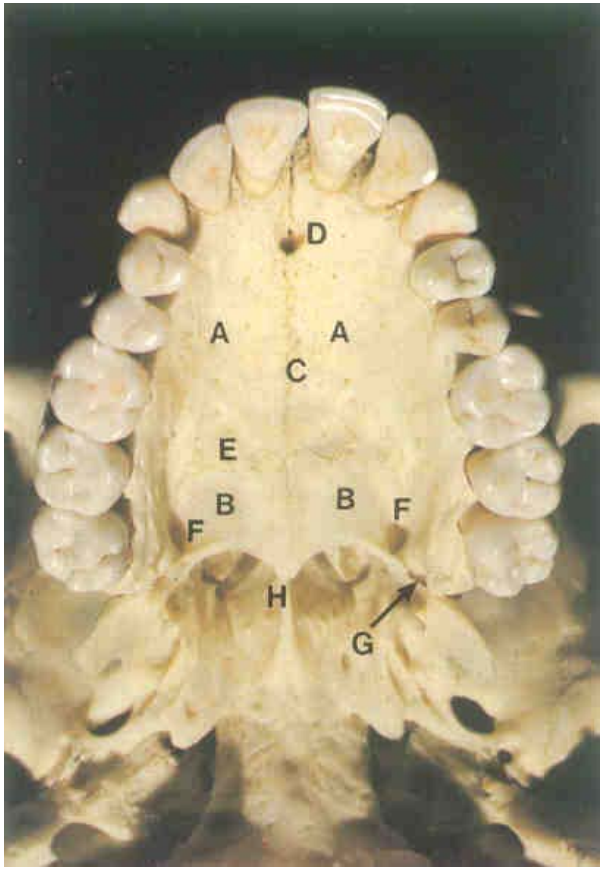
Epithelial sheath of Hertwig





Range of developmental questions

- Position
- Number
- Character
- Individual tooth morphogenesis



Understanding tooth development

- Tissue recombinations allow cellular interactions to be investigated

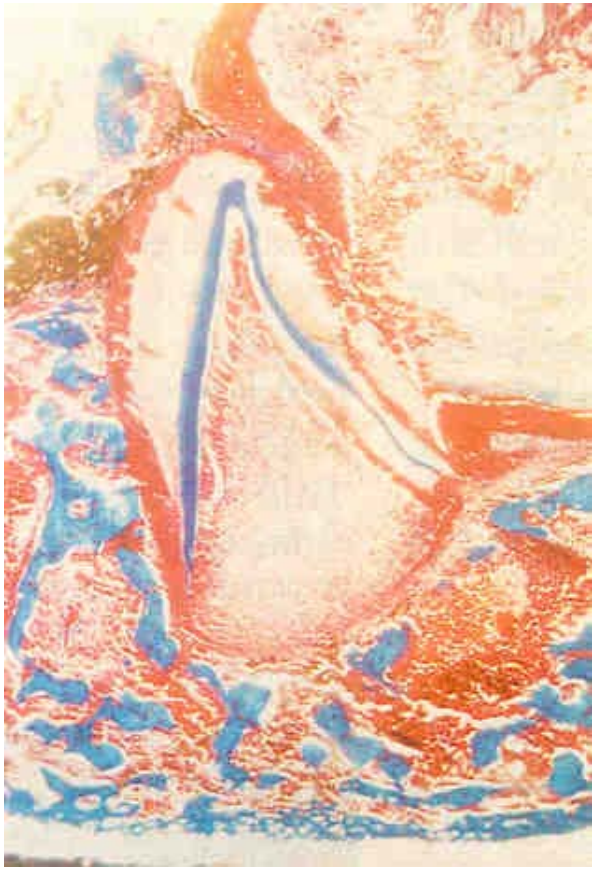
- result of culture of whole tooth germ at cap stage



Instructive role of mesenchyme

- Dental papilla mesenchyme with developing foot pad epithelium

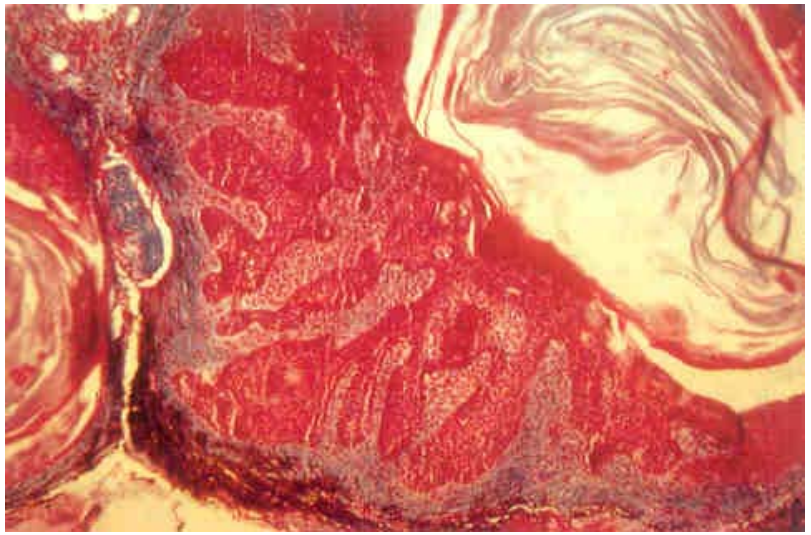
- hence dental papilla has instructive information



Instructive role of mesenchyme II

- Enamel organ with mesenchyme from foot

- - no tooth develops



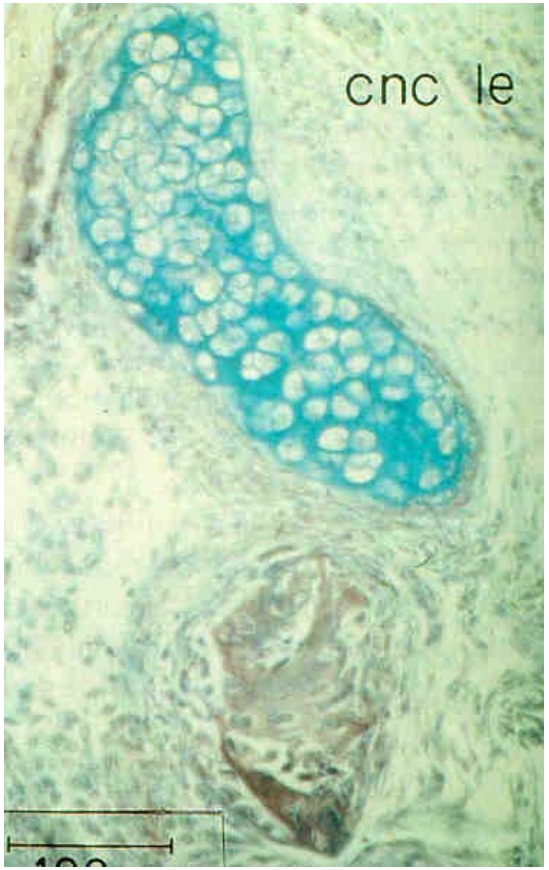
Control of tooth development

- Lineage labelling shows that neural crest contributes to developing tooth germ
- neural crest origin proven for odontoblasts, dentine matrix, pulp, cementum and periodontal ligament
- ability of mesenchyme to carry instructions depends on stage of experimentation

Early Importance of epithelium

- Combinations of premigratory neural crest with limb epithelium

- results in islands of bone and cartilage



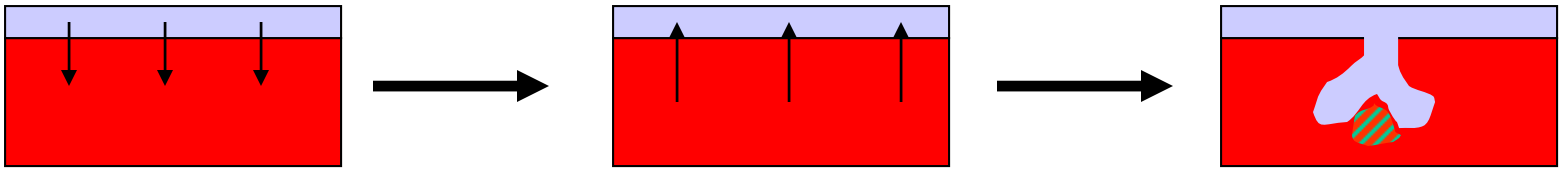
Early Importance of epithelium II

- Premigratory neural crest cultured with oral epithelium

- defined tooth germs are formed



Early role for epithelium and later role for mesenchyme



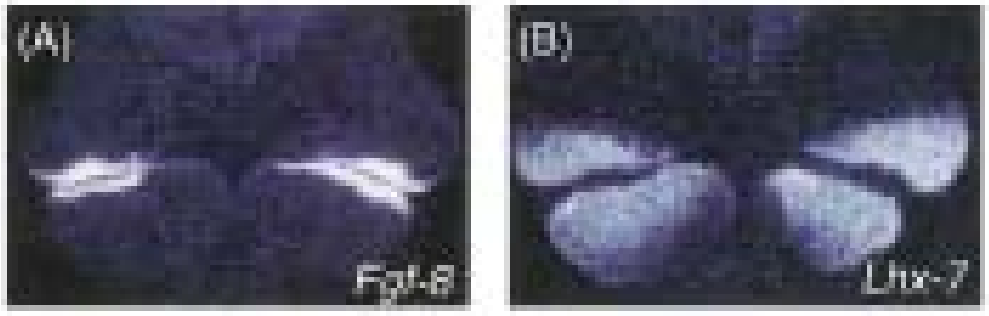
Epithelial signals induce mesenchymal response

Mesenchymal signals trigger epithelial invagination

Epithelial invagination confers new properties on mesenchyme

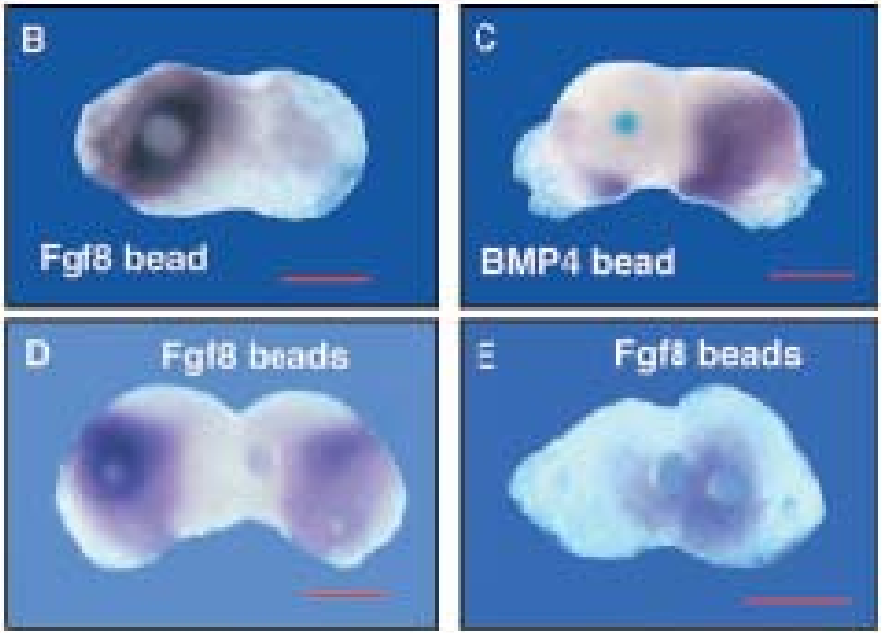
Patterning the jaws

- Regional differentiation of surface ectoderm shown by expression of growth factors - FGF-8



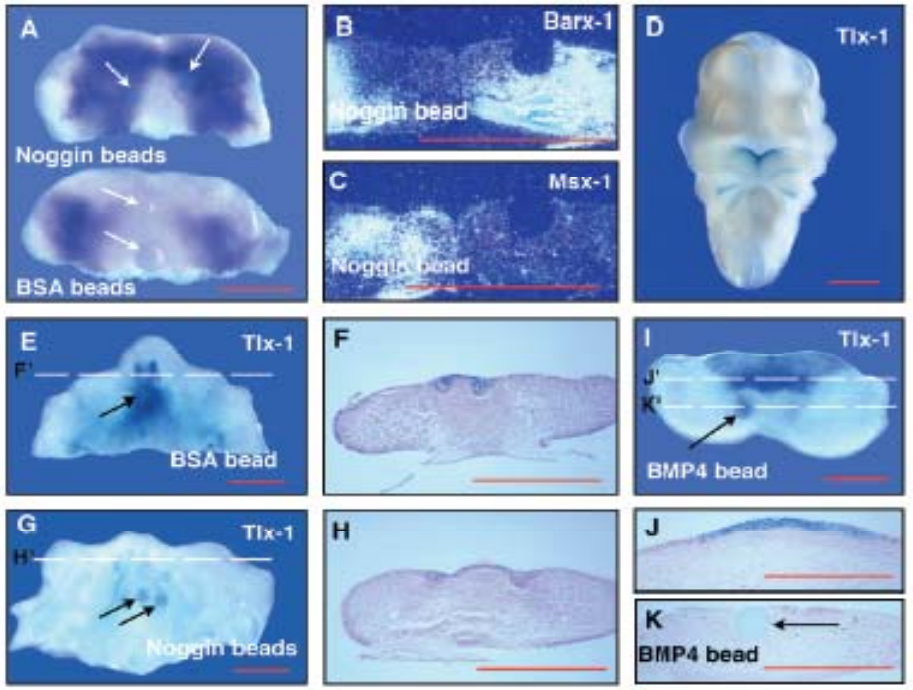
Regulation of jaw pattern

Barx-1
induced
by FGF-8
repressed by
BMP-4

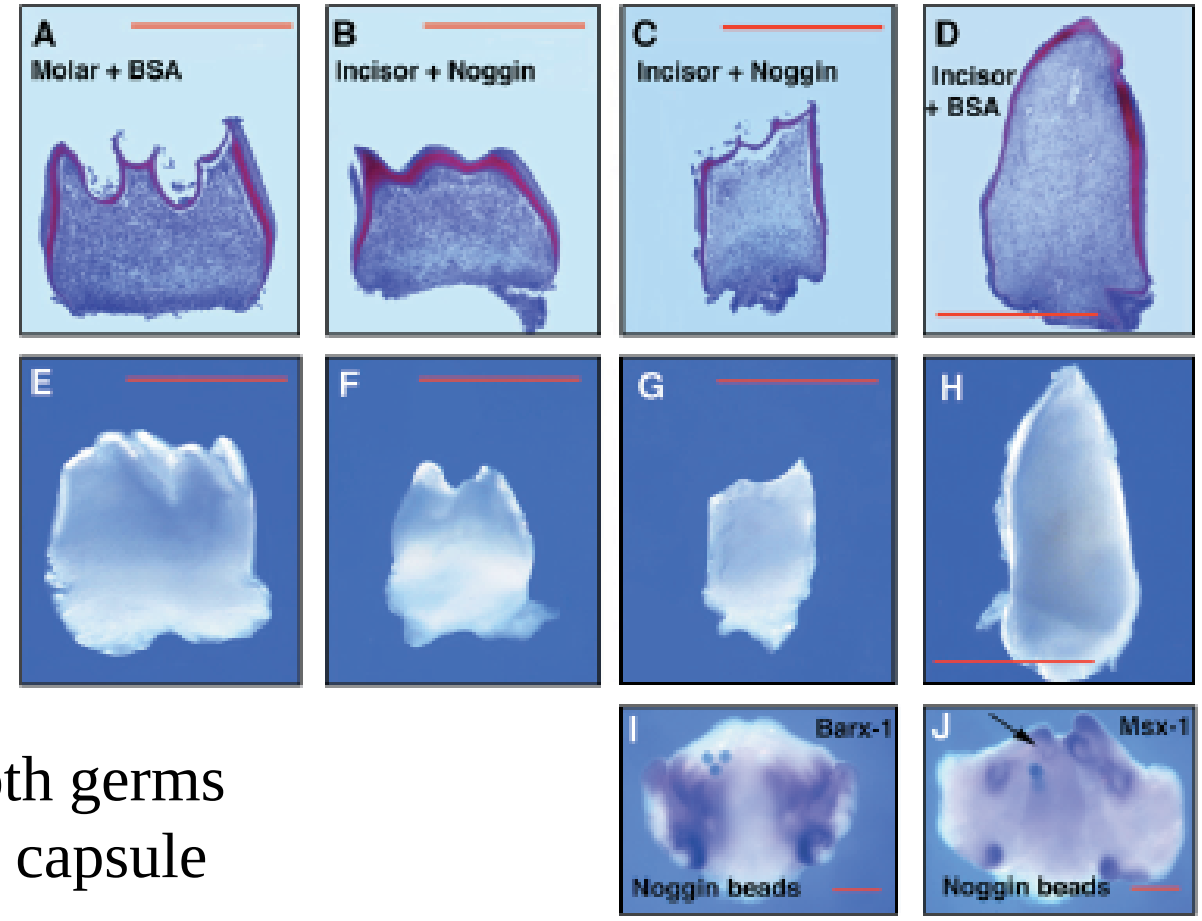


Level of BMP directs Barx-1 expression

Apply noggin as a BMP antagonist



Switch incisor to molar with noggin!



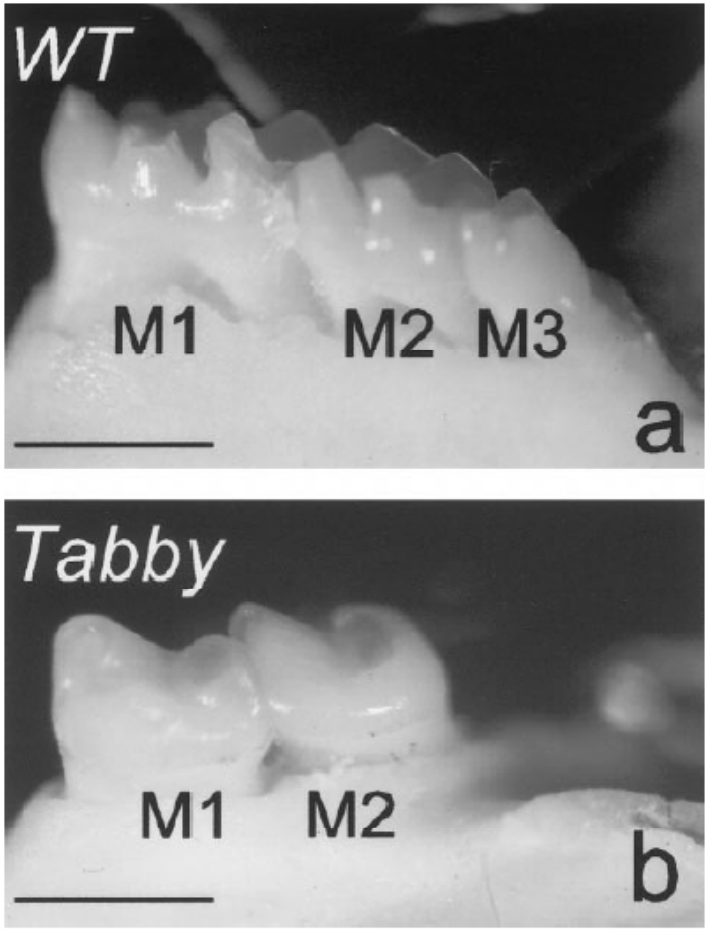
Grow tooth germs
in kidney capsule
for 14 days

Enamel knots

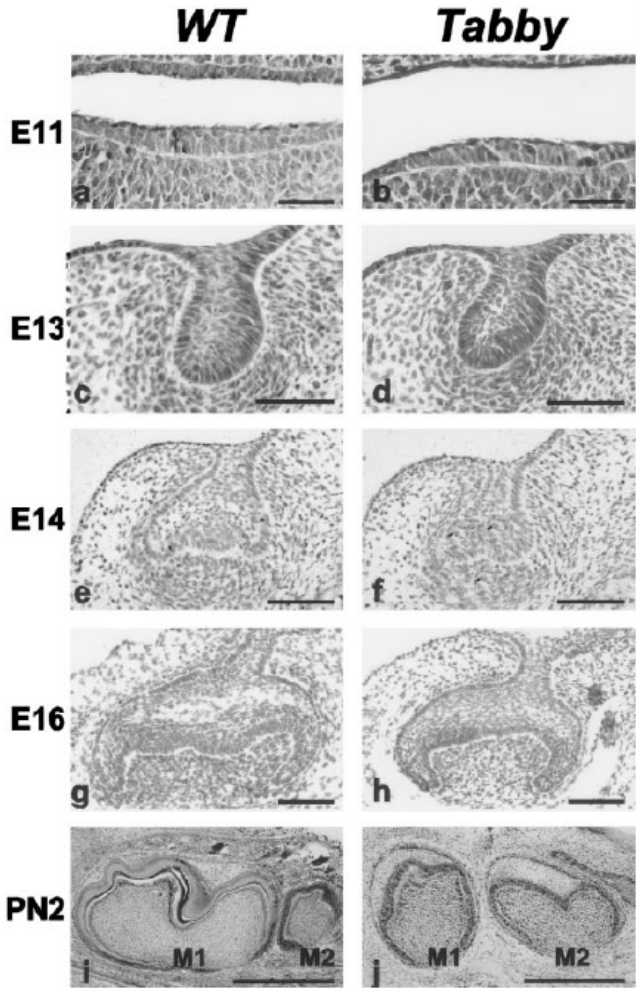
- Transitory structures in enamel organ
- Play a role in controlling cusp shape
- This knot marks future central fissure



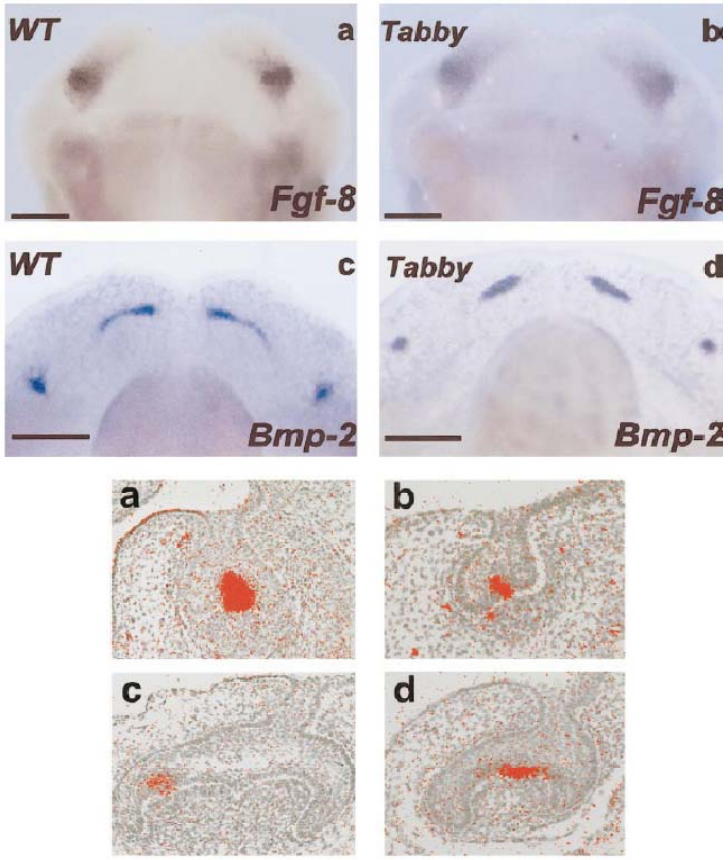
tabby mice mutant have abnormal tooth crowns



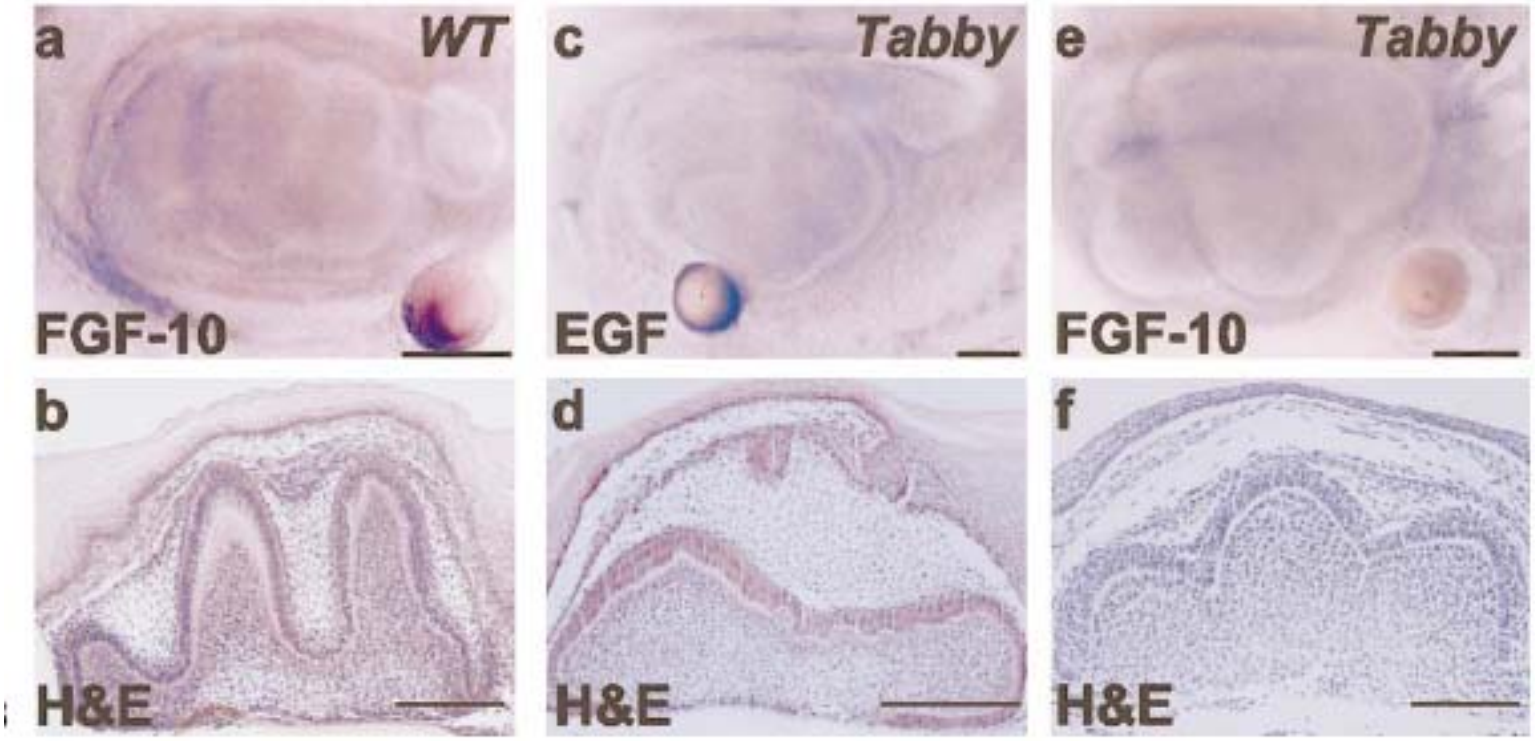
Defects appear to relate to enamel knot formation

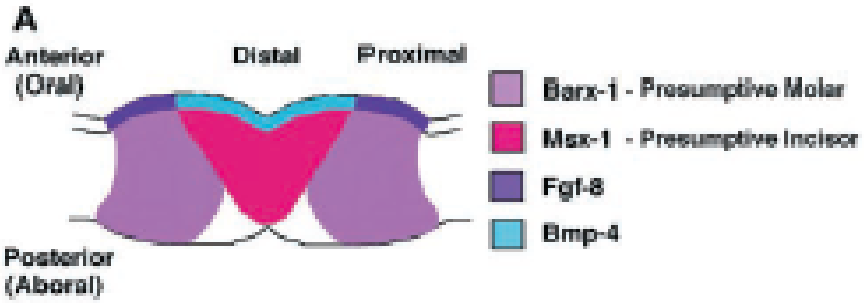


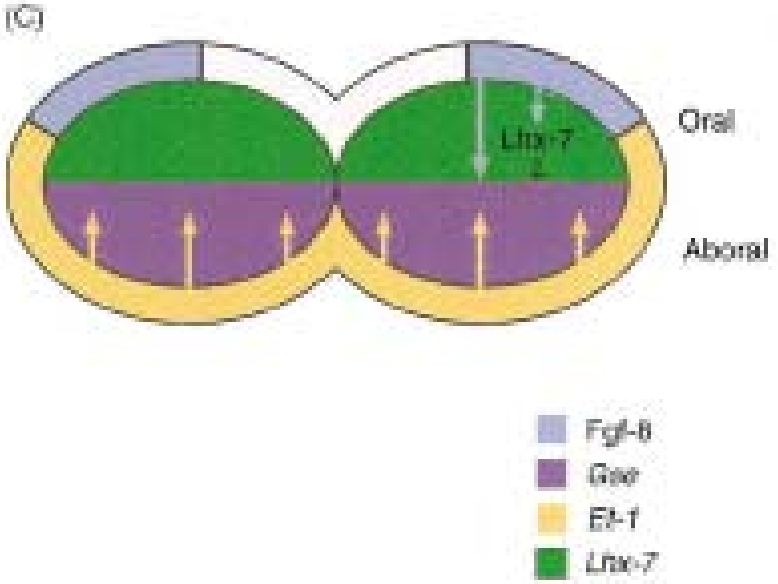
Reduced expression of FGF-8 in *tabby* mutant

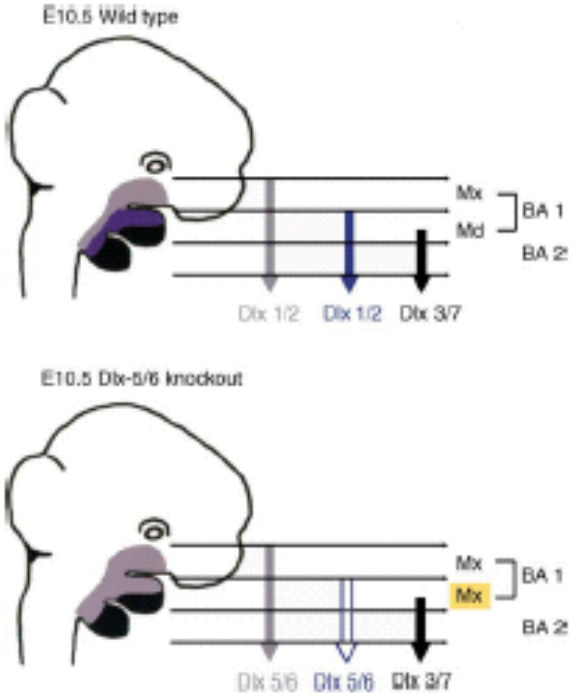


Culture with FGF results in a partial rescue




A
Anterior
(Oral)
Distal
Proximal
Posterior
(Aboral)
Barx-1 - Presumptive Molar
Msx-1 - Presumptive Incisor
Fgf-8
Bmp-4


(c)
Lhx-7
Oral
Aboral
Fgf-8
Gsc
Et-1
Lhx-7

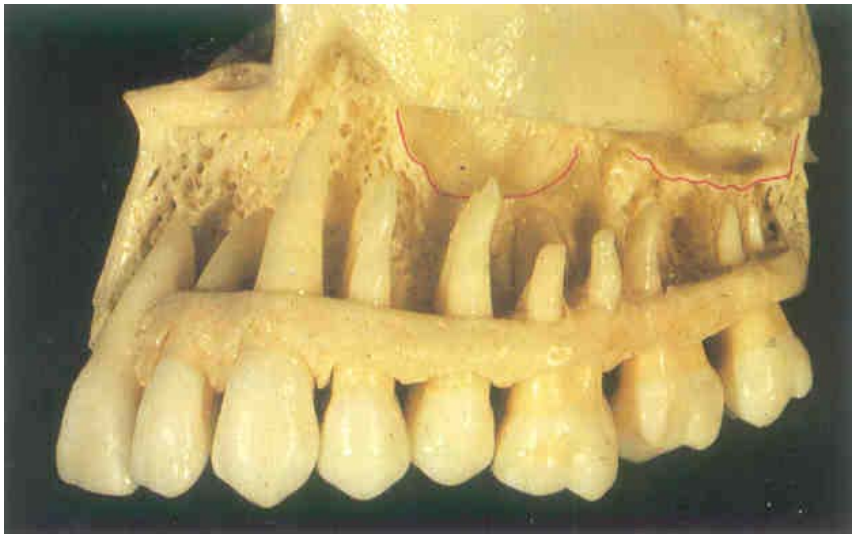

E10.5 Wild type
Mx
Md
BA 1
BA 2
Dlx 1/2
Dlx 1/2
Dlx 3/7
E10.5 Dlx-5/6 knockout
Mx
Mx
BA 1
BA 2
Dlx 5/6
Dlx 5/6
Dlx 3/7

Development of the periodontium (prospects for regeneration?)

Tooth development

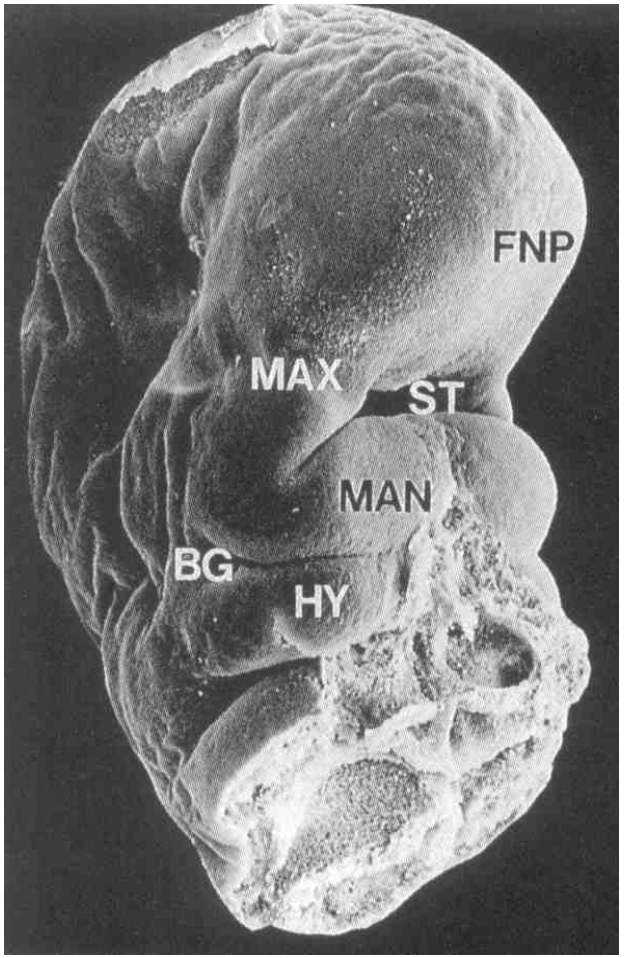
- Organised structures
- Regionally patterned
- Each tooth distinct by position and shape

- Clear example of developmental problem



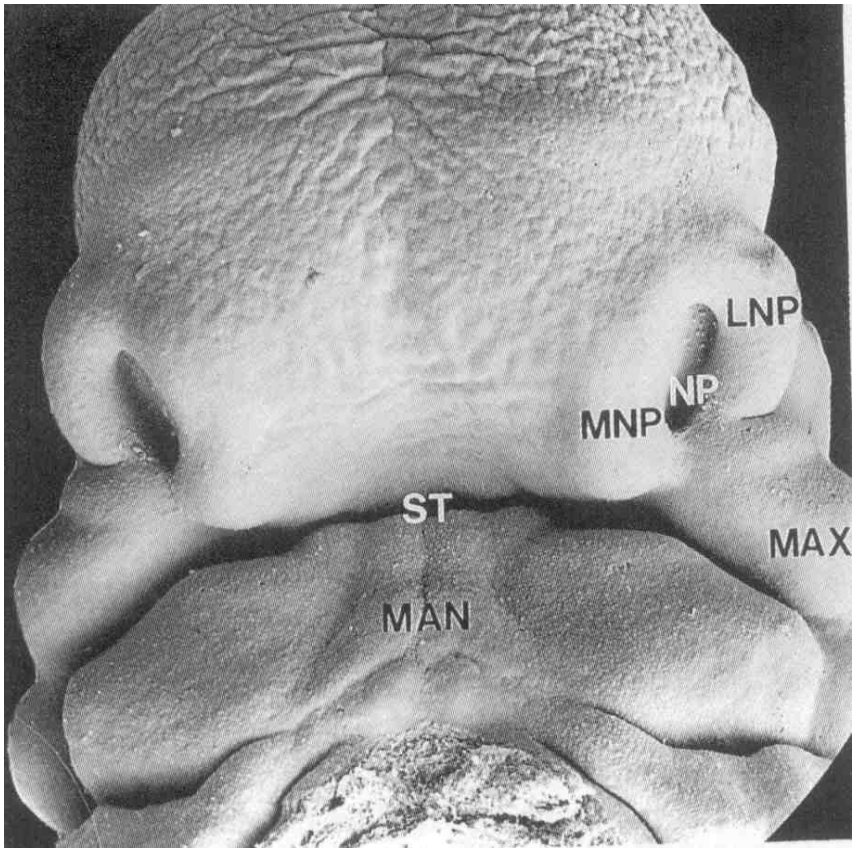
Tooth Origins

- First arch structures
- Combination of oral epithelium and underlying cranial neural crest
- Cranial crest distinct from trunk crest
- picture 26 day human embryo



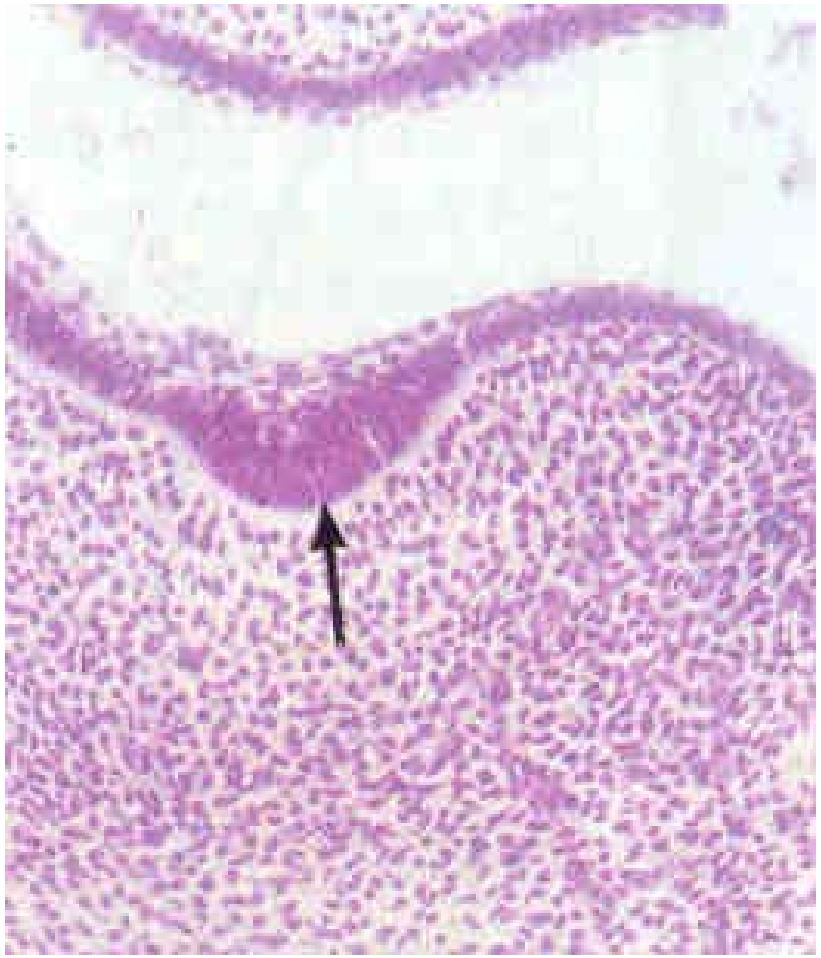
Oral Tissues

- embryo 37 days old
- mandibular and maxillary processes evident
- tooth formation started



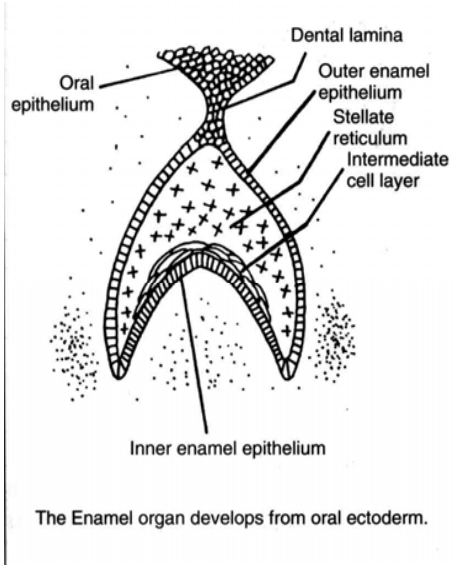
Primary epithelial band

- 6 weeks epithelial thickening in presumptive tooth forming region
- Consider possible developmental abnormalities

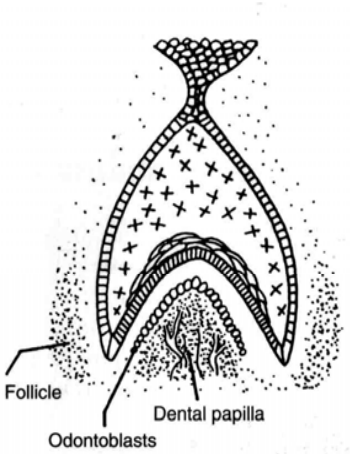


Structure of enamel organ

- At about 12 weeks enamel organ develops regional structure - the cap stage
- Separation of epithelium into inner and outer layers - future fates differ!
- Mesenchyme from jaw forms the dental papilla



The Enamel organ develops from oral ectoderm.



The tooth germ consists of the enamel organ, the follicle and the dental papilla.

Histology of cap stage

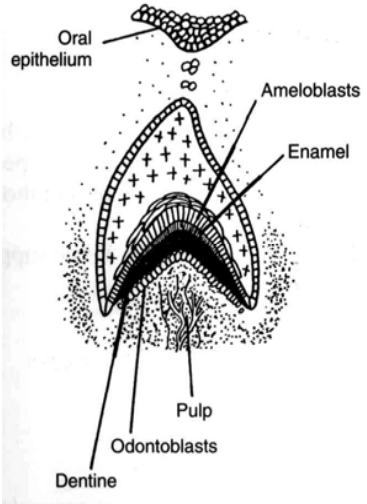
- Late cap stage 12 weeks
- A: Stellate reticulum
- B: Outer enamel epithelium - cuboidal
- C: Inner enamel epithelium - columnar
- D: Dental papilla
- E: Dental follicle

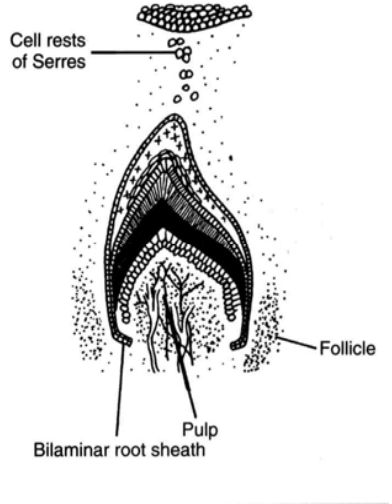


Cell differentiation

- Connection to oral epithelium severed

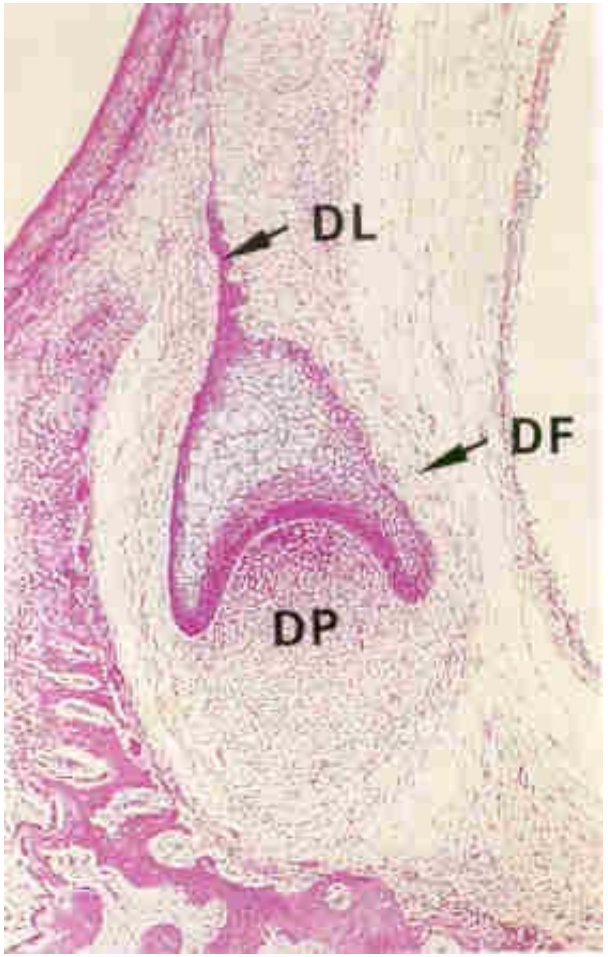
- inner enamel epithelium $\rightarrow$ ameloblasts
- dental papilla $\rightarrow$ odontoblasts + pulp
- deposition of enamel and dentine starts



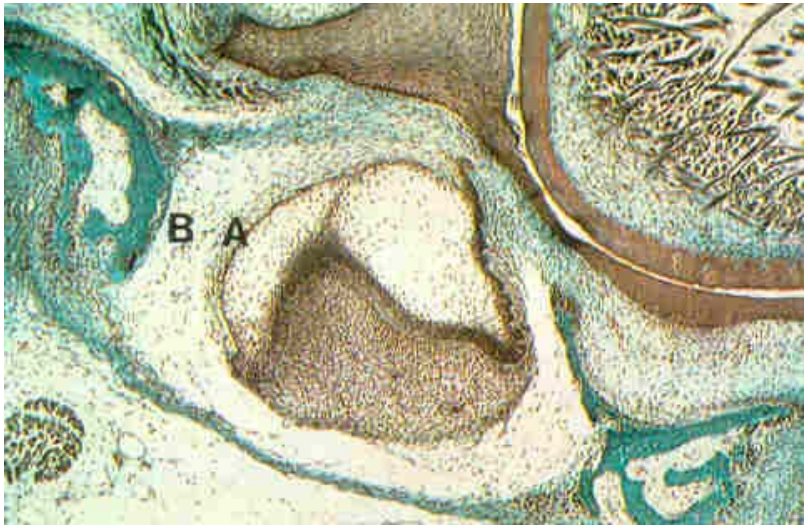


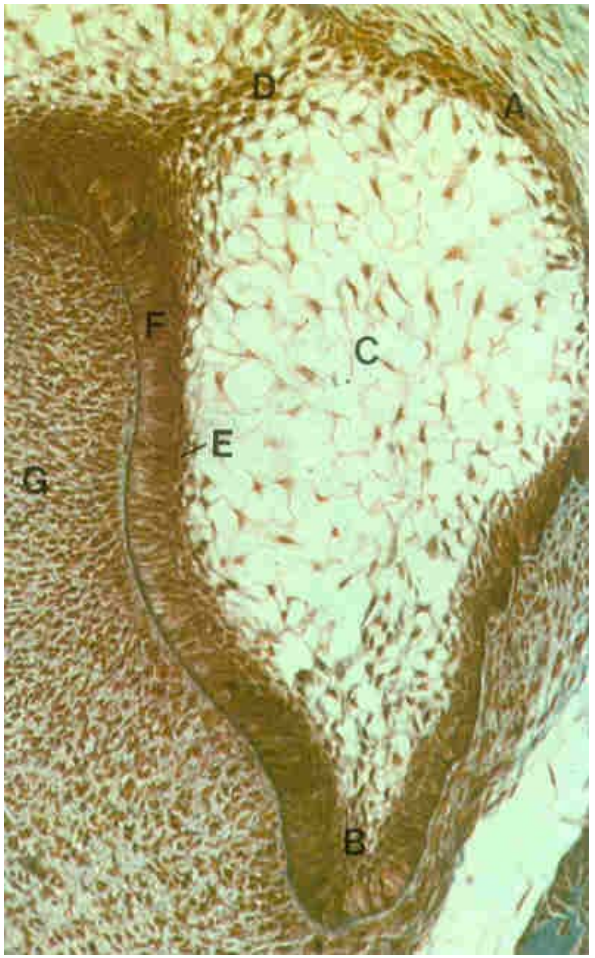
Early bell stage

- Breakdown of dental lamina
- Dental papilla becoming organised
- Condensation of mesenchyme form the dental follicle



Early bell stage



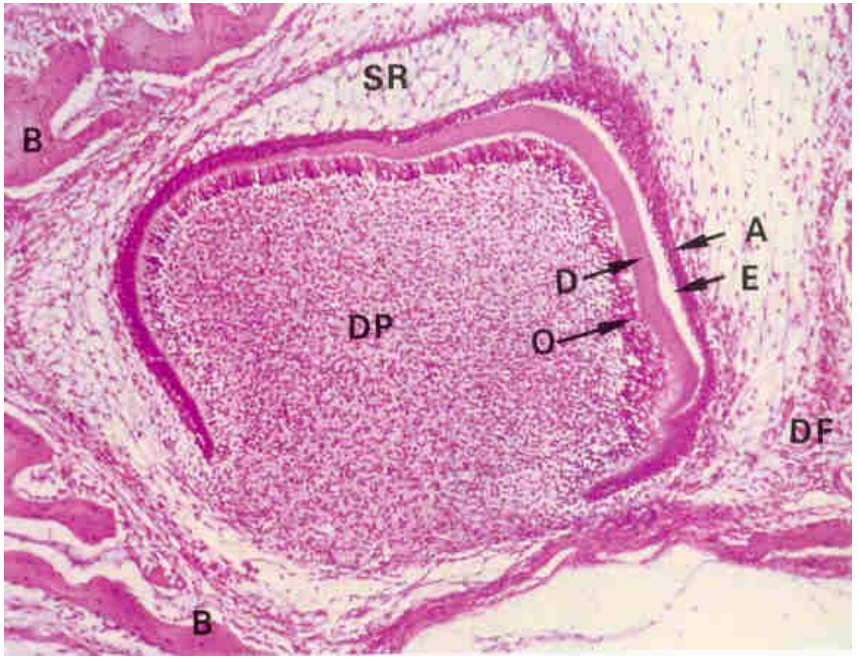


14 weeks - cusps defined
- organised patterns of cell differentiation

Bell stage

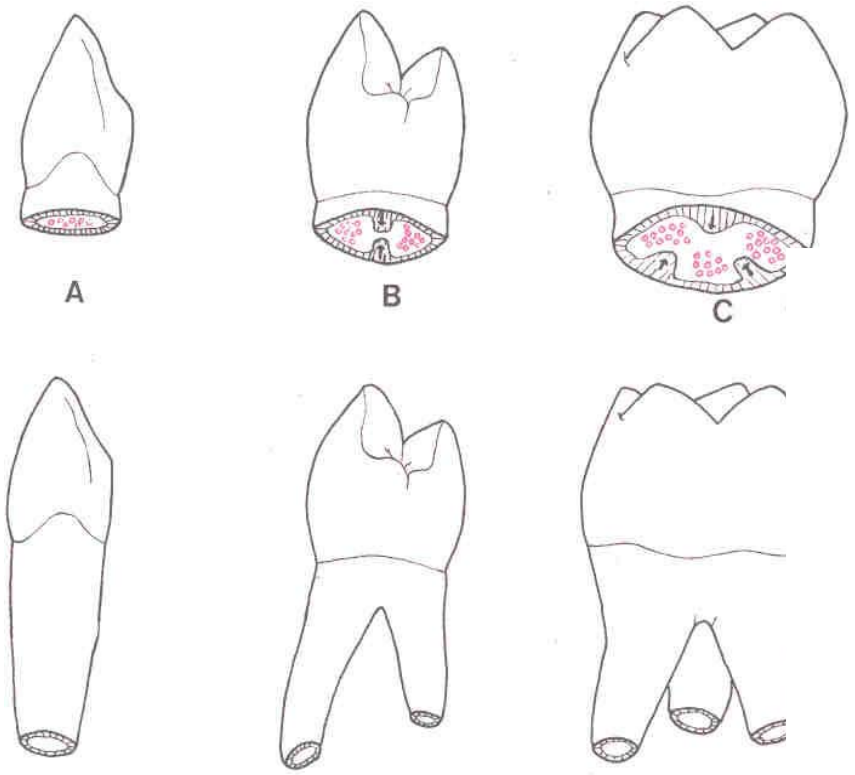
- Overt differentiation
- Dentine first to be laid down
- Organised layers of ameloblasts and odontoblasts evident

- Period of crown morphogenesis



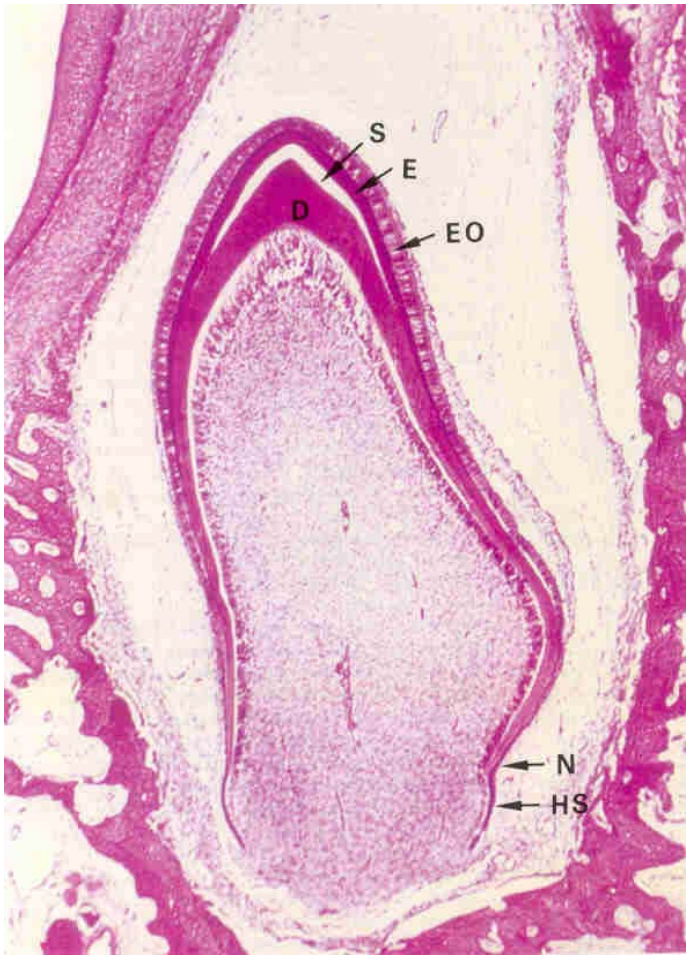
Root formation and patterning

- during late stages of tooth formation and during eruption root formation continues
- characterised by an absence of enamel and development of periodontal attachment



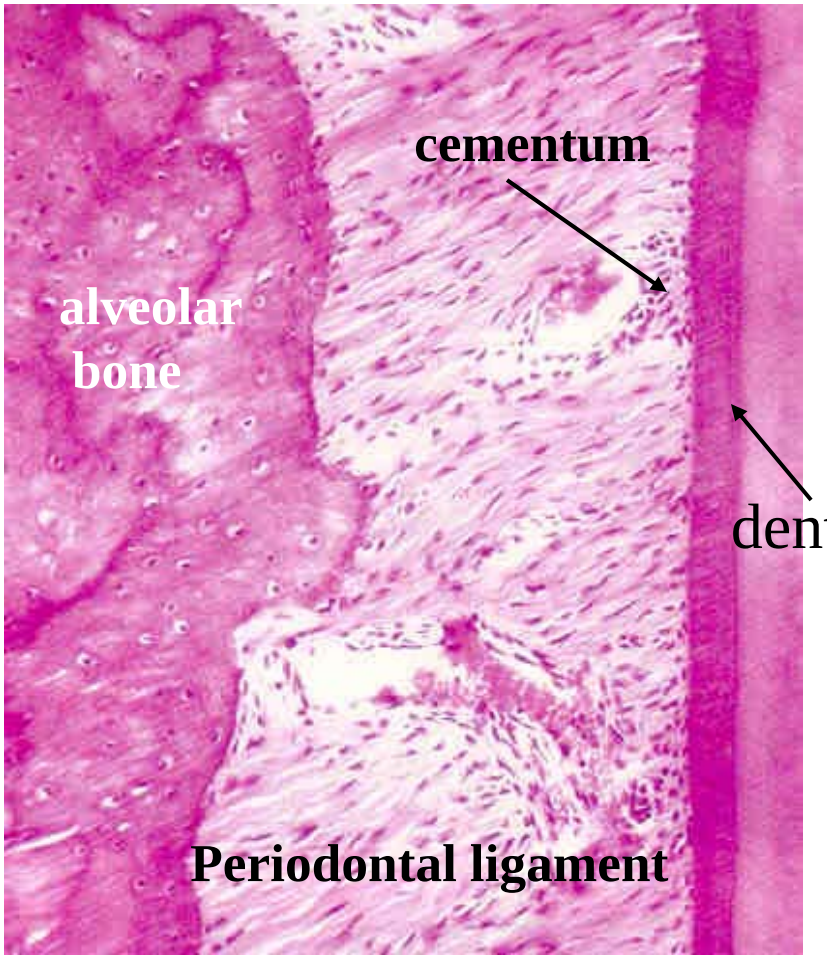
Root formation I

- root formation started root sheath evident
- distinct organisation of cells forming cementum and ligament



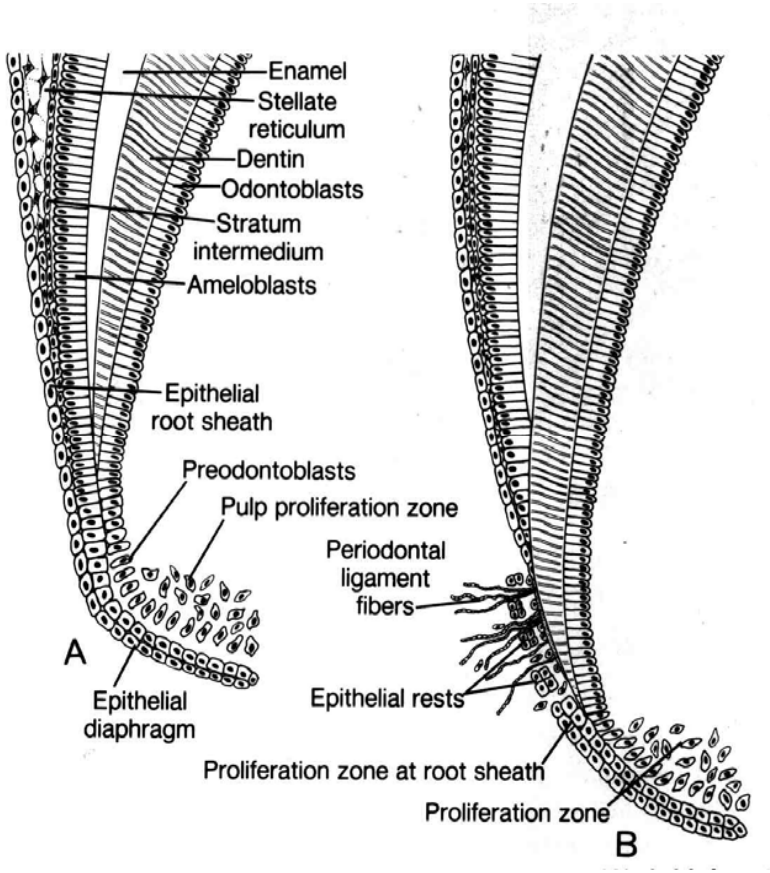
Root formation II

- Root dentine covered in bone-like cementum
- originates from cementoblasts
- ligament cells attach tooth to alveolar bone



Epithelial sheath of Hertwig





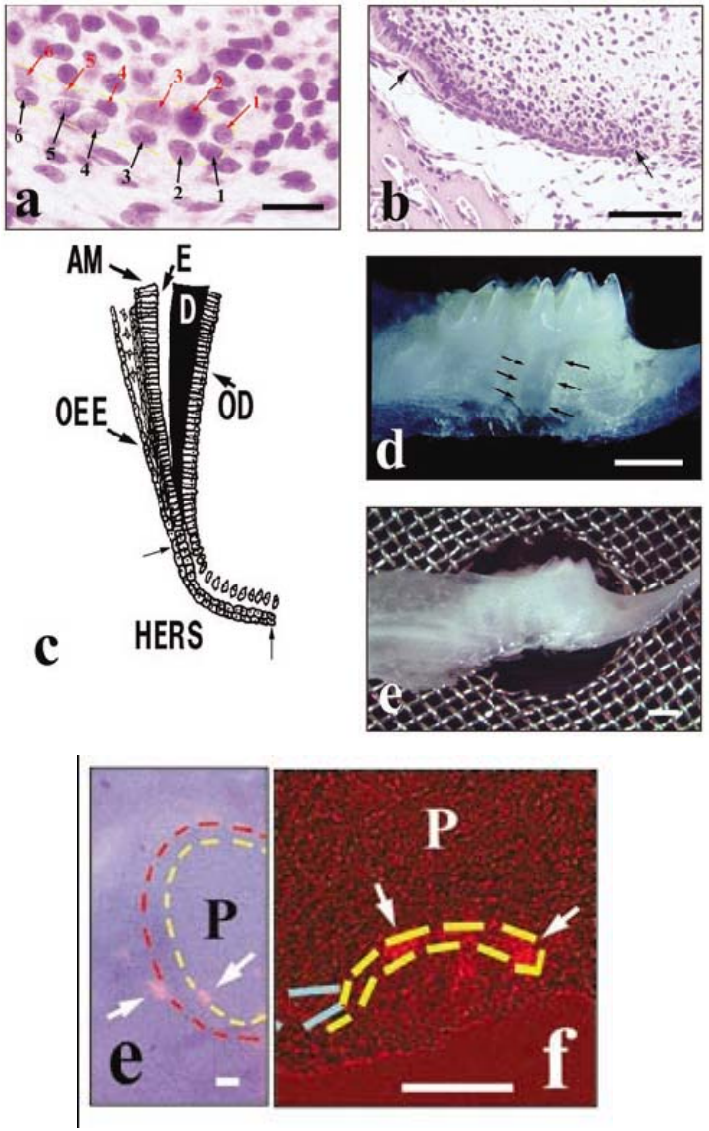
Function of HERS

- Patterning of the root structure
- Divide dental papillae from dental follicle
- Determine cessation of root growth?

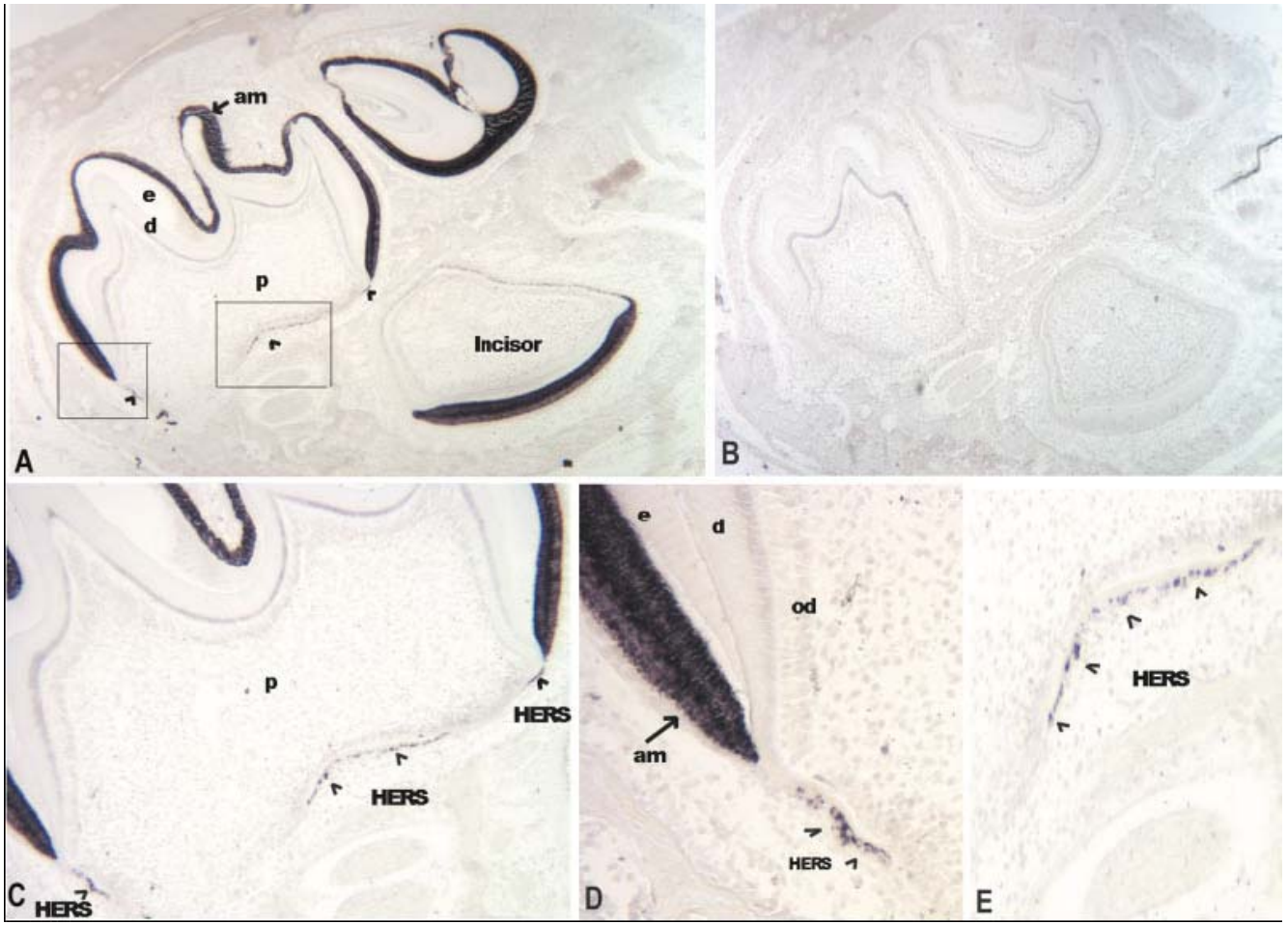
- Cellular origins:
  - Ligament
  - Cementum
  - Epithelial cell rests of Mallassez

Cells of the HERS

- Studies using organ culture show little cell migration
- Expression of BMPs 2 and 4 suggests a possible role as a signalling centre



Expression of ameoblastin in HERS cells



Regeneration of Periodontium

4 components

- Gingiva
- Alveolar Bone
- Periodontal Ligament
- Root cementum

Cementum Types

- Cellular intrinsic fibre cementum (CIFC)
- Acellular extrinsic fibre cementum (AEFC)

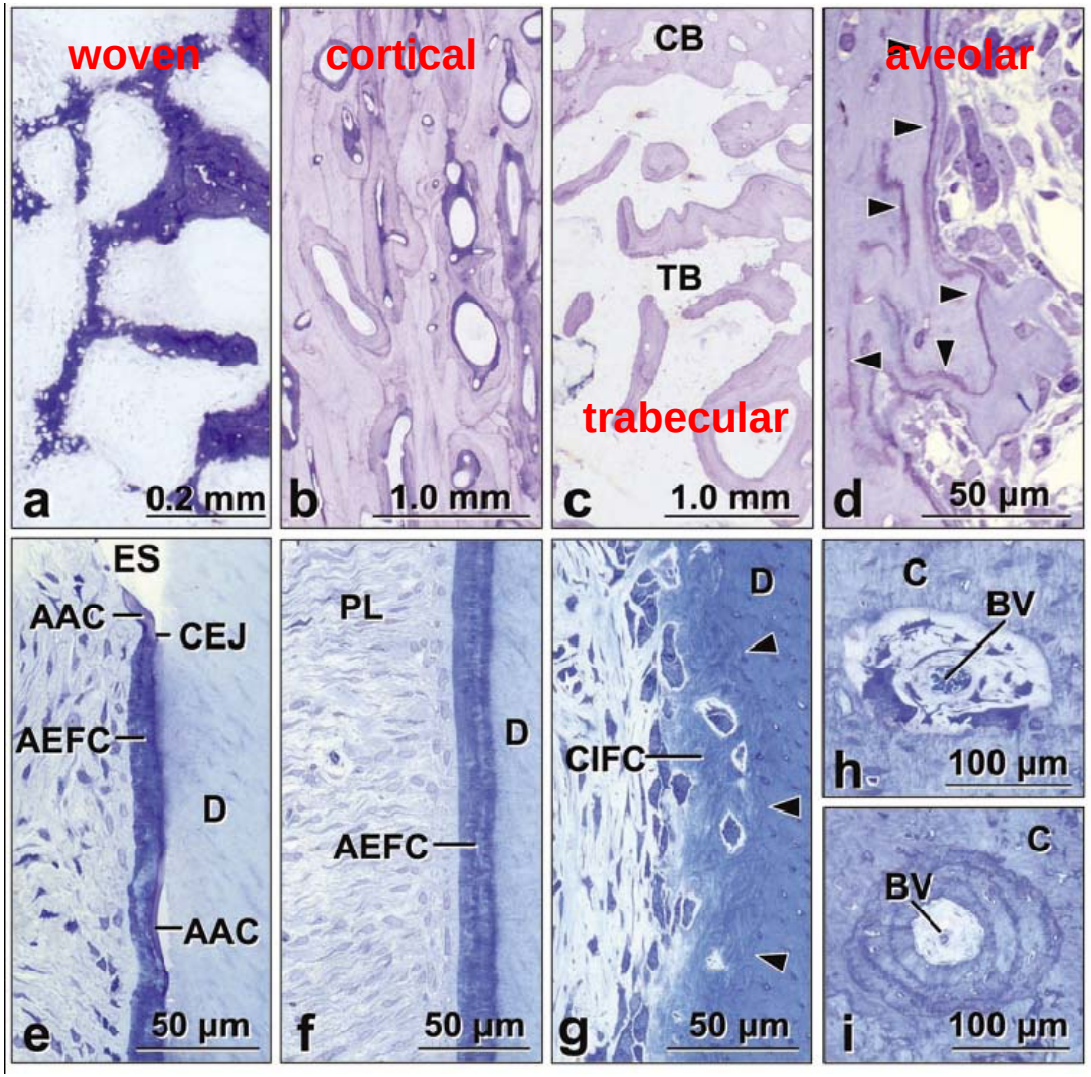
CIFC – bone like

AEFC – associated with stronger bonding

Comparison of cementum and bone

BONE

CEMENT

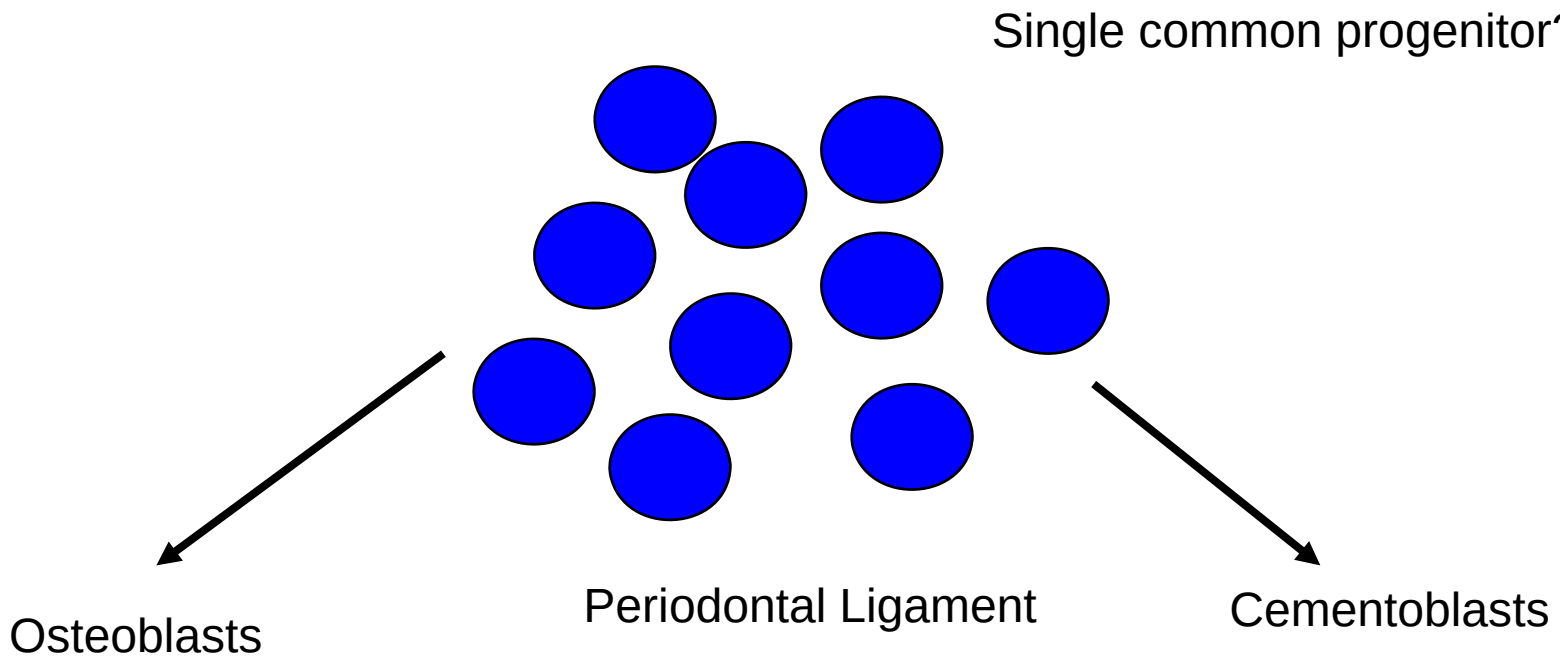


AAC
acellular afibrillar

AEFC
acellular extrinsic fibrillar

CIFC
cellular intrinsic fibrillar

Progenitor Cells



Cementoblasts – real or a positional osteoblast?

- Acellular afibrillar cementum (AAC) – same matrix as acellular extrinsic fibrillar cementum (AECF)

- AECF contains Sharpey's fibres
  - upto 30,000 fibres/mm^2
  - slow incremental growth
  - may include areas of CIFC – represent bone-like inclusions without an attachment role

Cementum characteristics

- AEFC grows slowly 0.005μm/day
- Lamellar bone 1-2μm/day
  - some bones even faster

- Cellular origins electron microscopy suggests that AEFC come from cells with a different morphology – not a single layer.

Matrix composition

Table. Extracellular Matrix Constituents and Sequestered Growth Factors of Bone and Cementum

Molecule (Abbreviation)	Bone Matrix and/or Osteoblasts and/or Osteocytes	CIFC and/or Cementoblasts and/or Cementocytes	AEFC and/or Cementoblasts	Cementum Type Not Specified or Different from AEFC and CIFC
Collagen types I, III, V, VI and XII	+[a]			+
Bone sialoprotein (BSP)	+	+	+	
Osteopontin (OPN)	+	+	+	
Dentin matrix protein 1 (DMP1)	+	+	+	
Dentin sialoprotein (DSP)	+	+	+[b]	
Dentin phosphoprotein (DPP)	+	n.d.[d]	n.d.	
Osteoblast/osteocyte factor 45 (OF45 or MEPE)	+	n.d.	n.d.	
Fibronectin (FN)	+	?[c]	?	
Tenascin	+	?	?	
Osteocalcin (OC), Bone Gla protein (BGP)	+	+ (*)	+ (*)	
Bone acidic glycoprotein-75 (BAG-75)	+			+
Cementum attachment protein (CAP)	-			+
Vitronectin (VN)	+	?	?	+ (repair cementum)
Lumican	+ (but weaker than in CIFC)	+	-	
Fibromodulin	+ (but weaker than in CIFC)	+	-	
Versican	+	+	-	
Decorin	+	+	-	
Biglycan	+	+	-	
Osteoadherin (OSAD)	+			+
Amelogenin (AMEL)	n.d.	+ (intra-/perilacunar)	+ (extension of crown enamel over root)	
Amelin/ameloblastin (AMB)	n.d.	+ (intra-/perilacunar)	+ (extension of crown enamel over root)	
Albumin	+	+	+	
Alpha2HS-glycoprotein	+	+	+	
Transforming growth factor-β1 (TGF- β1)	+			+
Bone morphogenetic proteins (BMPs)	+			+
Platelet-derived growth factors (PDGF) a,b	+			+
Fibroblast growth factors (FGFs)	+			+
Insulin-like growth factors I (IGF-I) and II (IGF-II)	+			+
Cementum-derived growth factor (CGF)	-			+

Matrix composition in cement

- Elevated levels of:
  - Bone sialoprotein (BSP)
  - Osteopontin (OPN)
- Absence or reduced level of:
  - Dentine sialoprotein (DSP)
  - Osteonectin (ON)
  - Fibronectin/Tenascin
  - Proteoglycans including biglycan and decorin

- Only a partial osteoblast phenotype in AEFC?

Cementum specific proteins

- Cementum attachment protein
  - promotes cell attachment to ECM
- Enamel Matrix Proteins (EMP)
  - Define a specific interfacial matrix layer
  - Promote cementoblast differentiation
  - Trigger AEFC formation?

Origin of cementoblasts?

- HERS via a epithelial to mesenchymal transformation
- Common lineage with ligament fibroblasts
- Cell rests provide stem cell population?

- Evidence:
  - Keratin 19 seen in ligament, HERS and epithelial cell rests

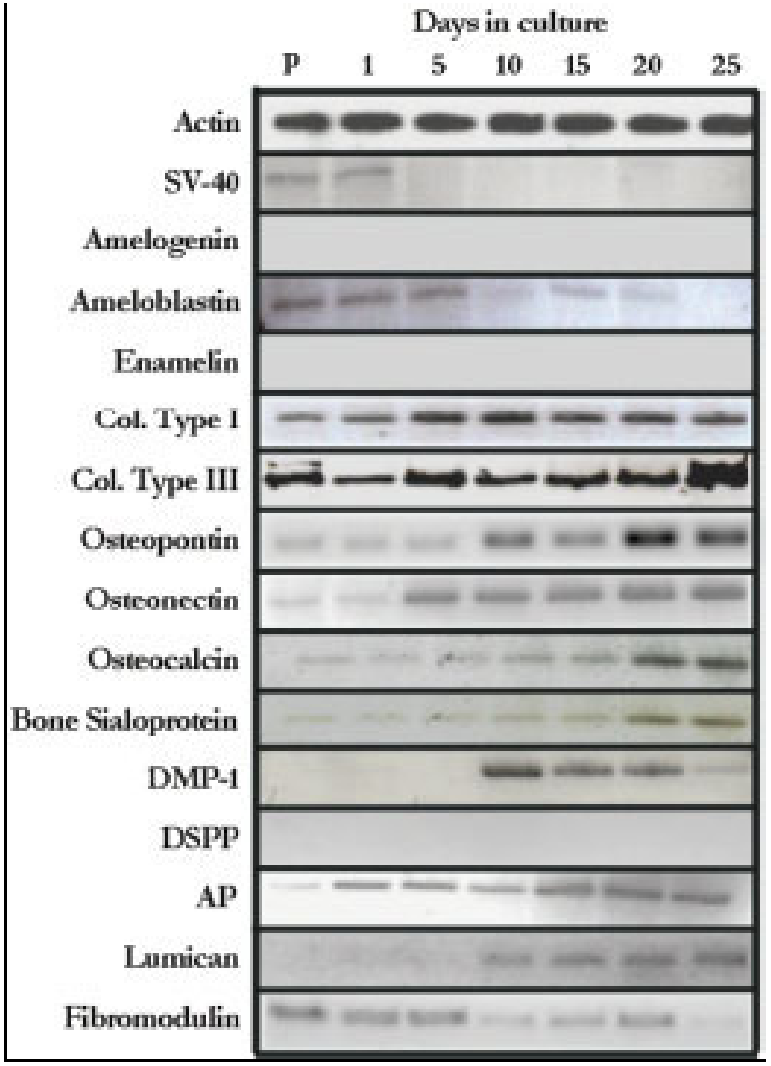
Study of HERS

- Immortomouse™

- Mouse carries a TS allele of SV40 large T
- Controlled under $H2K^b$ promoter
- Gene requires γ-interferon for expression

- Isolate cells grow in presence of IFN at 33ºC even if confluent

Characterisation of cultured HERS cells

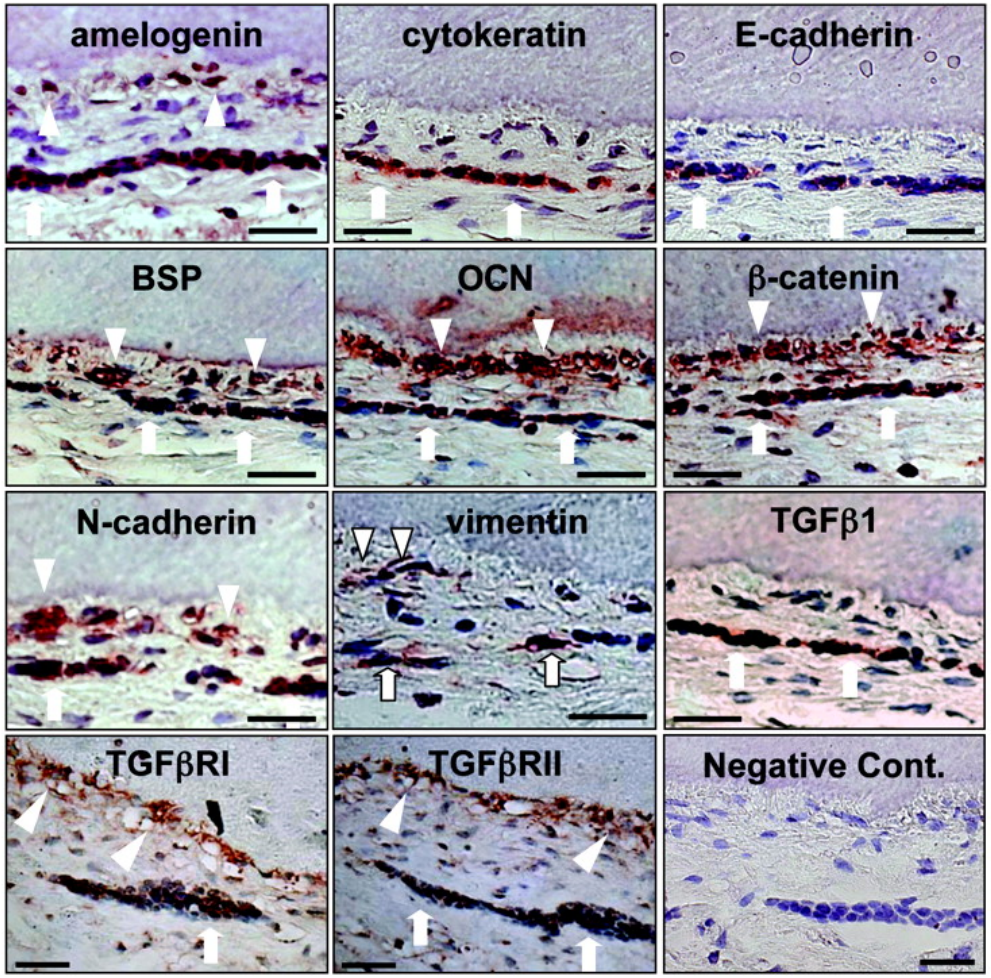


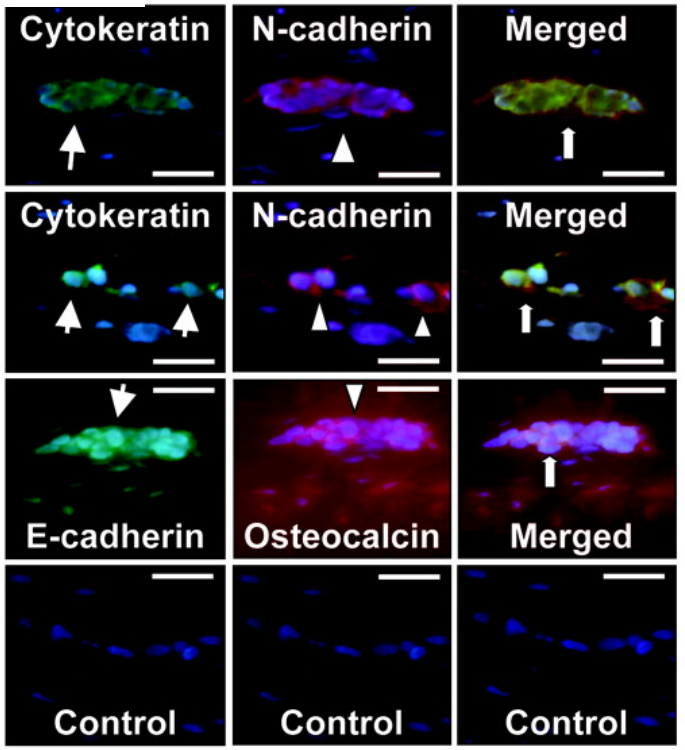
Differentiation of HERS cells

- Make bone-like nodules
- Express a range of bone markers
  - DMP-1, BSP, OSP, ON
  - Alkaline phosphatase
- Do not express DSPP hence not simply bone
- Make a matrix similar to accellular cementum

Expression of markers in the HERS cells in human

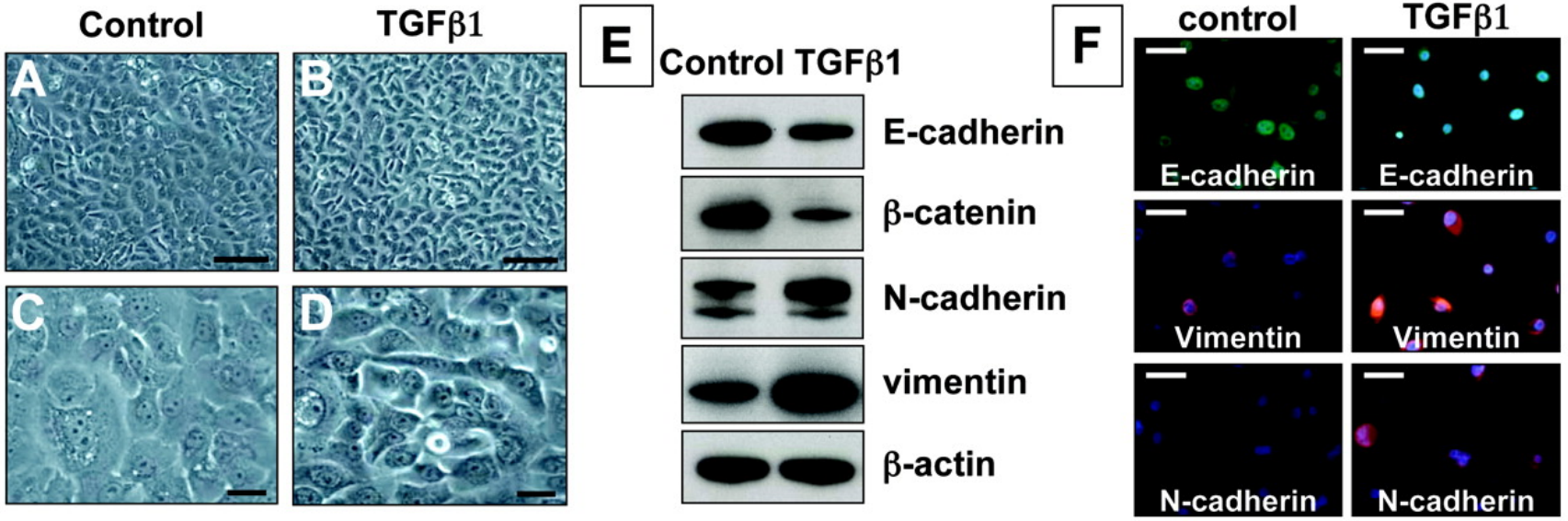
Range of cemento/osteogenic markers, cell adhesion molecules N and E cadherin and the mesenchymal marker vimentin





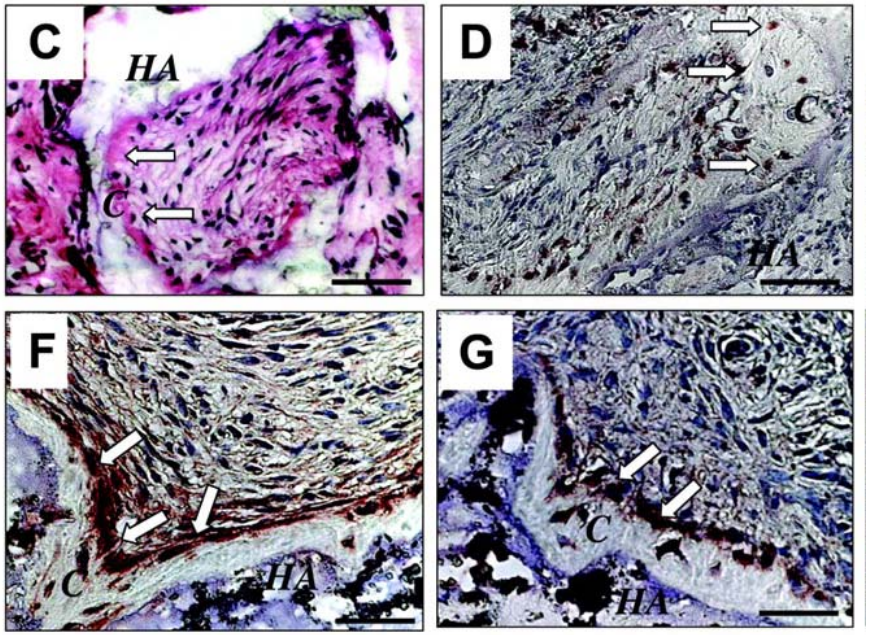
Co-localisation of different markers

Cells from impacted 3rd molars



Regulation of epithelial-mesenchymal transition by TGFβ1

- Add TGFβ and change cell morphology
- TGFβ induces mesenchymal markers and suppresses E-cadherin
- Co-localisation shows individual cells change expression of markers



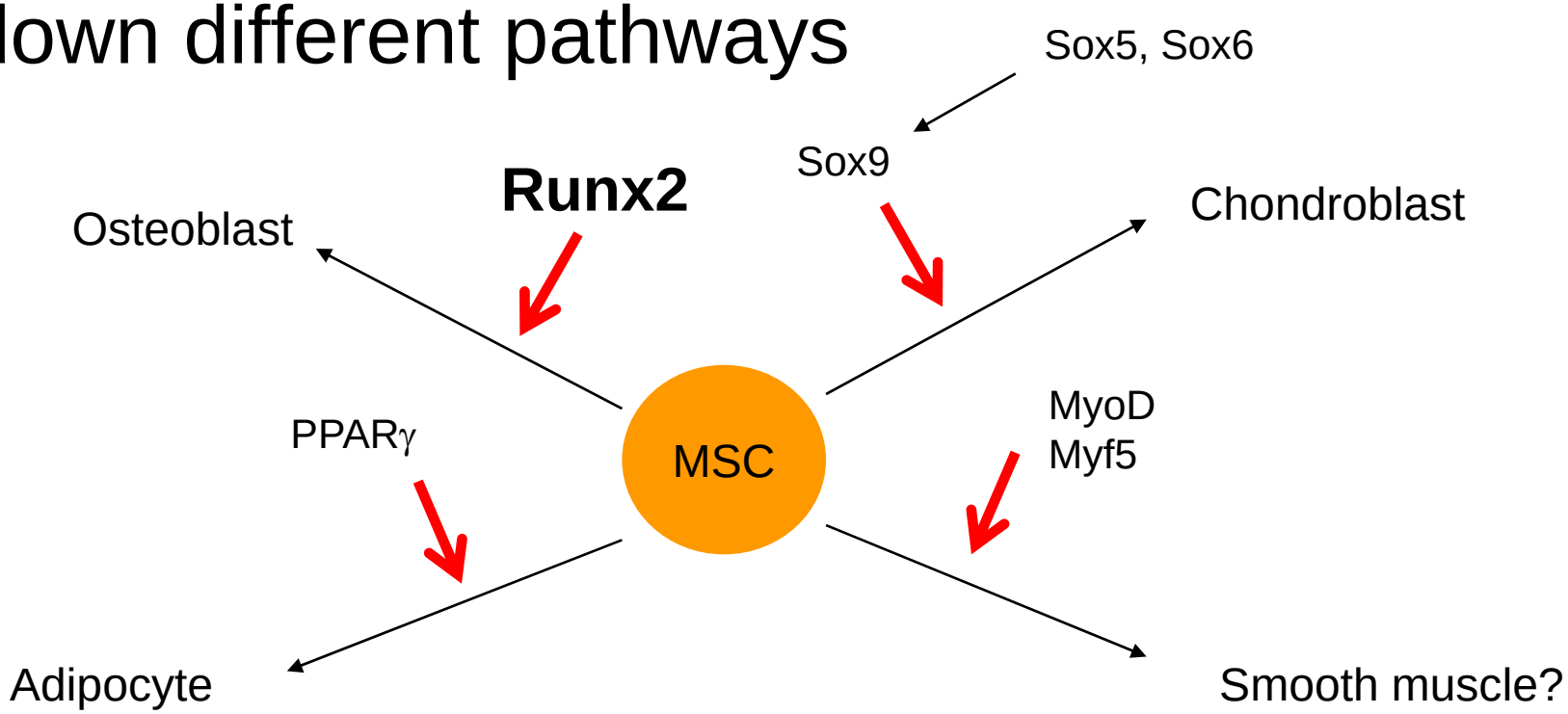
Treat cells for 48hrs with TGFβ-1, mix with a HA substrate and then implant subcutaneously into nude mice

Analyse after 8 weeks

Cementum-like tissue in C, derived from human cells D
Cementum tissue stains for BSP and ALP (panels F and G)

Bone cells and cell fate

- Cells of the skeleton become specified by master regulatory genes – direct cells down different pathways



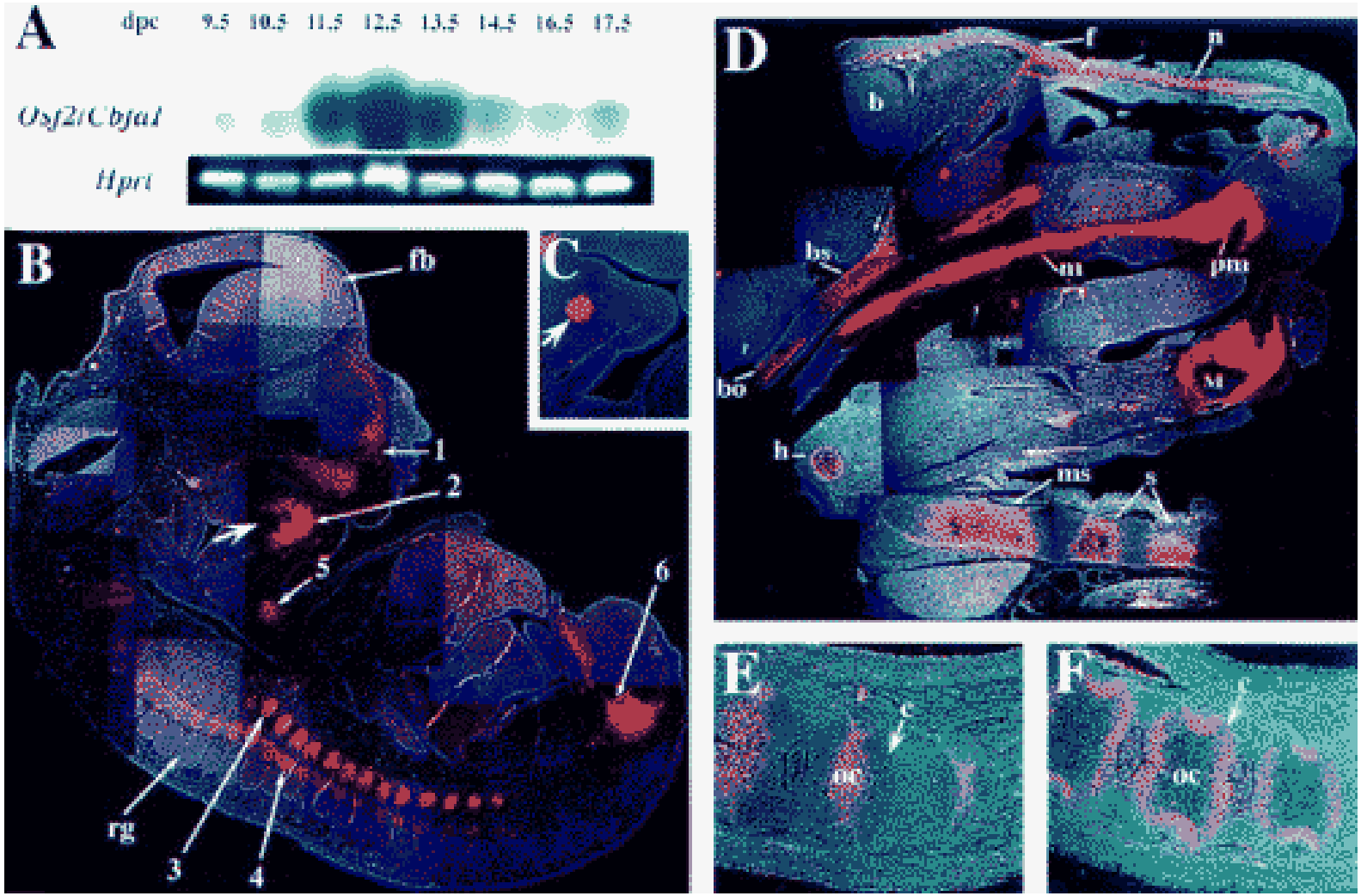
Hunting for osteoblast master regulators

- Osteocalcin – a bone specific protein
- Isolate promoter and use deletion analysis to identify putative binding sites
- This element suficient to drive expression in osteoblasts



OSE 2 binds proteins to mouse homologs of Runt, (Drosophila pair-rule)

Runx2 (cbfa1) is expressed in all developing bones



E12.5

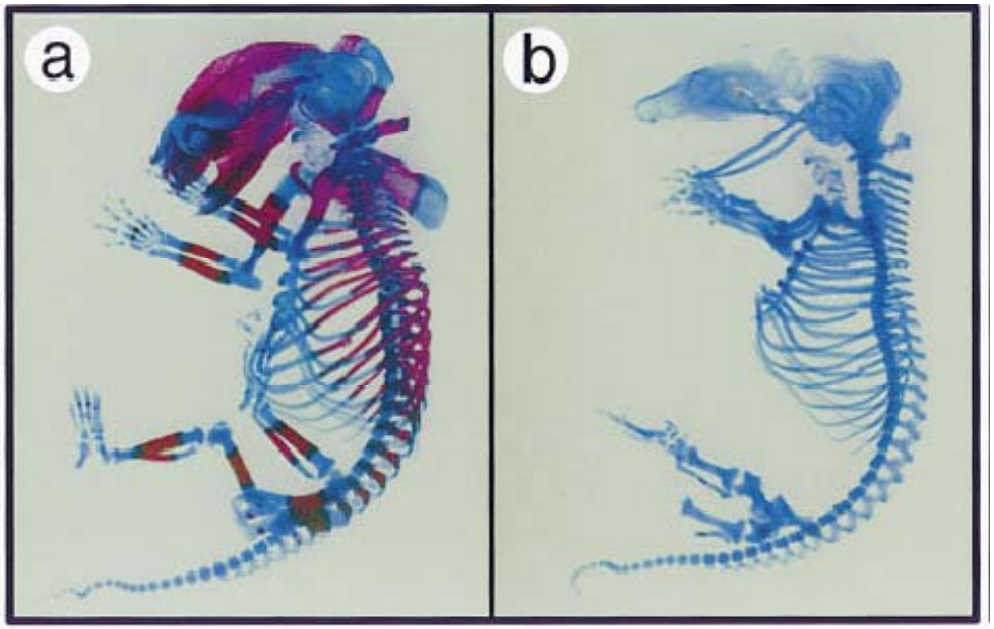
E16

C: Coll II in Meckel's cartilage

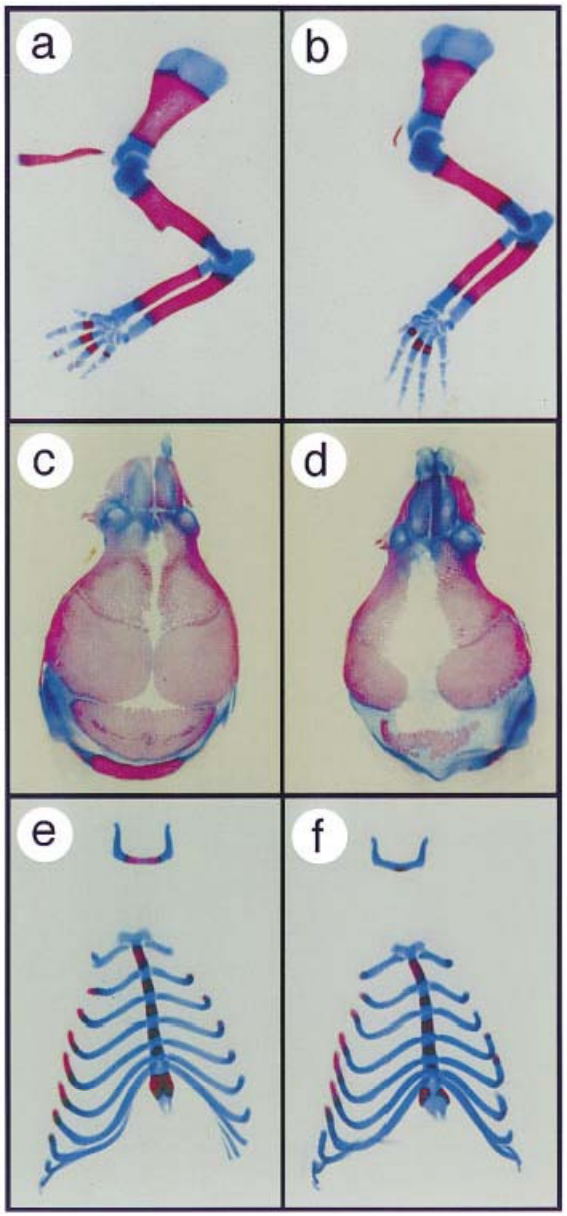
MGP

Runx2 activity

- Ectopic expression drives bone genes
- Knock-out leads to complete failure of osteoblast differentiation



Wild-type Runx +/-

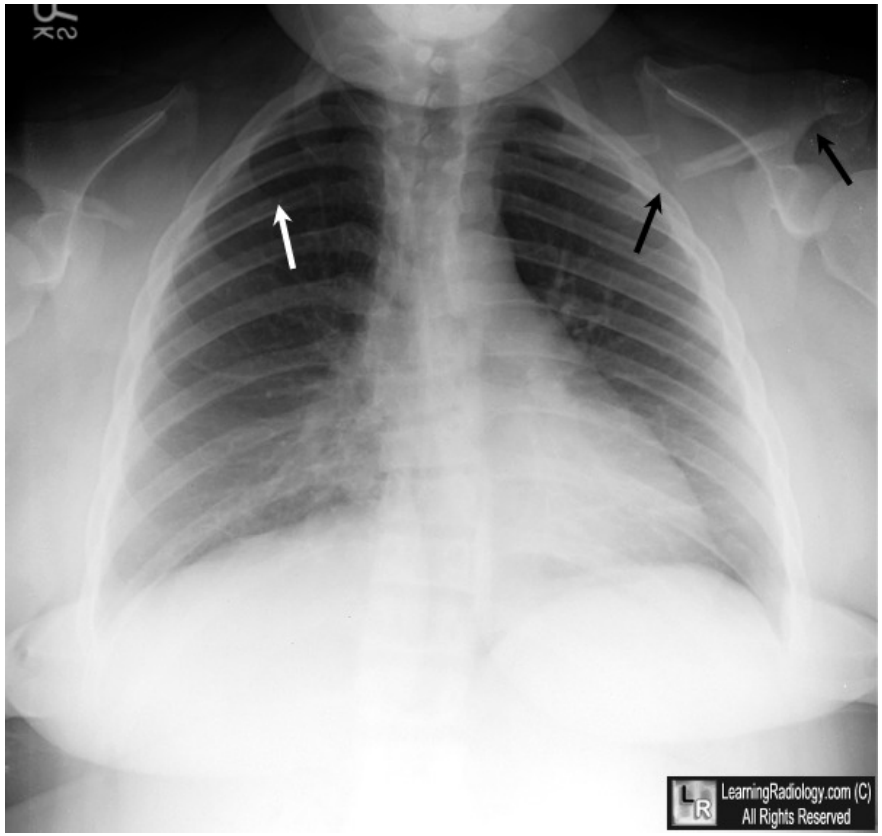


Mice heterozygous for Runx2

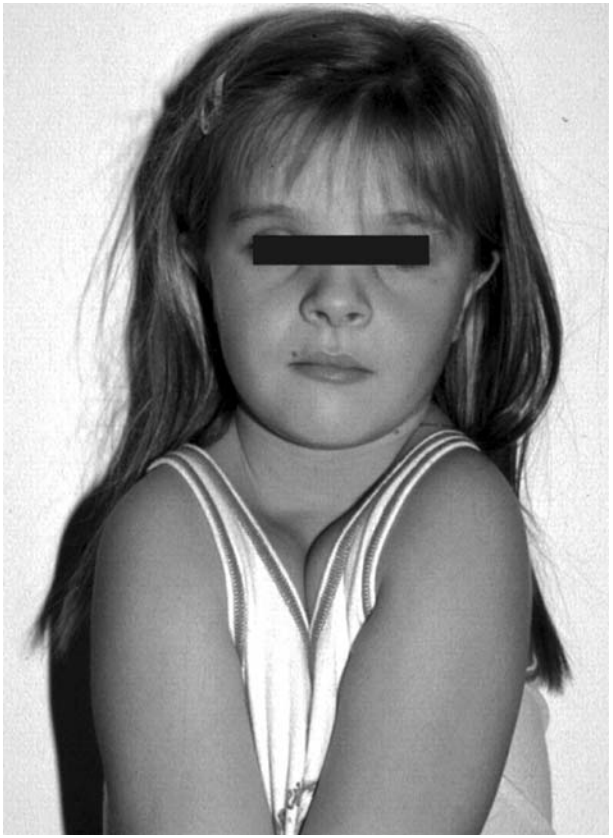
- Absence of clavicle
- Delayed skull formation
- Defects in hyoid bone and sternum
- Extra wormian bones

Human syndrome cleidocranial dysplasia

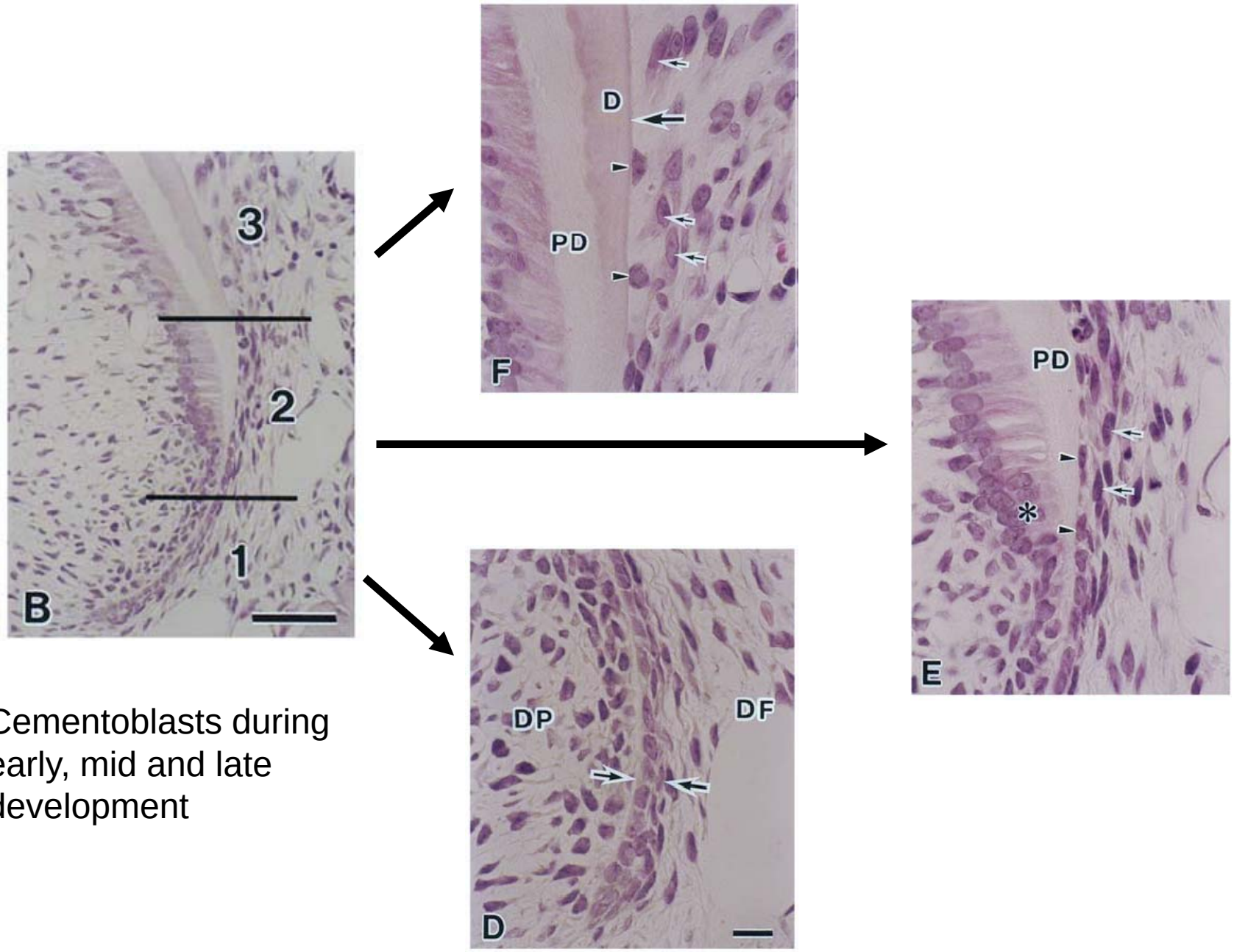
cleidocranial dysplasia (CCD)



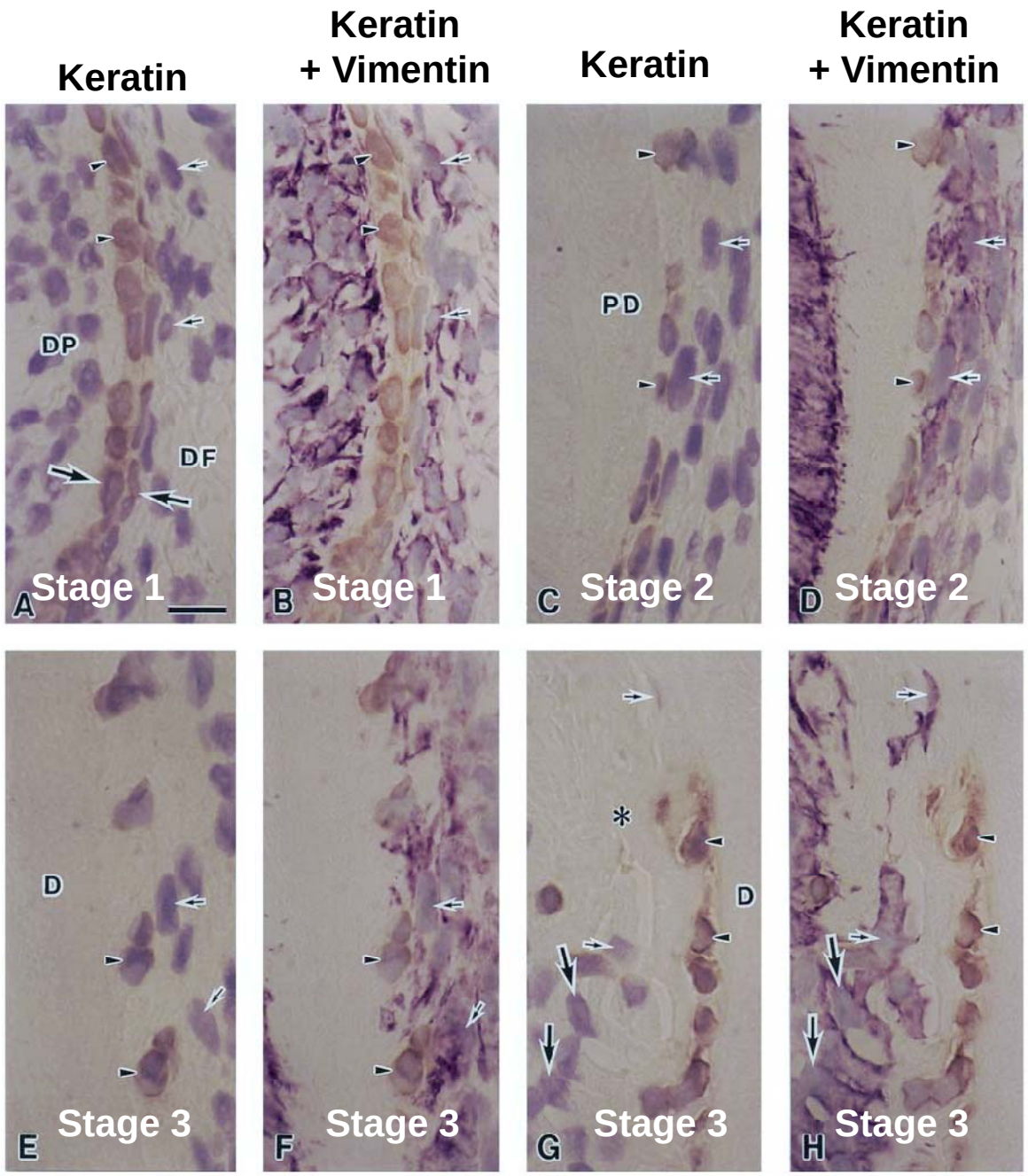
Clavicles reduced or absent (10% cases)



Hypermobile shoulders



Cementoblasts during early, mid and late development

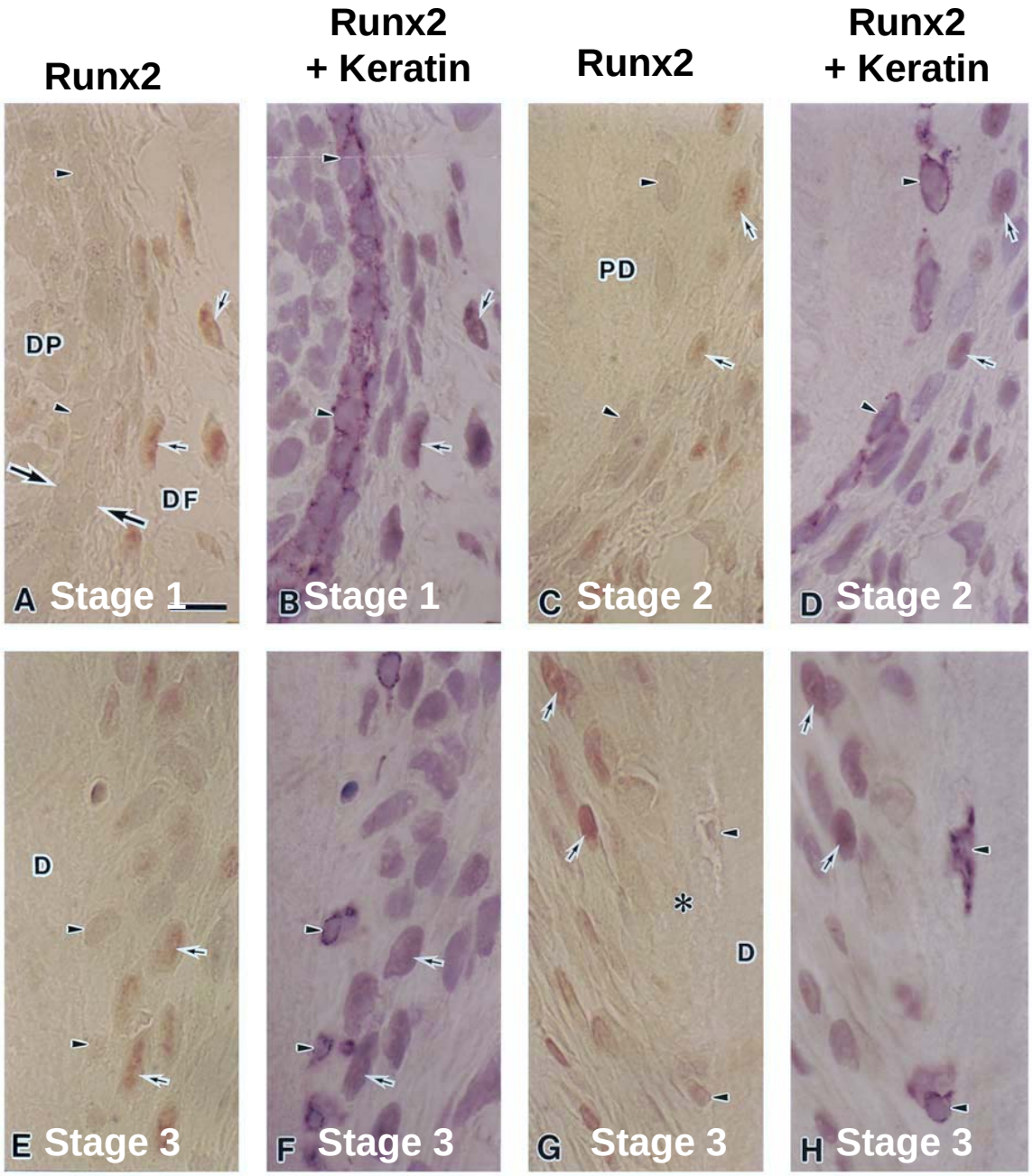


Keratin and vimentin Staining of HERS

Stages 1-3 accellular cementum formation A-F

Stages 3 cellular cementum formation G-H

No double labelled cells visible



Keratin and vimentin Staining of HERS

Stages 1-3 accellular cementum formation A-F

Stages 3 cellular cementum formation G-H

No double labelled cells visible

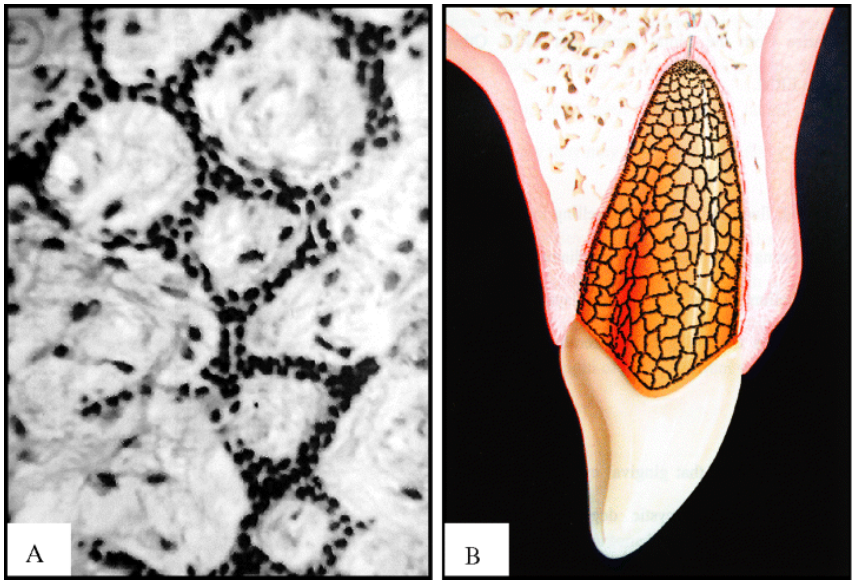
Cementoblasts appear to originate from surrounding mesenchyme

- No evidence that HERS cells undergo epithelial to mesenchymal transition

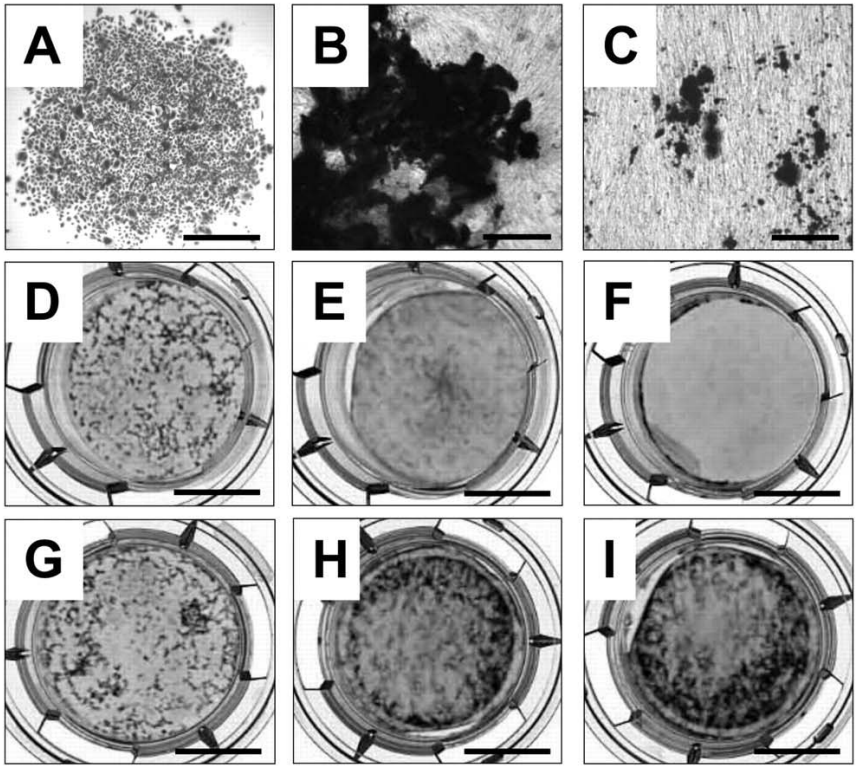
- Studies of PDL cultures are complicated by contaminating cells, osteoblasts and cell rests.

Epithelial cell rests as progenitors?

- Still open question
  - Express bone related markers including:
    - Osteopontin
  - Can differentiate into bone nodules unlike ligament cells
  - Proliferate during orthodontic tooth movement
- BUT many studies suggest a vestigial role



Co-culture of HERS with PDL cells leads to osteoblastic differentiation



Compare E with F

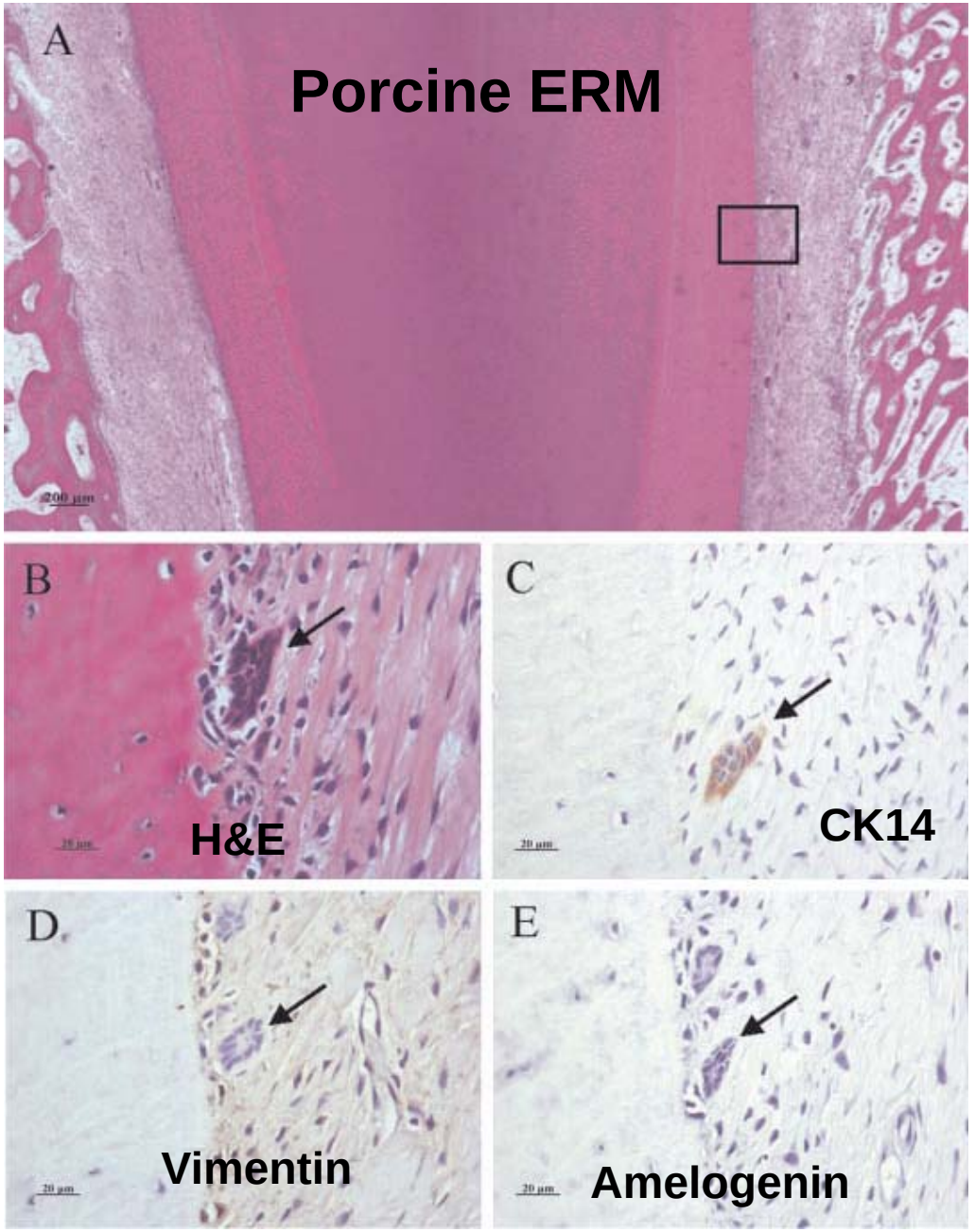
PDL cells with/without HERS cells

Staining shows ERM is:

Keratin positive

Vimentin negative

Amelogenin negative



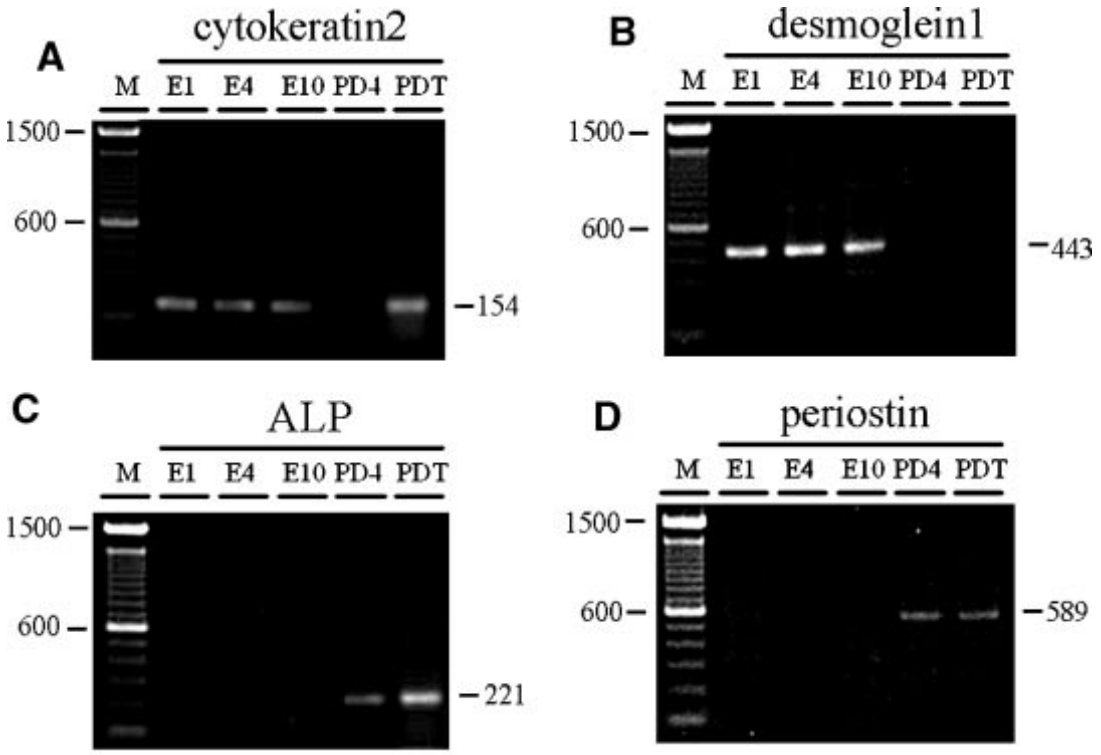
Epithelial Cell Rests

- Same lineage as enamel organ
- Use of feeder layer allows enamel markers to be expressed – some reports that amelogenin is expressed by ERM cells
- Combination with dental pulp cells allows overt differentiation of organised enamel

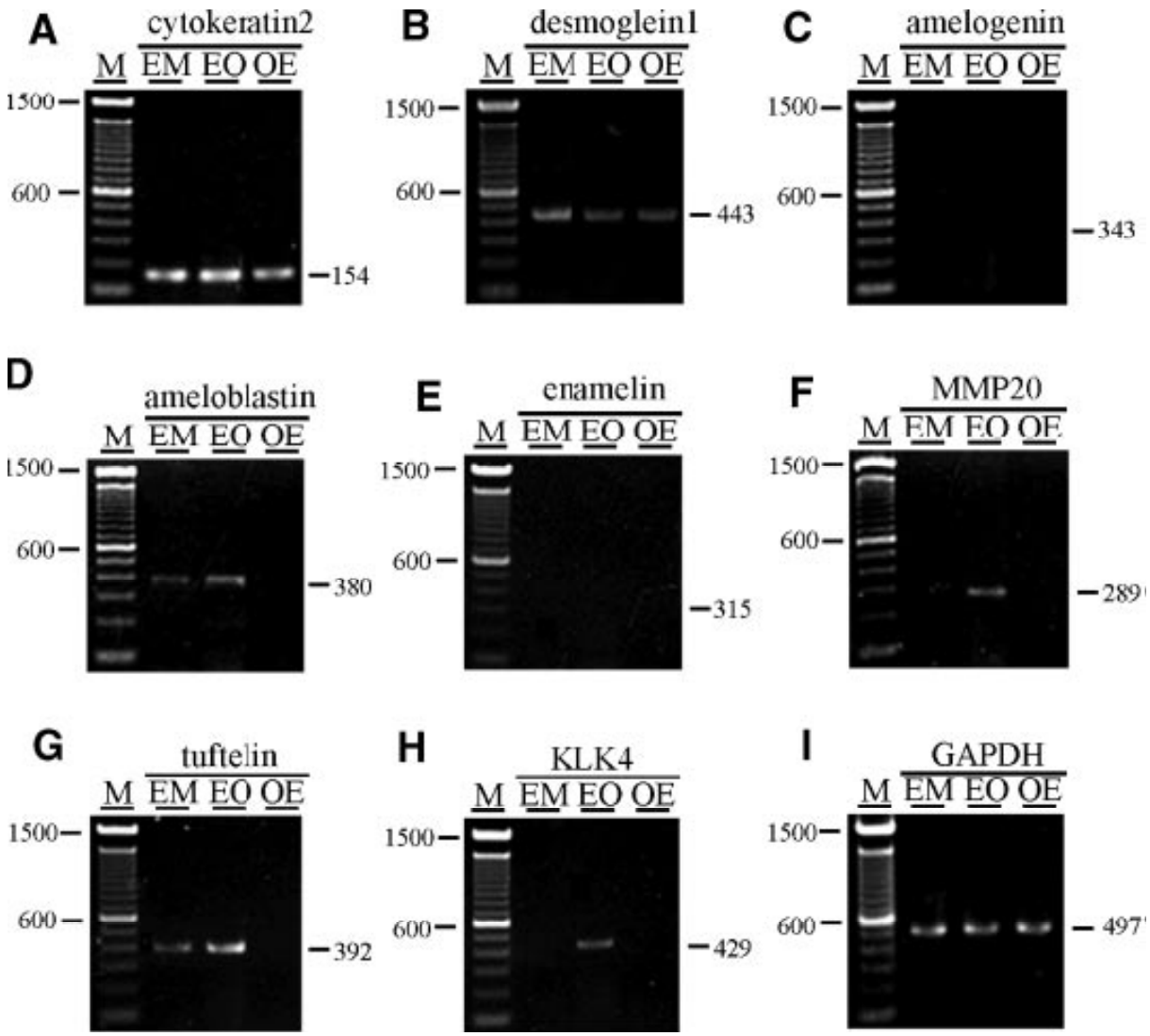
Characterisation of ERM cultures

ERM passage 1-10 express epithelial markers (E1, 4, and 10)

PDL cultures express osteogenic markers (PD4, PDT)



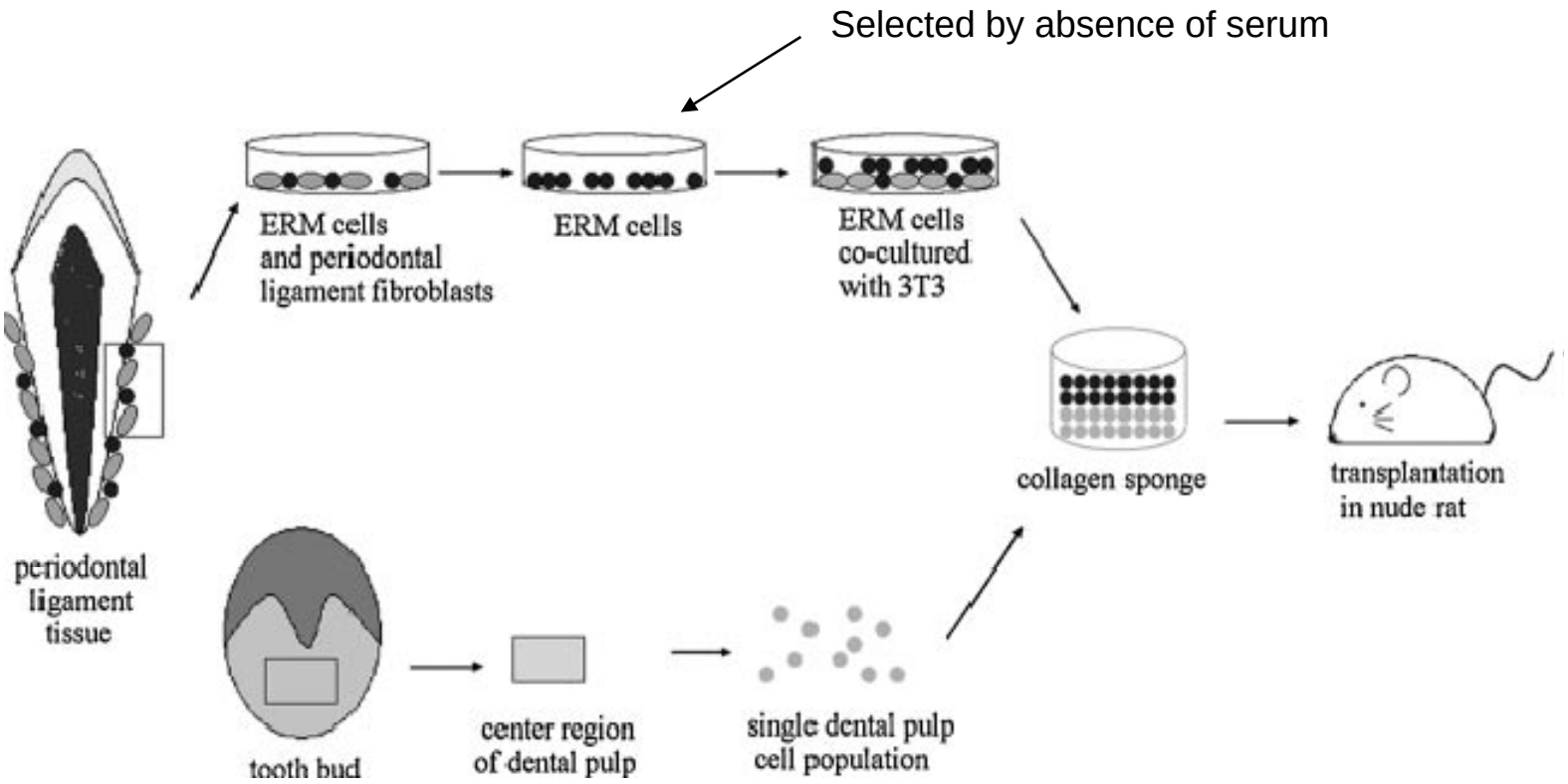
ERM cells are like enamel organ cells



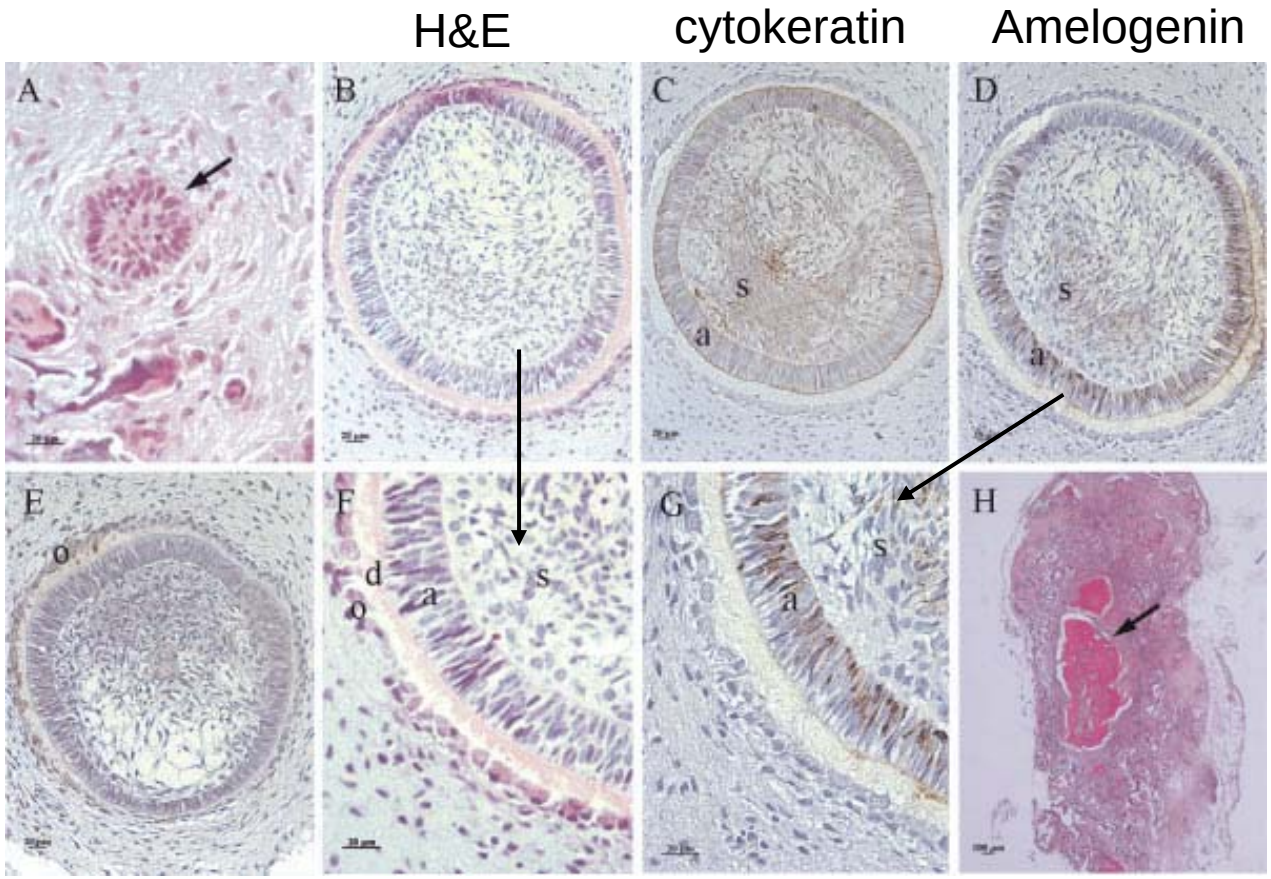
EM = ERM
EO = enamel organ
OE = oral epithelium

Isolate Epithelial Rests of Malassez (ERM) and test capacity to differentiate

Co-culture with fibroblasts and dental pulp cells



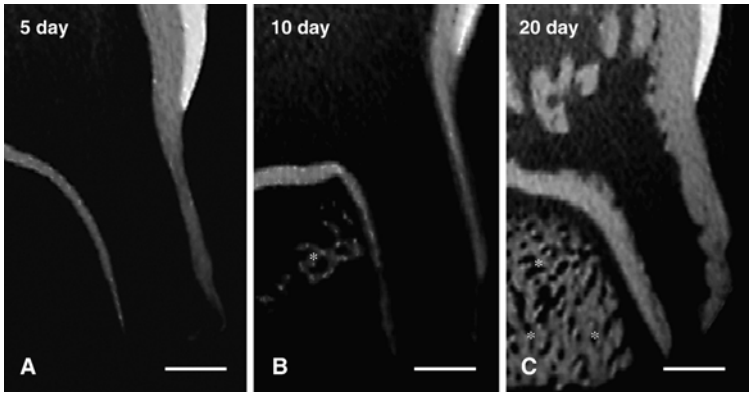
Differentiation capacity of ERM when implanted *in vivo*

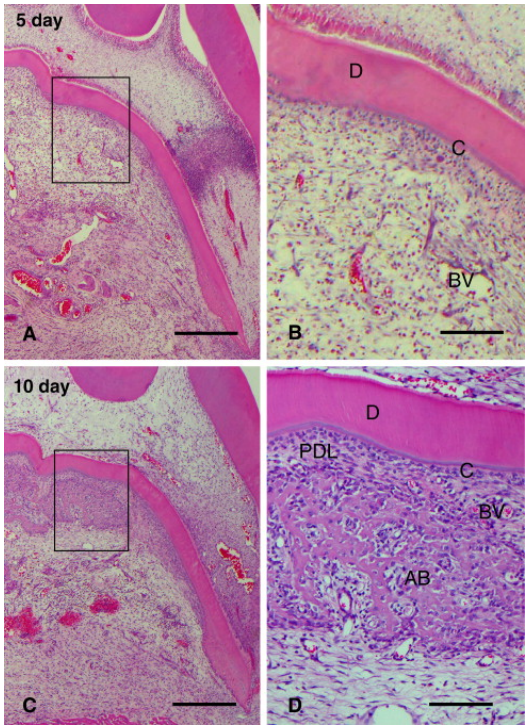


After 4 weeks (except A – 2 weeks)

Alveolar bone – originates from PDL?

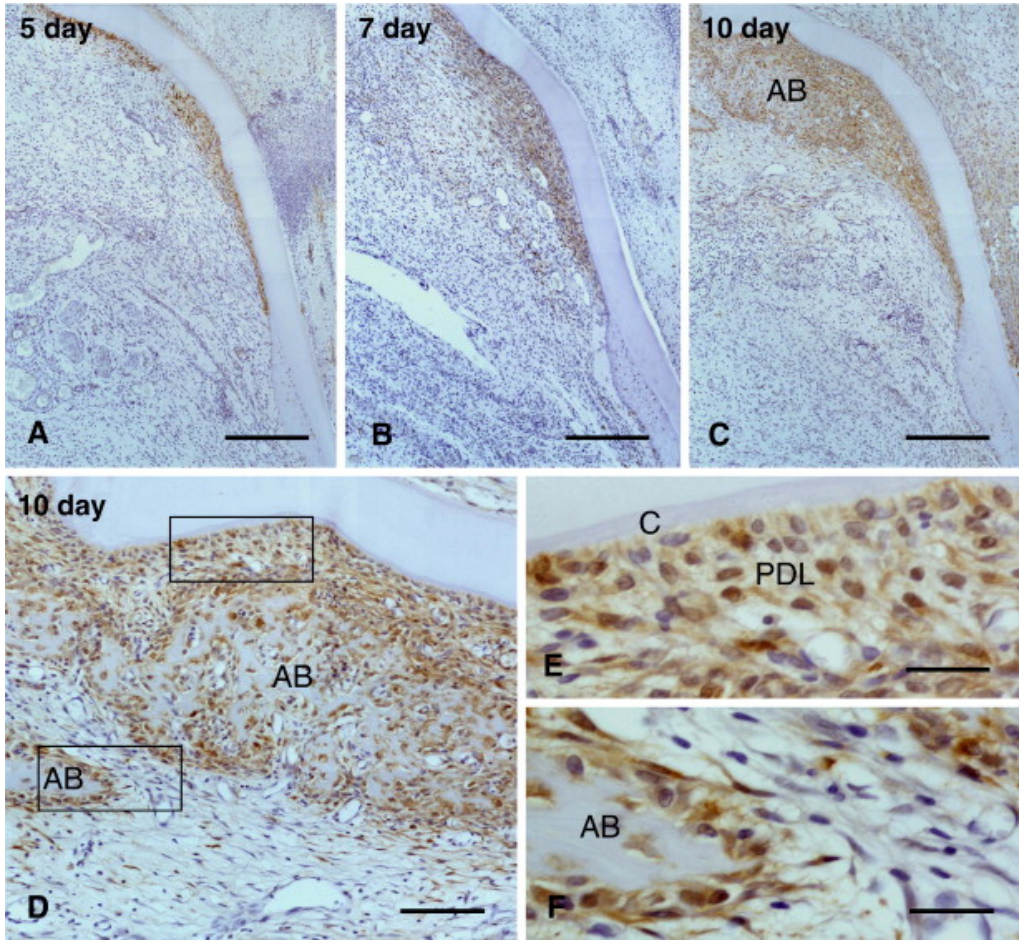
- Transplant labelled molars into subcutaneous sites
- Follow bone regeneration of transplanted tissue
- Expression of markers/cell proliferation suggests that basal layers contain multi-potential cells
- Cannot be absolutely sure this is not the result of contaminating osteoblasts…..





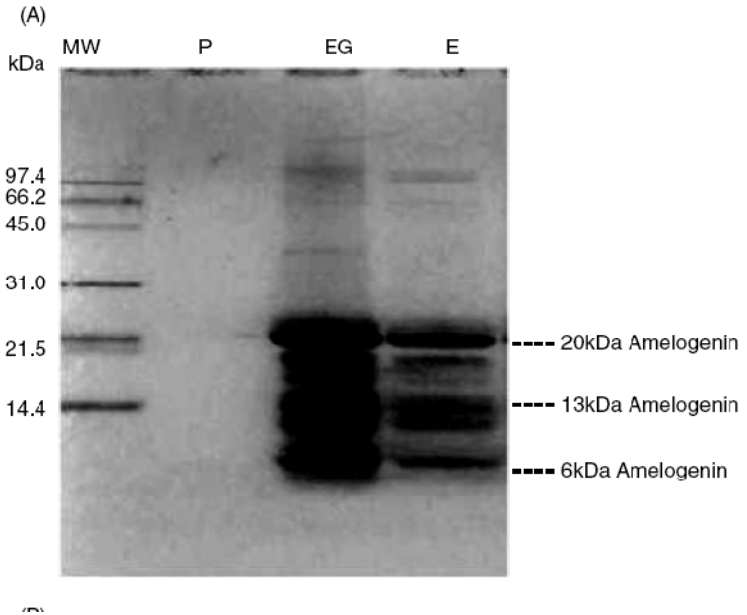
Trace origins of cells with Green Fluorescent Protein (GFP)

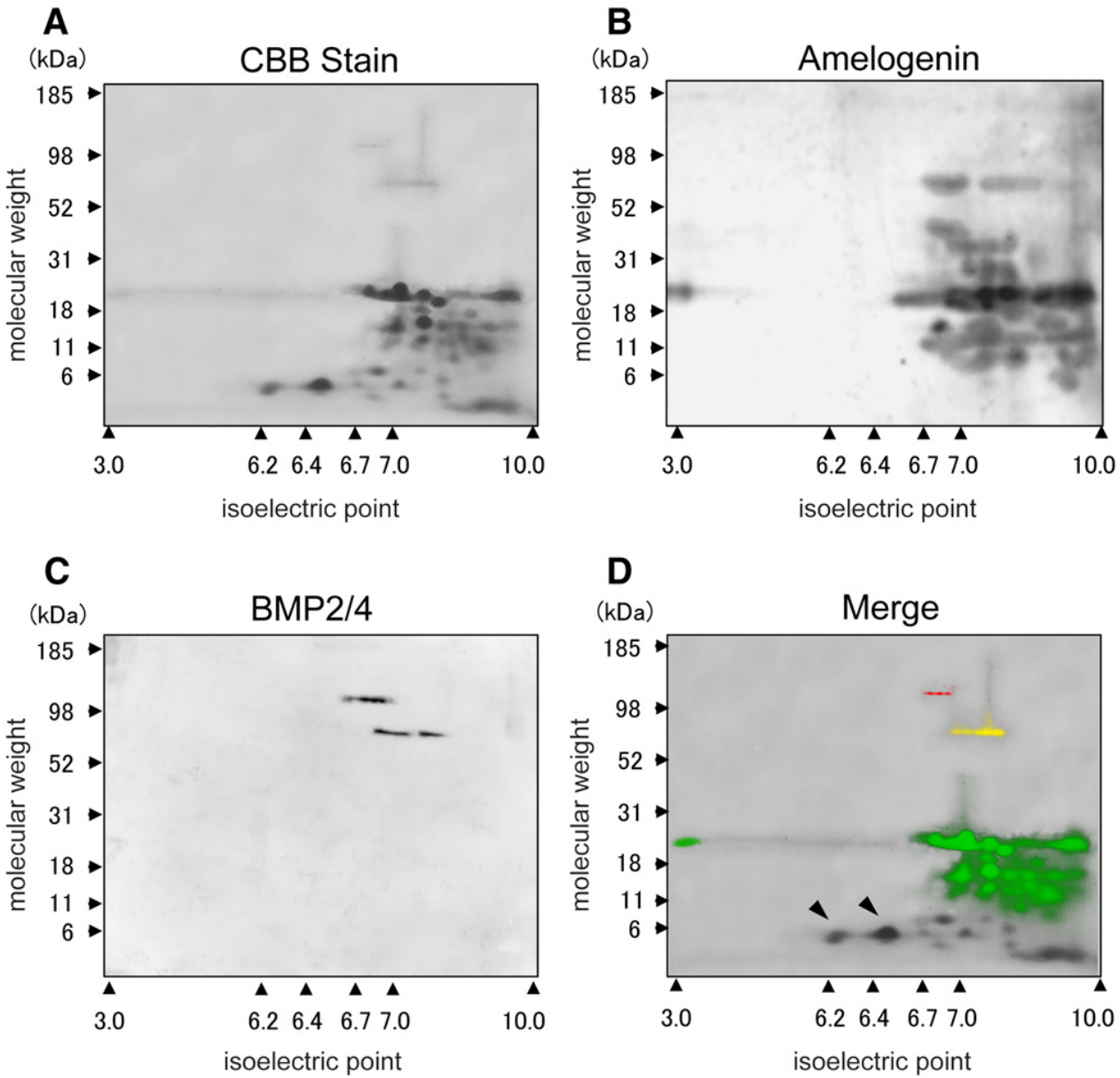
Immunolocalisation of GFP suggests that bone is derived from transplanted tissue



Emdogain

- Based on the observation of specific gene expression in HERS cells
- Used as a mixture of amelogenins, BSP and growth factors (TGFβ-like?)
- Many reports of responses but clearly these include cellular (bone-like) cementum





2D analysis of emdogain

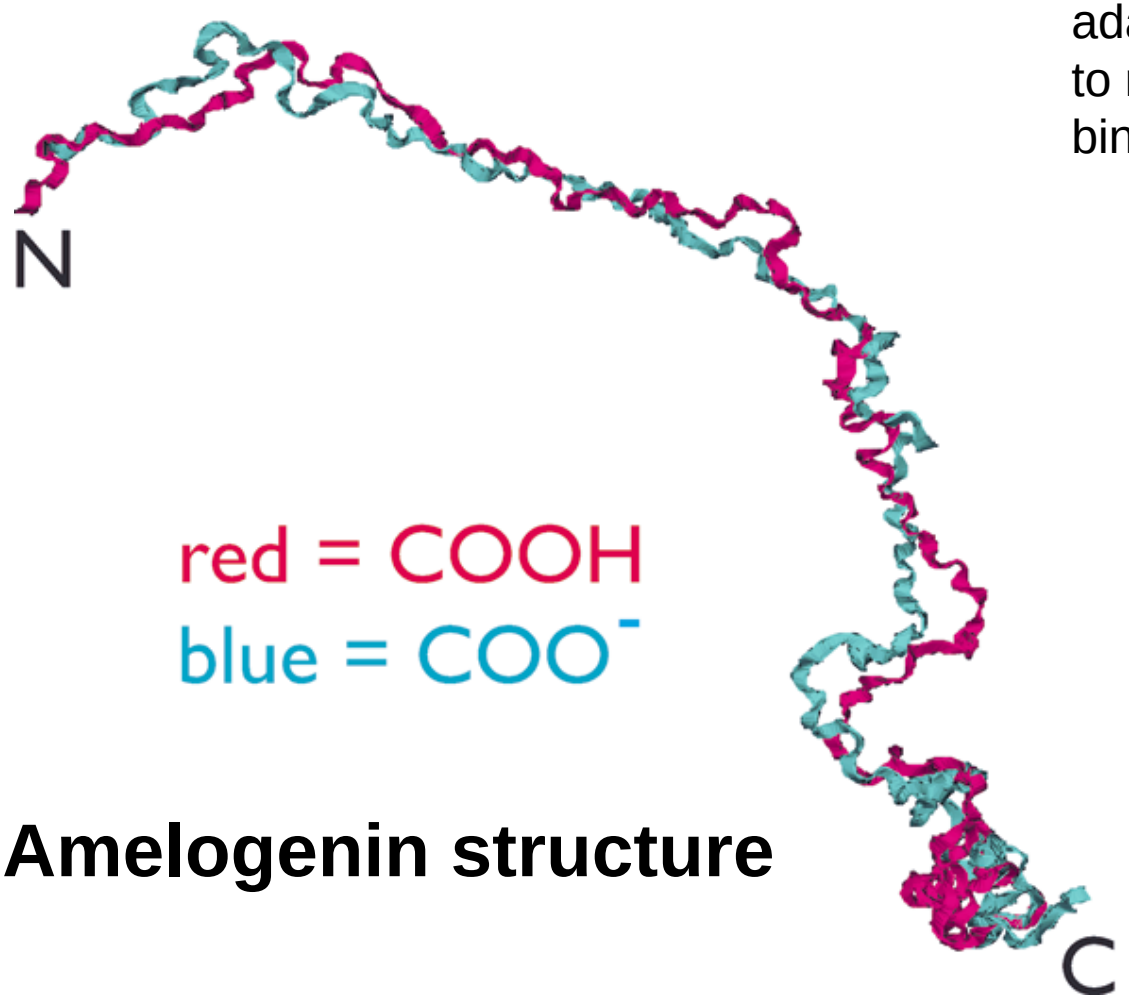
Contains BMP

90% amelogenin

No TGFβ

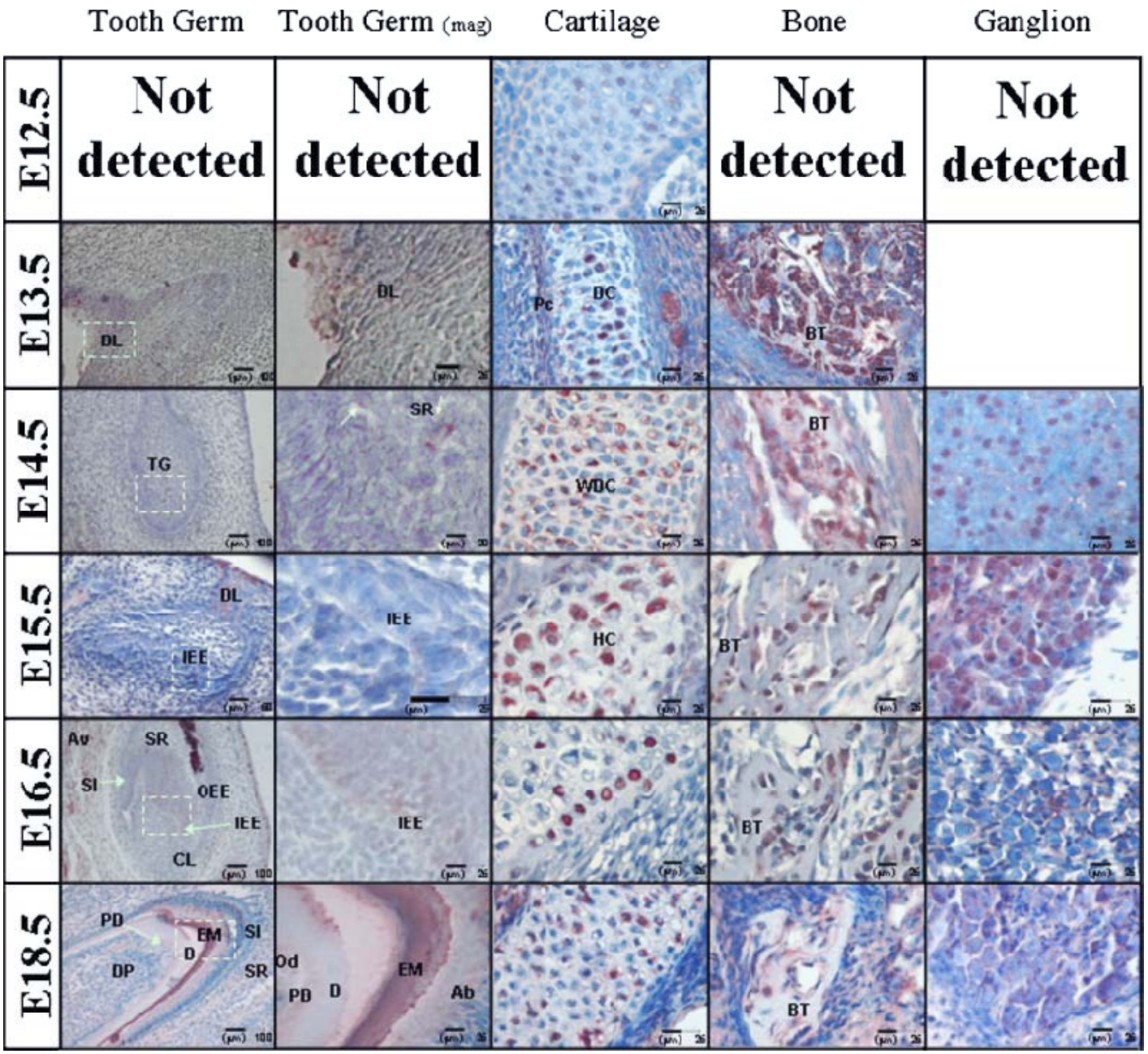
Amelogenin

- Key to biomineralization
- Essential for enamel development
- Mutations lead to defective crystal formation
- Interacts with many other matrix proteins
- Represent one of a class of proteins that are intrinsically disorganised



Amelogenin structure

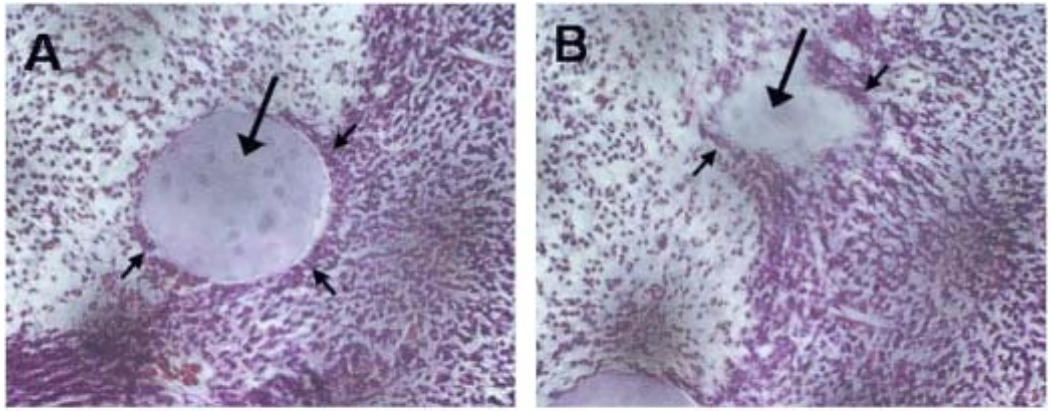
Protein shows extreme structural adaptability allowing conformation to match a diverse range of binding targets

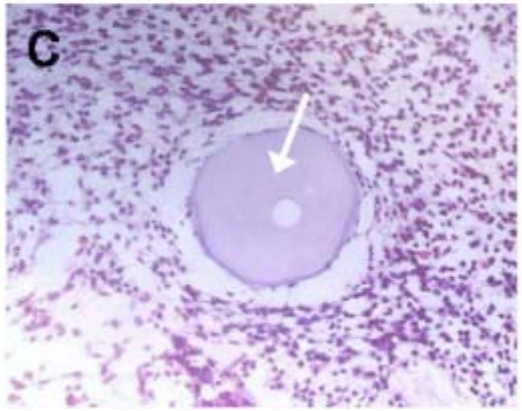


Amelogenin

Not tooth specific!

Amelogenin not just mineralization





Control

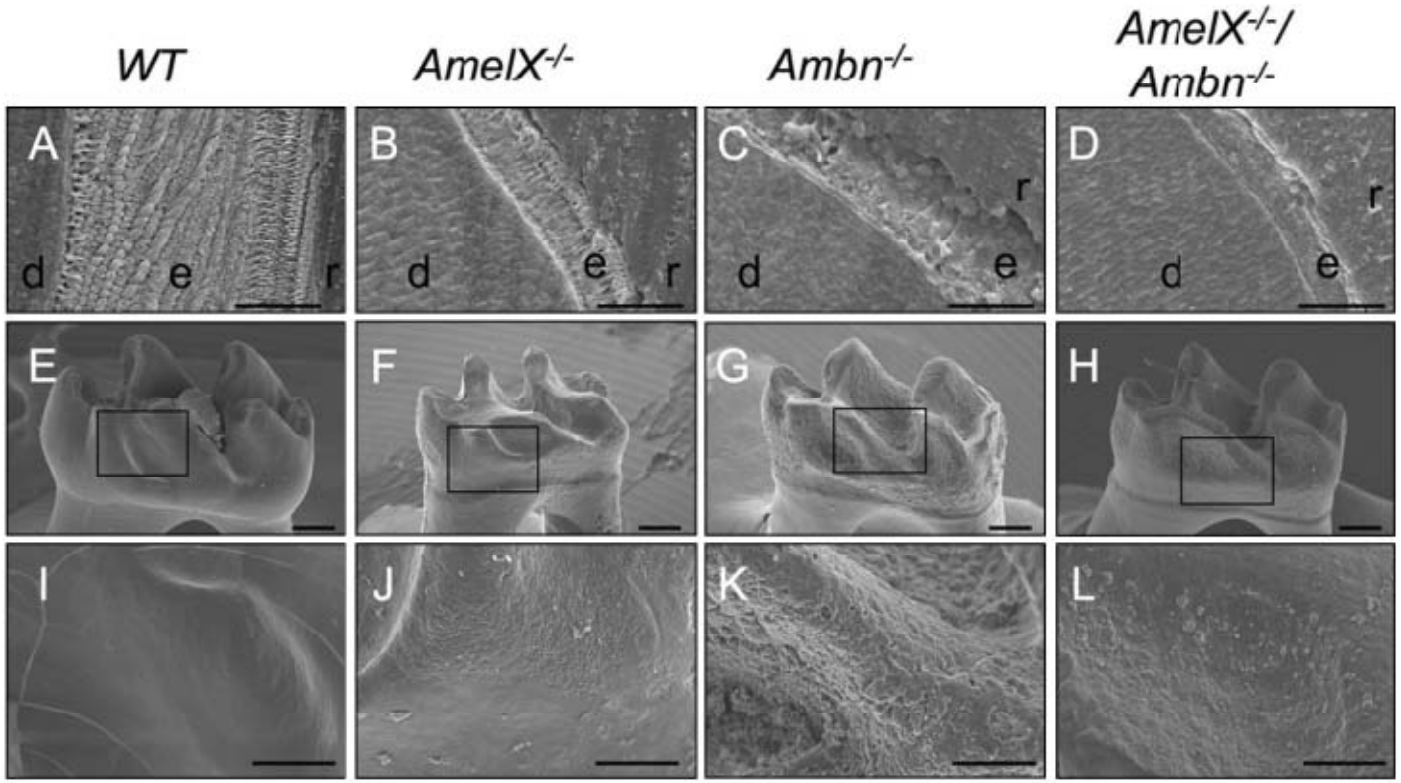
Amelogenin coated beads results in attraction of mesenchymal cells in developing mouse mandible

Effect after 24 hours

Absence of amelogenin

- 2001 mouse KO – enamel defect
- 2003 progressive cementum destruction and excessive osteoclast activity
- 2006 general loss of weight
- 2007 Role in bone formation and remodelling

Phenotype of amelogenin mutant

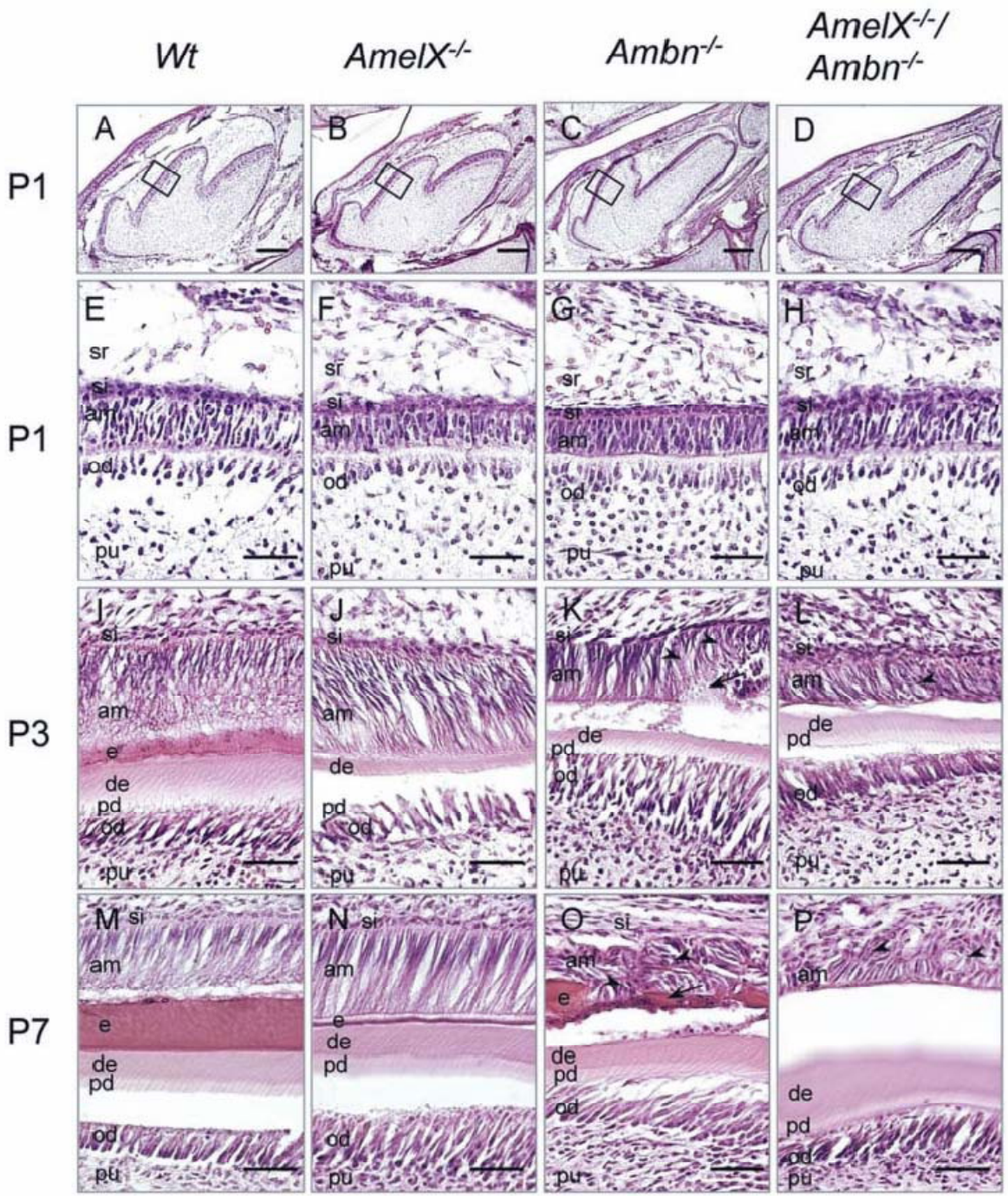


Incisor

Molar

enamel surface

All mutants present as amelogenesis imperfecta



Analysis of KO mice for:

Amelogenin
Ameloblastin
Double mutants

Single mutants make some enamel but not in double mutant

Which are the active components in emdogain?

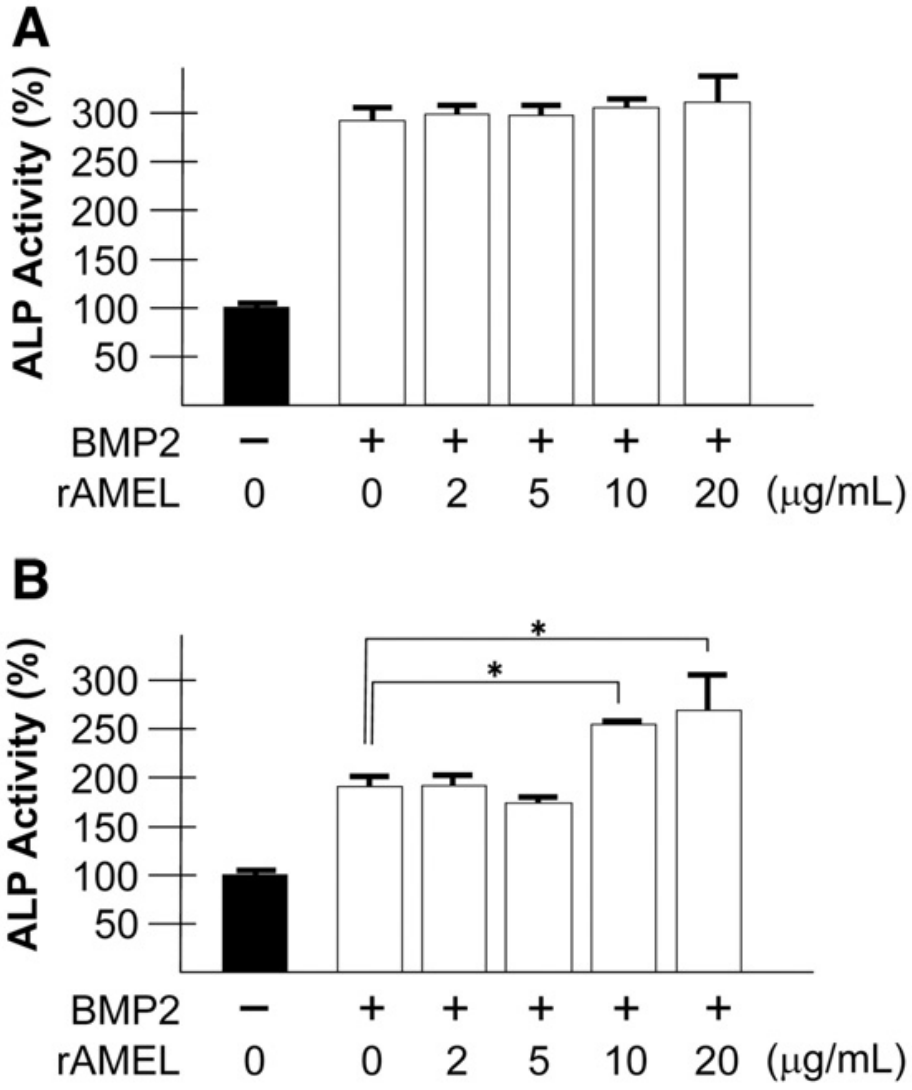
- Known to promote cementogeneis and ostegenesis
- 90% Amelogenin but also BMPs (c7%)
- Unable to stimulate Smad (BMP-signalling) in cultured cells.

Activities of amelogenin

- Binds to BMP – hence contamination of emdogain?

- Binds heparan sulphate an ECM component that also binds to BMP

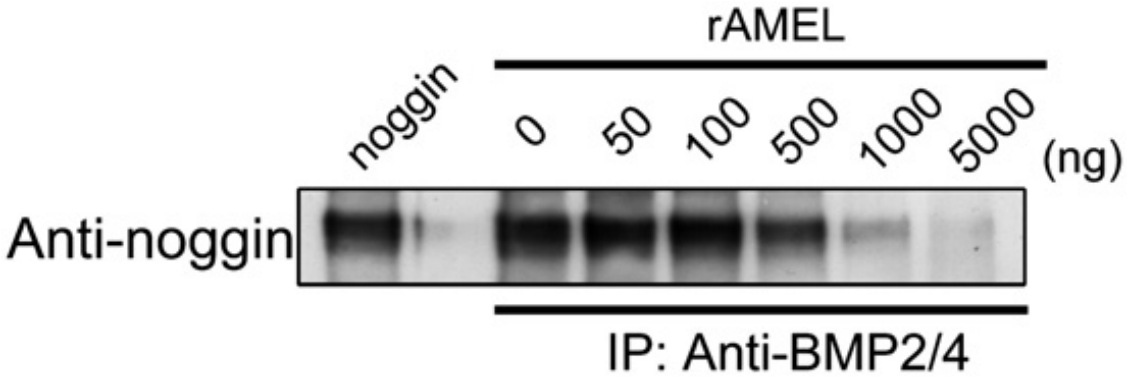
Antagonistic effect of noggin on BMP can be blocked by amelogenin



Without noggin

With noggin

Amelogenin blocks BMP binding to noggin



Emdogain what does it tell us?

- Amelogenin the active component
- Epithelial cell rests can express amelogenin under the correct circumstances
- Are epithelial cell rests and their stimulation key to successful regeneration?