

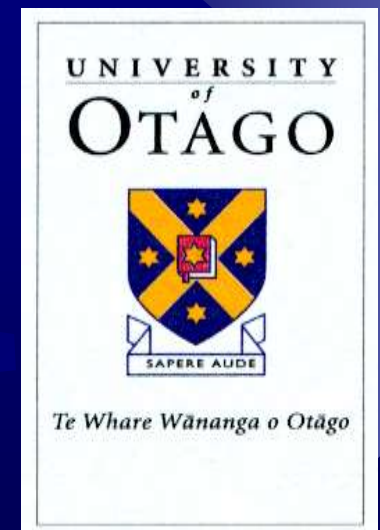
Development of the Dentition

By Murray C Meikle

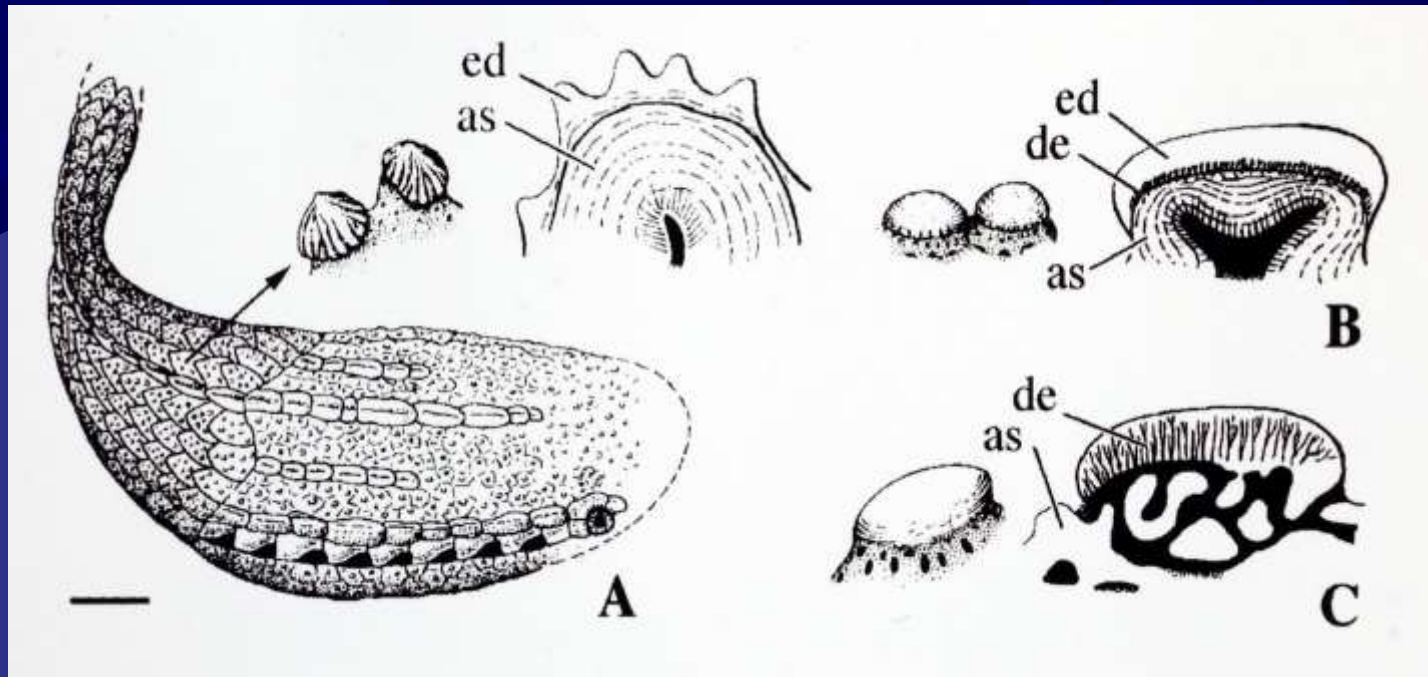
Biological Foundations of Orthodontics
and Dentofacial Orthopaedics

Seminar 7

2004

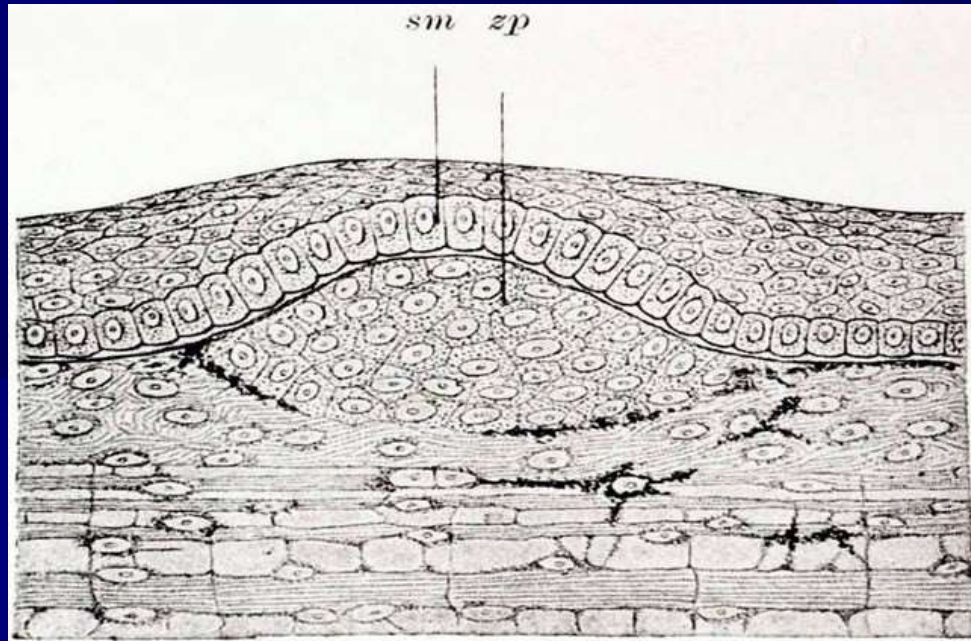


Odontodes: ancestors of teeth



- The fossil record suggests that the earliest attempts at biomineralization occurred more than 500 million years ago (Myr) in the form of a dermal exoskeleton. The surface ornamentation of early Ordovician vertebrates (circa 450 Myr ago) consisted of rows of small tubercles or odontodes composed of aspidin(as, a type of acellular bone) covered by enameloid (ed) and/or dentine (de)
- From Huysseune and Sire (1998) *European Journal of Oral Sciences* **106**, 437–481.

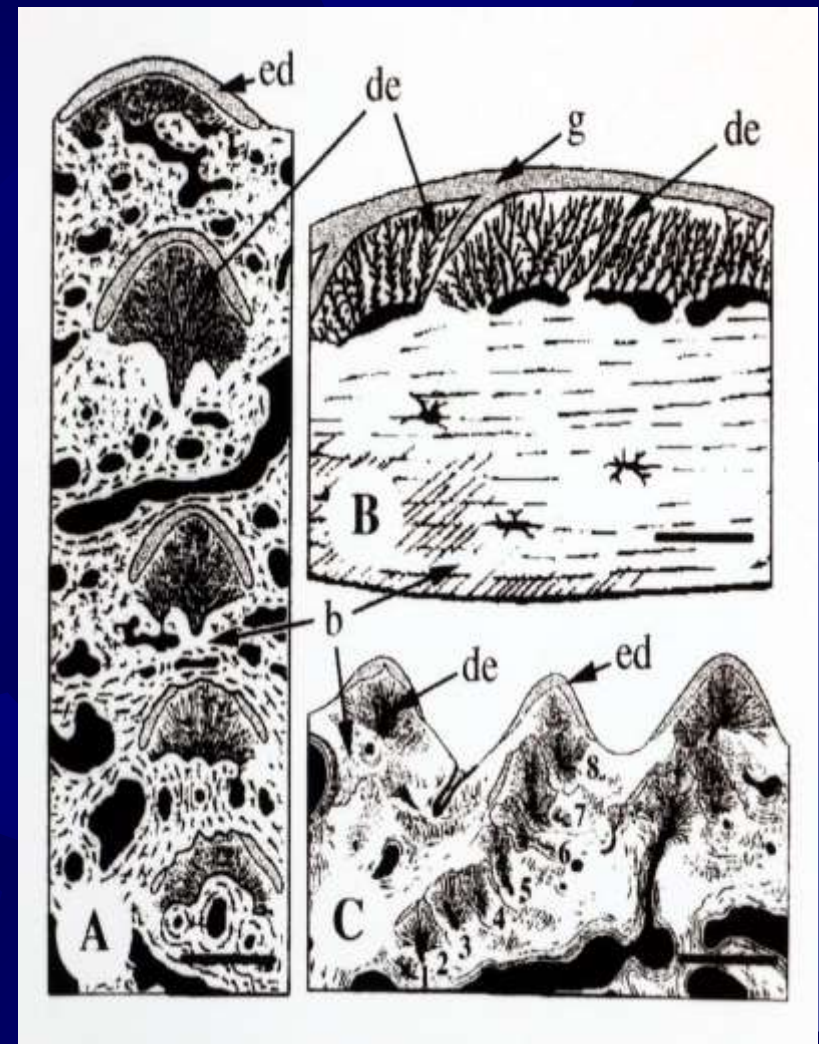
Development of odontodes



- During gnathostome evolution 450 Myr ago, odontodes in the oral cavity gave rise to teeth. They developed from reciprocal interactions between epithelium and mesenchyme, but differ in a number of respects from teeth.
- The dental organ did not invaginate, but consisted of a single layer of epithelial cells *sm* (Schmelzmembrane); the dental papilla *zp* (Zahnpapilla) arose in the superficial part of the adjacent mesenchyme.
- From Hertwig (1902), *Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbelthiere*.

Odontocomplexes

- Odontodes always formed in a superficial position and did not erupt, being covered by epidermis and possibly by the dermis as well.
- They were more likely to become submerged by the formation of other hard tissues on top (by bone and/or new odontodes) to form odontocomplexes.
- In section C eight generations of odontodes have accumulated in the dermal skeleton of the Jurassic actinopterygian *Scanilepsis*. G, ganoine; de, dentine; ed, enameloid.



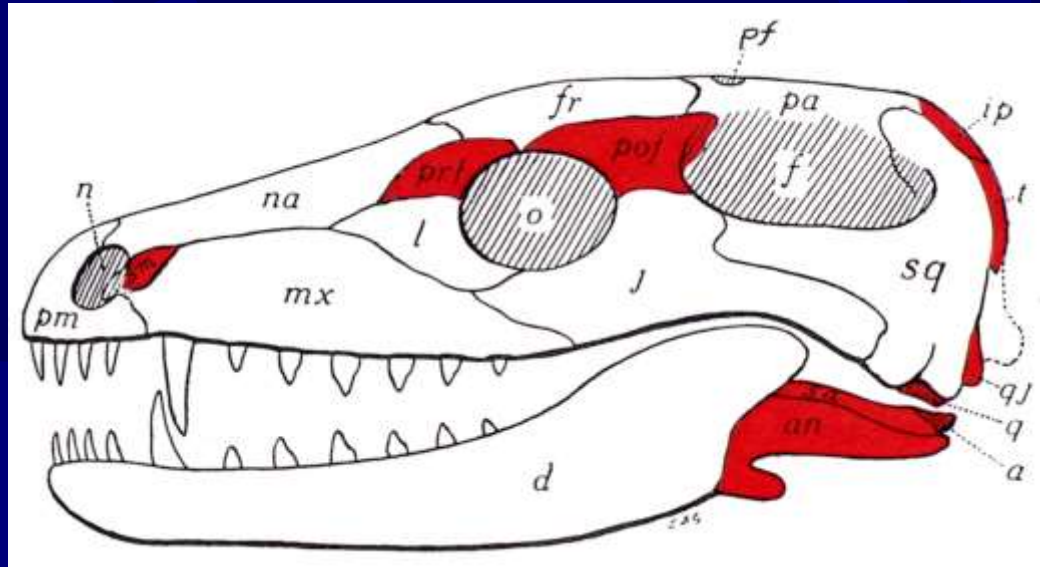
From Huysseune and Sire (1998). *European Journal of Oral Sciences* 106, 437–481.

Conodonts



- Recent research suggests that mineralization began not in the skin of fish, but in the mouths of conodonts that lived 220–510 Myr; a group of small eel-shaped chordates (approximately 15–20 mm in length) with a complex feeding apparatus composed of tooth-like structures..
- It has been proposed that the mineralized vertebrate skeleton first appeared as odontodes of dentine or dentine plus enamel in the conodont feeding apparatus.
- Bone appeared later, co-ordinated with the development of a dermal exoskeleton and appears to have been primitively acellular.
- From Donoghue *et al.* (2000). *Biological Reviews of the Cambridge Philosophical Society*.

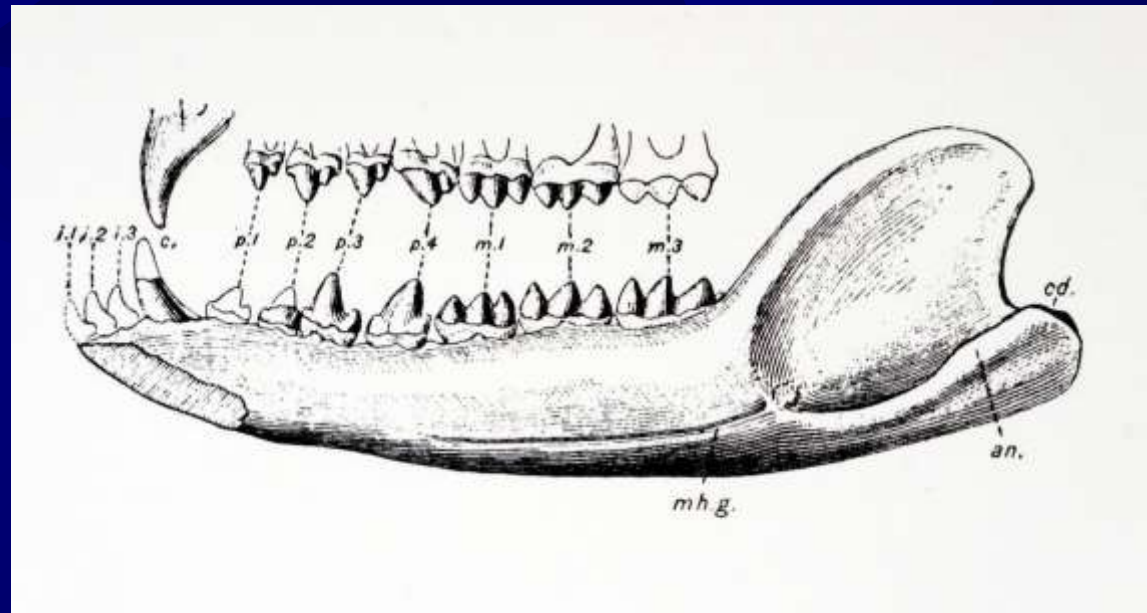
The reptilian dentition



- ✱ In the majority of fish and reptiles the teeth are homodont – a similar shape; they are also polyphyodont – being continually replaced throughout life.
- ✱ The ancestral reptilian tooth was a simple conical shape; the diversity of shape seen in modern mammals arose from the addition of lateral denticles or cusps to the original reptilian cone. Transitional stages are seen in the mammal-like reptiles; the therapsids (248–213 Myr) have a canine-like tooth separating the incisors from the cheek teeth.
- ✱ From Goodrich (1930), *Studies on the Structure and Development of Vertebrates*.

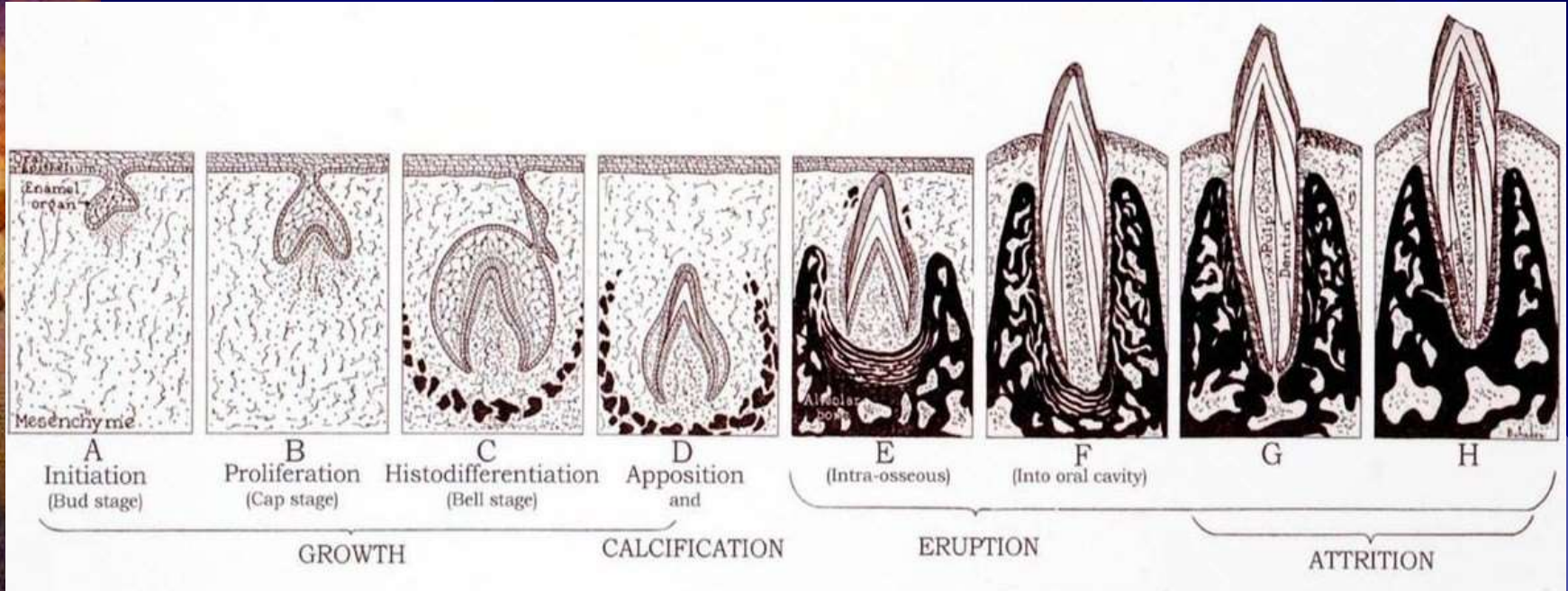
Evolution of the mammalian dentition

From Osborn (1907),
*Evolution of Mammalian
Molar Teeth.*



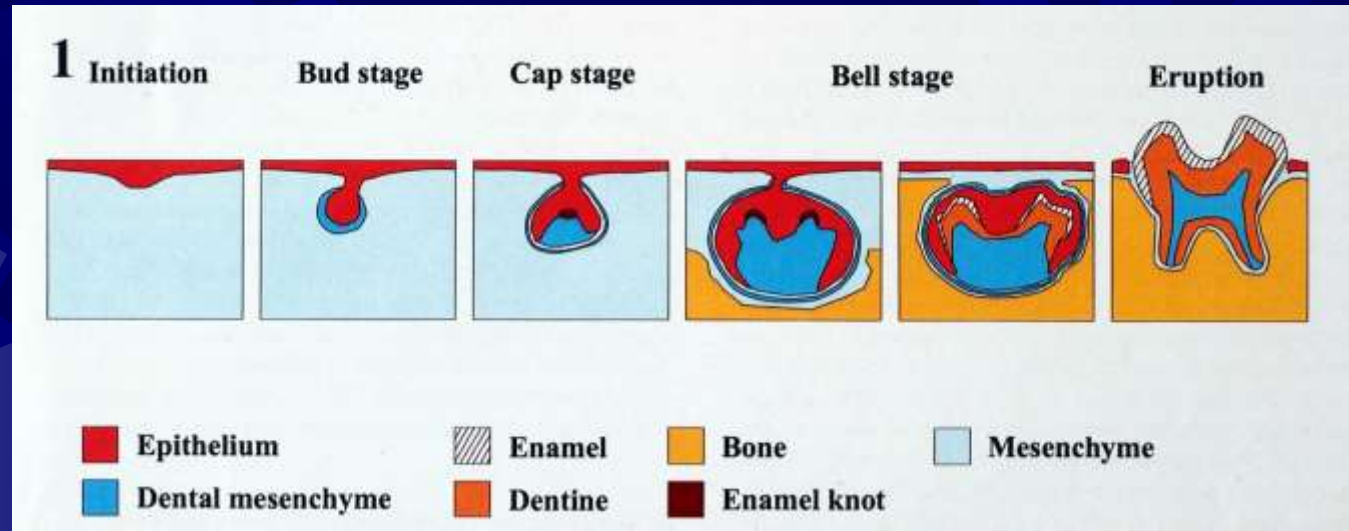
- In mammals the teeth are sequentially arranged, serially homologous structures restricted to a single row along the margins of the maxilla and mandible. They are heterodont, consisting of divergent shapes grouped into incisors, canines, premolars and molars.
- Mammalian teeth are also diphyodont being replaced no more than once. The illustration shows the upper teeth and lower jaw from *Triconodon ferox* a Mesozoic mammal from the Upper Jurassic (actual length of mandible 35 mm).

Life cycle of a tooth



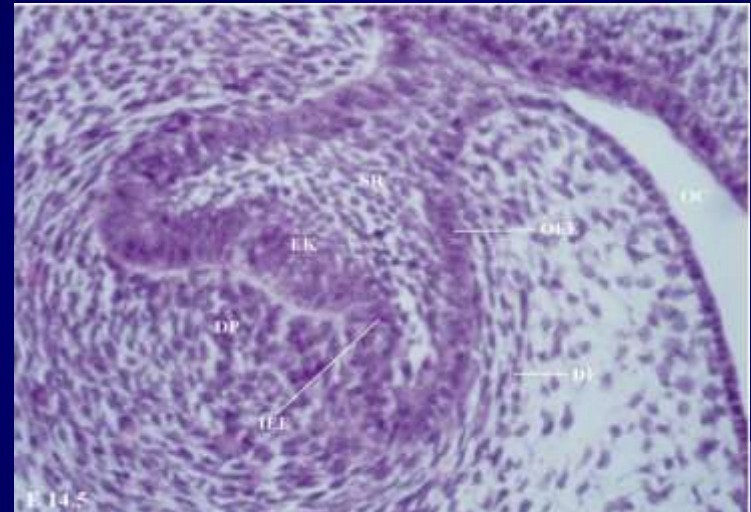
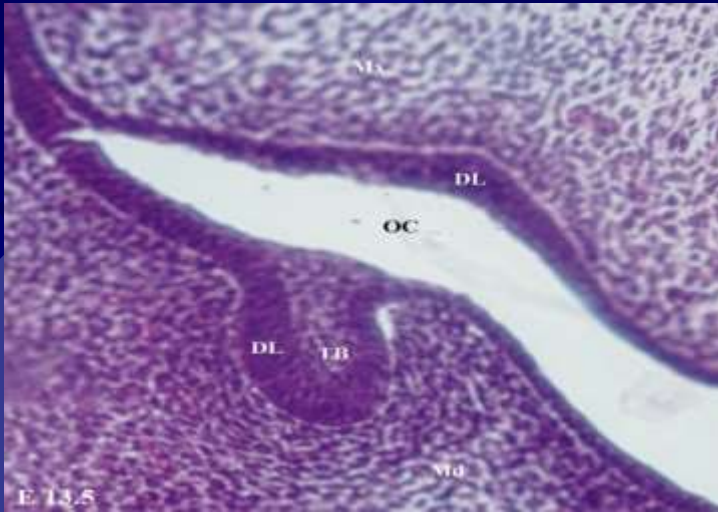
- Diagrammatic representation of the development and life cycle of a tooth. Figure 1 from the classic paper on tooth development by Schour and Massler (1940).
- The following slides are intended as an abridged version of tooth development based on the stages proposed in the above figure, followed by a brief description of the molecular control of odontogenesis.
- From Schour and Massler (1940). *Journal of the American Dental Association* **27**, Part I 1778–1793; Part II 1918–1931.

Odontogenesis



- Teeth are epithelial appendages derived from a series of epithelial-mesenchymal interactions (a characteristic they share with hair, feathers, scales and dermal bones) between the oral epithelium and underlying neural crest-derived mesenchyme.
- The first sign is a thickening of the oral epithelium to form the dental lamina. The epithelium proliferates and invaginates to form a bud around which mesenchymal cells condense; the partially enclosed mesenchymal condensation forms the dental papilla, which gives rise to the tooth pulp and progenitors for the odontoblast cell lineage.
- From Thesleff and Sharpe (1997). *Mechanisms of Development* 67,111–123.

Histodifferentiation and morphogenesis



- Left. Early tooth development in the mouse. E 13.5 shows the dental lamina (DL) in the maxilla; in the lower arch the epithelium has invaginated into the ectomesenchyme to form a tooth bud.
- Right. E 14.5. During the cap and bell stages the epithelium undergoes histodifferentiation and folding morphogenesis establishes the shape of the crown. The epithelial structure is known as the enamel or dental organ and is divided into an inner enamel epithelium (IEE) and outer enamel epithelium (OEE). DP, dental papilla; DF, dental follicle; EK, enamel knot; SR, stellate reticulum.
- Courtesy of M Foxworthy.

Dentinogenesis and amelogenesis



- ✦ Odontoblasts and ameloblasts differentiate during the late bell stage, a process regulated by interactions between the two tissues.
- ✦ The IEE first induces adjacent cells in the dental papilla (DP) to differentiate into odontoblasts (Od) which synthesize a layer of predentine (arrows). Dentine acts as a signal to IEE cells to differentiate into ameloblasts (Ab) and begin secreting enamel matrix (E); enamel will not form in the absence of dentine.
- ✦ Courtesy of M Foxworthy.

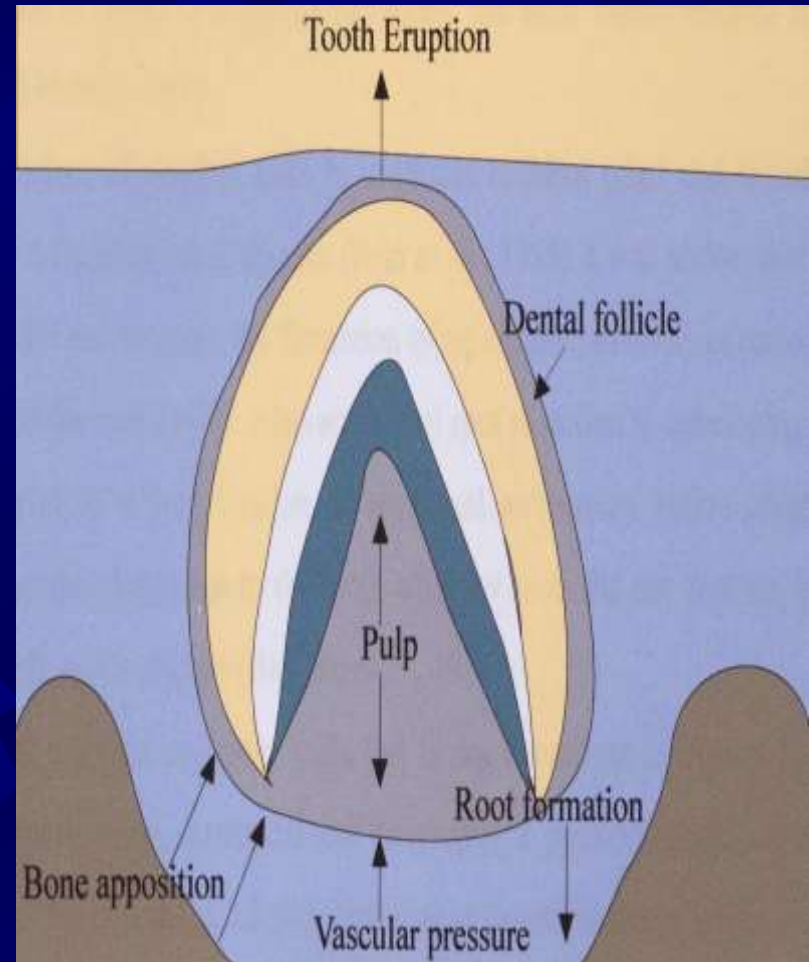
Root formation

- ✦ The junction between the inner and outer epithelia constitutes the cervical loop (CL), which will elongate into Hertwig's epithelial root sheath; this maps out the future morphology of the root and induces further differentiation of odontoblasts.
- ✦ The more peripheral cells surrounding the developing tooth form the dental follicle (DF). Degeneration of the root sheath exposes follicular cells to dentine and their differentiation into cementoblasts. Some remnants of the root sheath may persist as the epithelial rests of Malassez.



Tooth eruption

- The tooth and every structure in the periodontium have been implicated as the primary eruptive mechanism. Theories from vascular pressure beneath the tooth, pulp pressure from within the root, to root formation and bone deposition have been suggested, but are not primary mechanisms.
- In rodents the dental follicle theory for teeth of limited eruption (molars) and the periodontal ligament for continuously erupting teeth (incisors) have been shown to be critical.
- From M Foxworthy (2003), *The role of c-fos in the development and eruption of the murine dentition*. PhD Thesis, University of London.



The role of the dental follicle in tooth eruption

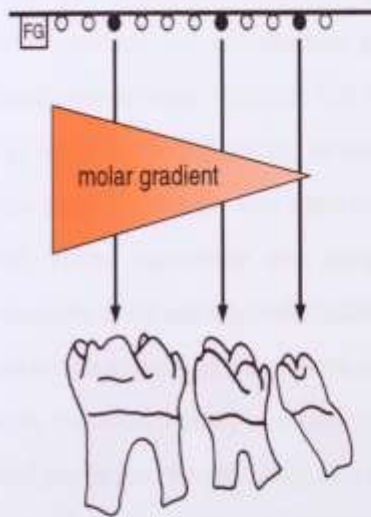
- ✿ During tooth eruption osteoclasts widen the gubernacular canal to create an eruption pathway. This is dependent upon an abundant supply of osteoclast precursor cells within the dental follicle (TRAP⁺ve mononuclear cells), and which are concentrated coronally to the crown of the tooth.
- ✿ Tooth eruption is a complicated multifactorial process relying on alveolar bone resorption coronally, bone formation apically, renewal of the periodontal membrane, and the formation of an epithelial attachment between the tooth and gingival tissues. The periodontal membrane does not play a major role in the eruption of the mammalian dentition.

Molecular control of odontogenesis

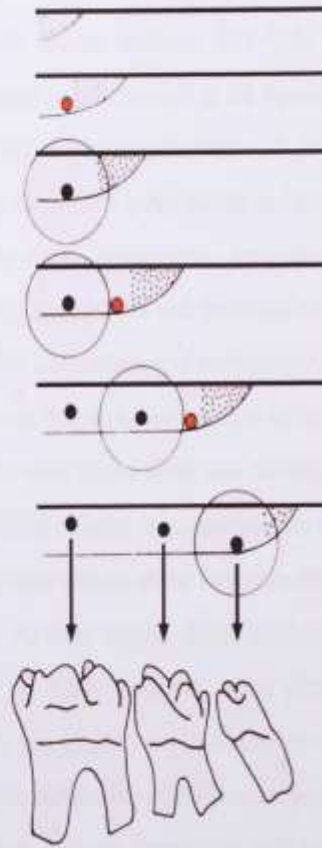
- Two theories have been proposed to explain differences in segmental patterning and shape between teeth.
- The field or gradient model (Butler, 1939) assumes that concentrations of chemical morphogens regulate the development of initially identical tooth primordia. The field model implies that the epithelium controls patterning.
- In the clone model tooth shape and pattern are self-generated rather than controlled from the outside (Osborn, 1978). An updated version has been proposed by Sharpe (1995) and termed the odontogenic homeobox code.

Theories of tooth patterning

A. Regional Field Theory



B. Clone Theory

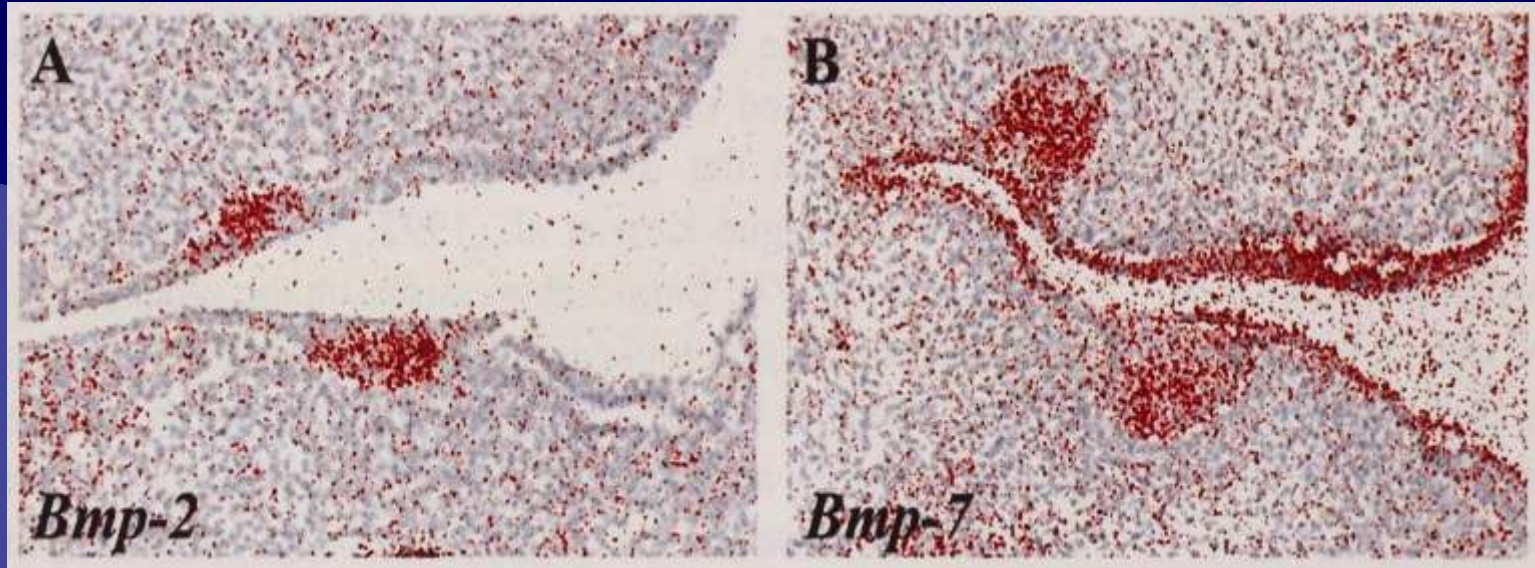


- According to the regional field model, identical tooth primordia (black dots) are acted upon by a morphogenetic substance (orange) generated by a field generator (FG).
- In the clone model all teeth develop from a single clone of mesenchymal cells. Mesenchyme enters the jaw from the neural crest and following interaction with the oral epithelium, a clone of cells is established for a specific tooth class (black dots). Surrounding the clone is a zone of inhibition (circle); once the growing clone has escaped this zone of inhibition a new primordium (red dots) is initiated.
- From M Cobourne (2002), *The role of sonic hedgehog signalling during early tooth development*. PhD Thesis, University of London.

The initiation signal comes from the epithelium

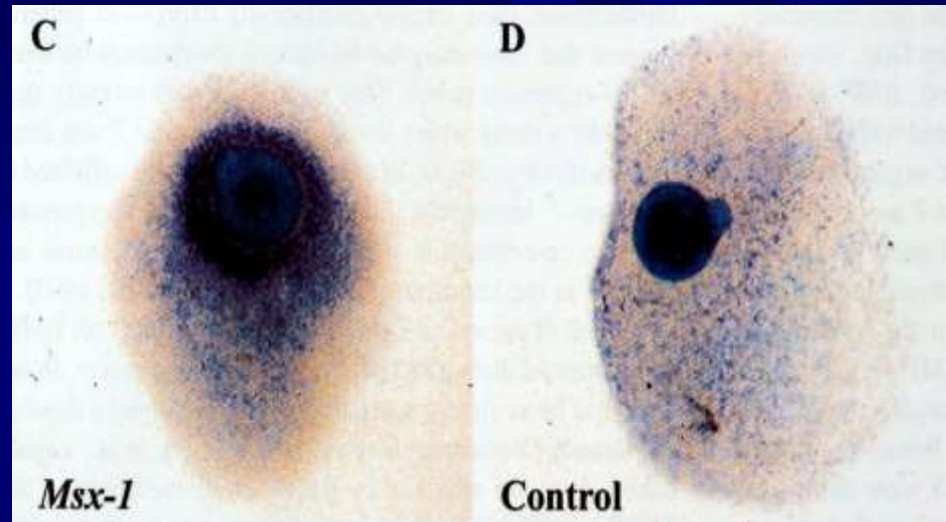
- Heterotropic tissue recombination experiments in mice have shown that prior to the bud stage, the oral epithelium is instructive for tooth germ initiation (Mina and Kollar, 1987), provided the mesenchyme is neural-crest-derived (Lumsden, 1988).
- After the bud stage the inductive potential shifts to the mesenchyme (about E12.5 in mice), with odontogenic mesenchyme being capable of inducing tooth formation when combined with non-odontogenic epithelium (Kollar and Baird, 1970).
- Numerous secreted molecules (which can be regarded as endogenous morphogens) have been shown to be associated with epithelial-mesenchymal signalling during tooth development. These include members of the BMP, FGF and Hh families which are expressed in the dental epithelium during early tooth development.

BMP signalling during early tooth morphogenesis



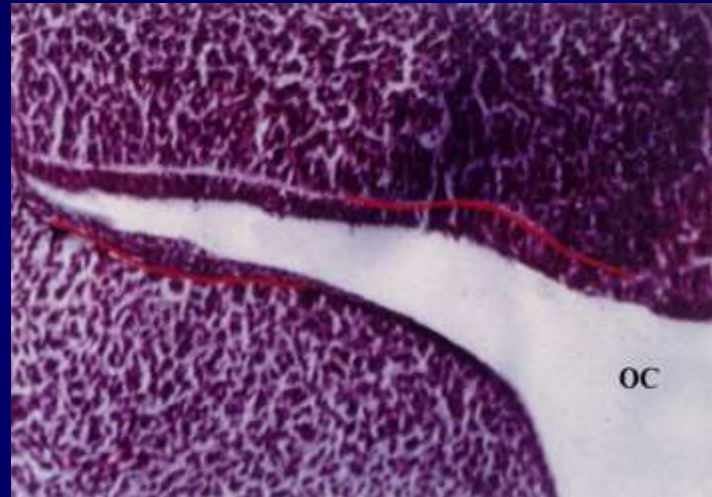
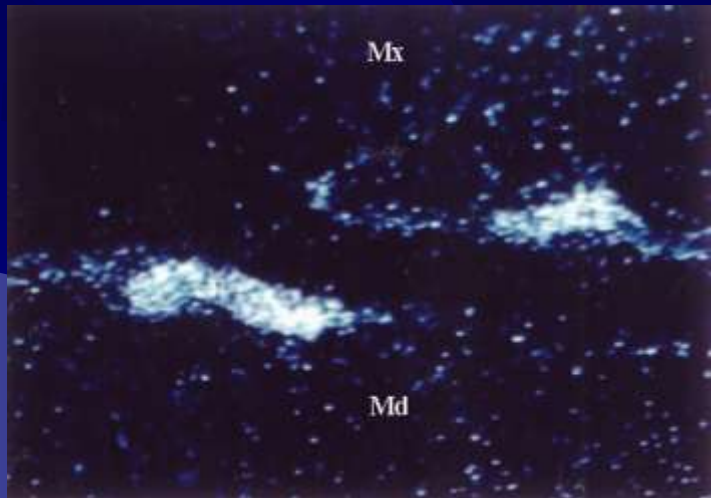
- BMPs (bone morphogenetic proteins) were the first signals to be identified in the transmission of inductive interactions between the dental epithelium and the mesenchyme.
- *Bmp-2* and *Bmp-4* as well as *Bmp-7* are expressed early in the dental epithelium; however, the expression of *Bmp-4* shifts to the mesenchyme when the instructive capacity shifts from the epithelium.
- From Thesleff and Sharpe (1997). *Mechanisms of Development* 67,111–123.

BMPs stimulate *Msx-1* and *Msx-2* expression



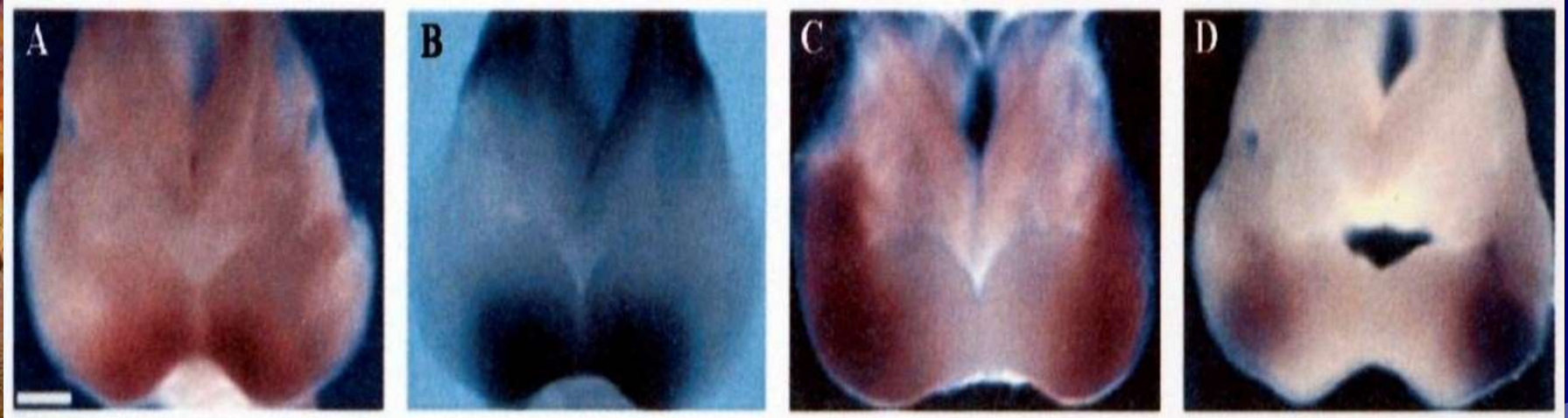
- BMP-2 and BMP-4 have been shown to mimic some effects of the presumptive dental epithelium on mesenchyme at a time when the instructive capacity still resides in the epithelium. In particular, they stimulate the expression of the homeobox-containing transcription factors *Msx-1* and *Msx-2*.
- In this illustration a bead releasing BMP-2 protein has induced the induction of *Msx-1* in cultured E11 dental mesenchyme. On the right is a control culture with a BSA-containing bead. BMPs and FGFs play important roles in dental patterning (see later).
- From Thesleff and Sharpe (1997). *Mechanisms of Development* 67,111–123.

Sonic hedgehog expression



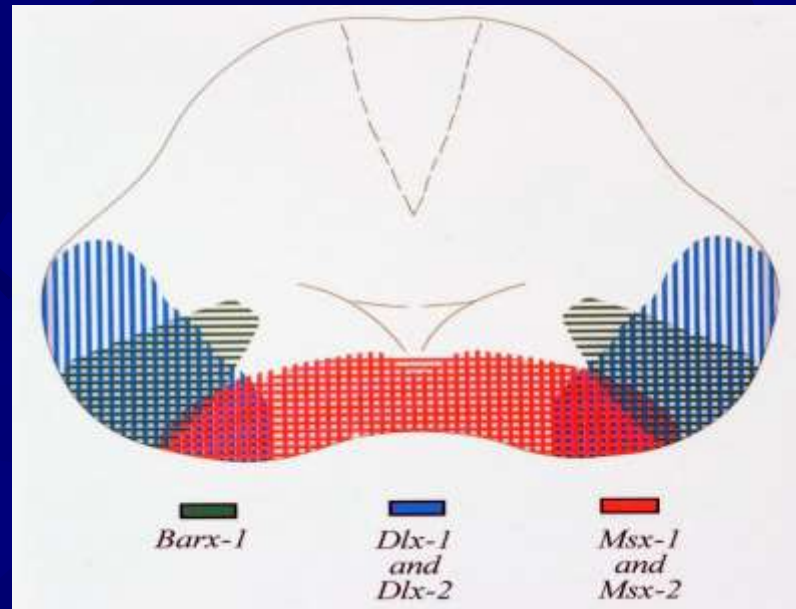
- *Shh* is expressed in epithelial thickenings at sites where teeth are required; in mice *Shh* signalling is inhibited in the diastema region where teeth are not formed (mice do not have canines or premolars). *Shh* expression appears to follow those of *Fgf-8* and *Fgf-9*, suggesting that FGFs may be upstream regulators of *Shh* signalling.
- Left. ^{35}S radiolabelled *in situ* hybridization of *Shh* in primary molar thickenings in a 13.5 day *pc* mouse. Right. H&E stained adjacent section; the red line represents the junction between the epithelium and ectomesenchyme.
- Courtesy of M Cobourne.

The odontogenic homeobox code



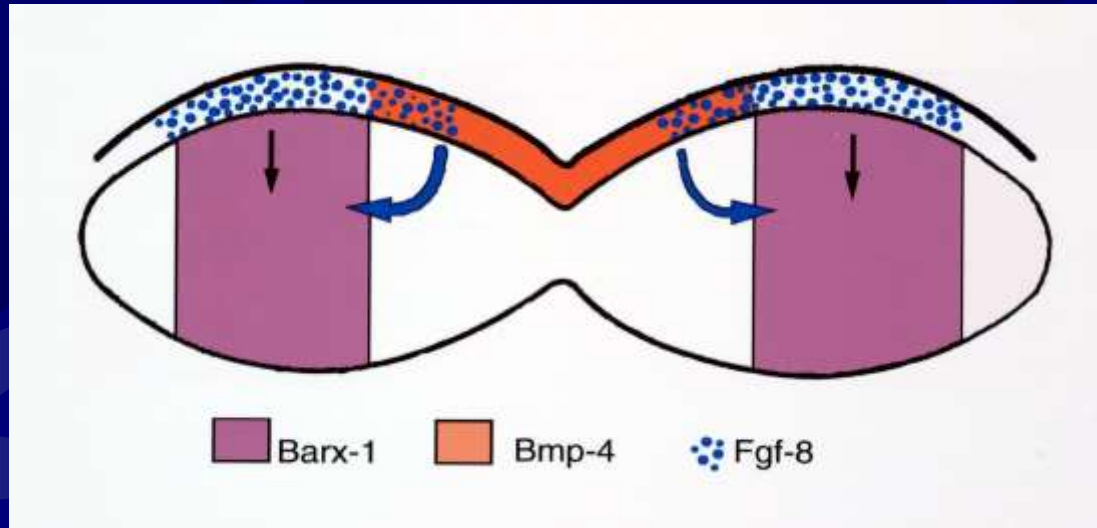
- The odontogenic homeobox code proposes that variation in tooth morphogenesis is specified by the combinatorial action of different homeobox genes expressed in neural crest-derived mesenchyme, and implies that tooth development in different regions of the jaws involves independent genetic control.
- These 4 images show the expression patterns of *Msx-1*, *Msx-2*, *Dlx-2* and *Barx-1* in the developing mouse mandible. Whole mount *in situ* hybridization of E10.5 mouse embryos; the mandible has been dissected free and is viewed from the oral aspect.
- From Thomas and Sharpe (1998). *European Journal of Oral Sciences* 67,111–123.

The odontogenic homeobox code



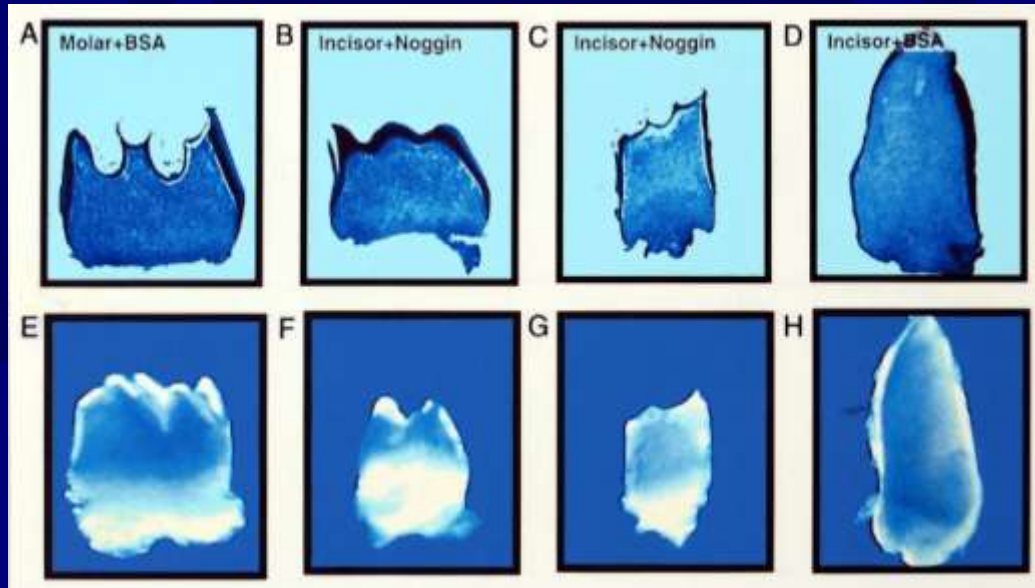
- ✿ Diagrammatic representation of the previous slide. During the initial stages of development, *Msx-1* and *Msx-2* are co-expressed in the ectomesenchyme of the presumptive incisor region, while *Dlx-1* and *Dlx-2* cover the proximal (molar) part.
- ✿ Another homeobox gene *Barx-1* is expressed specifically in the ectomesenchyme of the molar region within the *Dlx*-positive domain.
- ✿ Courtesy of P T Sharpe.

Incisor and molar specification



- ✦ Signalling molecules involved in dental patterning are BMP-4 and FGF-8 which are expressed in the oral epithelium. At the initiation of tooth germ development, BMP-4 activates *Msx-1* expression in the mesenchyme of the presumptive incisor region.
- ✦ At the same stage FGF-8 is expressed in the epithelium covering the presumptive molar region. Expression of *Barx-1* in mesenchymal cells occurs in the region of the epithelium showing positive expression of FGF-8 and is negatively regulated by BMP-4.
- ✦ Courtesy of P T Sharpe.

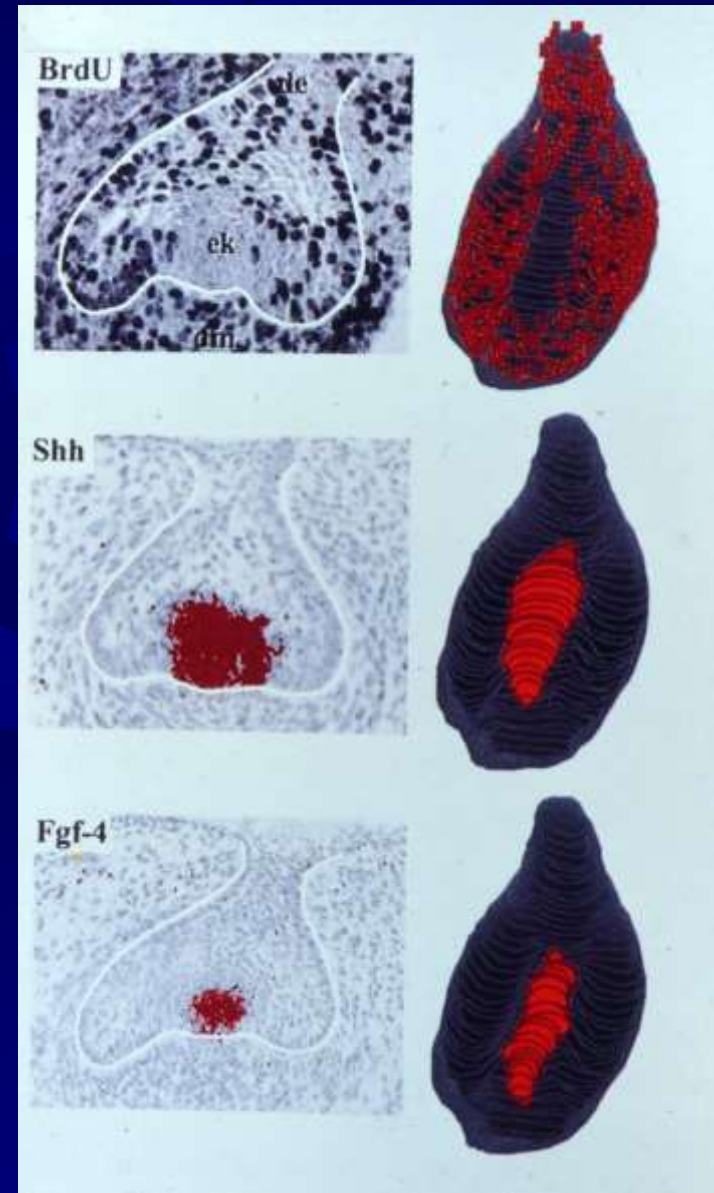
Transformation of tooth identity



- ✿ Inhibition of BMP-4 signalling with exogenous Noggin protein results in the ectopic expression of *Barx-1* in the presumptive incisor ectomesenchyme; the outcome is transformation of tooth identity from incisor to molar in E10 tooth germs. A gain-of-function change in gene expression has produced an alteration in tooth phenotype.
- ✿ E11 explants treated in the same way are unaffected, showing that ability to alter tooth patterning is limited to a small temporal window of development.
- ✿ From Tucker *et al.* (1998). *Science* **282**,1136–1138.

The enamel knot as an organizing centre

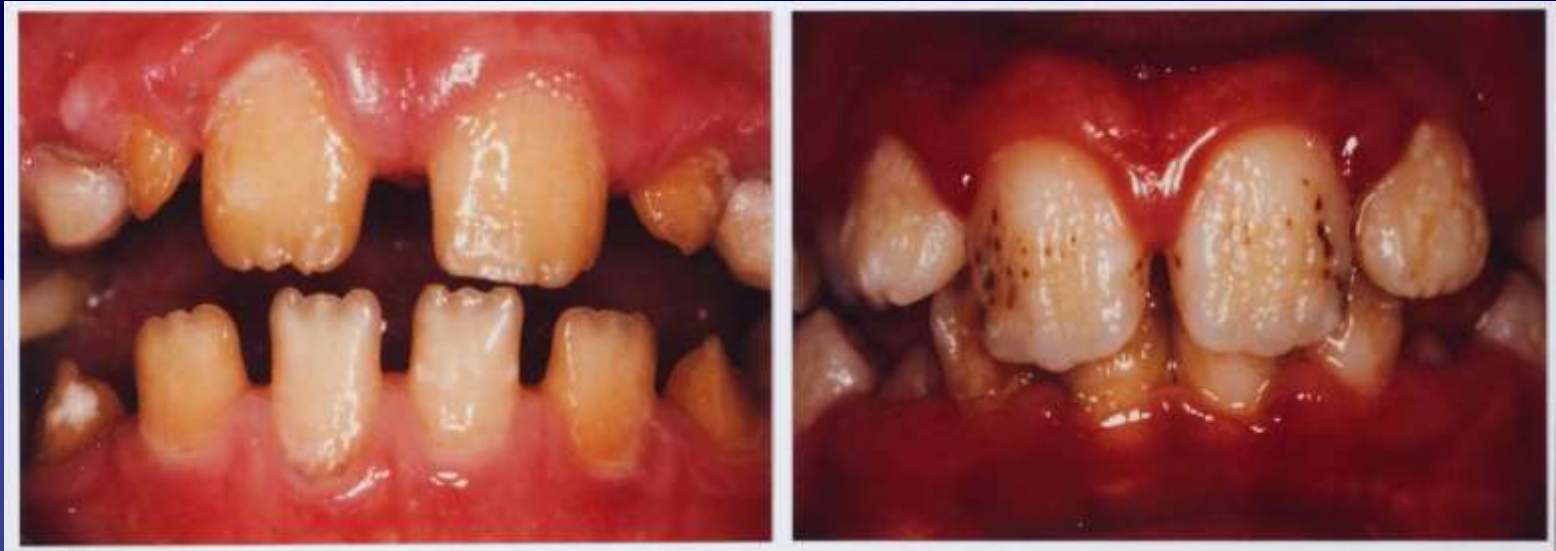
- The enamel knot (ek) is a population of non-dividing epithelial cells that appear during the late bud stage. It has been proposed that the enamel knot acts as a signalling centre responsible for directing cell proliferation and cuspal patterning in the enamel organ.
- Left, cells in the ek have left the cell cycle (lack of BrdU uptake) and are non-proliferative in contrast to surrounding cells which continue to divide. Ek cells express *Shh*, *Fgf-4* and *Bmp-2*, *Bmp-4* and *Bmp-7*.
- Right, 3D computer generated reconstructions of cap stage mouse first molars.
- From Thesleff and Sharpe (1997). *Mechanisms of Development* 67, 111–123.



Enamel matrix proteins

- During amelogenesis the extracellular matrix of enamel, which is initially protein-rich, loses most of the protein at the expense of calcium and phosphorus and becomes the most highly mineralized tissue in the body (more than 98 % mineral in the form of hydroxyapatite).
- The uniqueness of dental enamel is reflected in the tissue specificity of its matrix components, and to date the primary structure of four enamel matrix proteins have been derived from cDNA sequences.
- Amelogenin comprises 80–90 % of the total organic material. In humans the amelogenin gene is expressed on the short arms of both the X and Y chromosomes; however, the level of expression on the Y chromosome is only 10 % of that on the X chromosome.
- Non-amelogenins are grouped under the umbrella term enamelines (which includes tuftelin, ameloblastin/sheathlin and enamelin). While amelogenins are selectively lost during mineralization, enamelines are partially retained.

Amelogenesis imperfecta

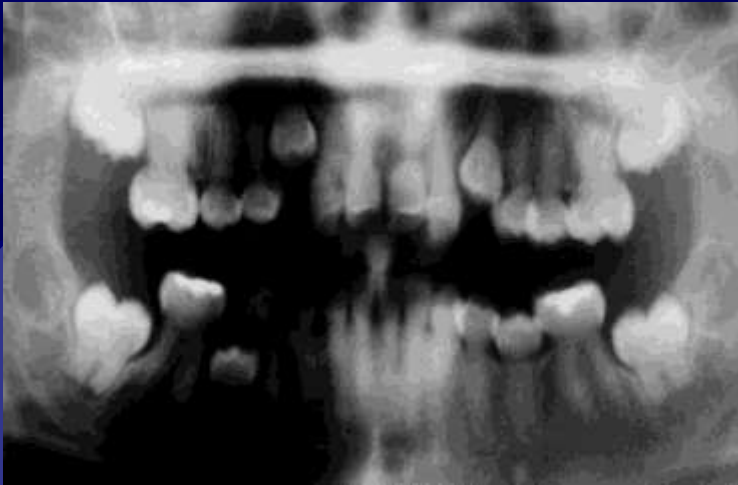


- X-linked amelogenesis imperfecta. Various mutations in the *AMELOGENIN* gene have been found in families with the X-linked form. Two loci have been identified AIH1 and AIH3. Less than 5 % of families with AI show an X-linked pattern of inheritance.
- The molecular basis of the remaining 95 % autosomal forms are less well understood. However, the enamel specific genes *AMELOBLASTIN*, *TUFTELIN*, and *ENAMELIN* are all potential candidate genes.
- Autosomal dominant forms of AI (type IA; smooth hypoplastic variety) and (type IC; pitted hypoplastic variety). Photographs courtesy of E Sheehy.

Dentine matrix proteins

- The main structural component of dentine matrix, like that of bone is type I collagen with lesser amounts of minor collagens. Other proteins and proteoglycans that it shares with bone include osteocalcin, osteopontin, osteonectin/SPARC, bone sialoprotein, decorin and biglycan. There are, however, two proteins that are unique to dentine.
- Dentine phosphoprotein (DPP, also called phosphophoryn), after type I collagen the most abundant organic constituent of dentine matrix, and dentine sialoprotein (DSP), which accounts for 5–8 % of dentine noncollagenous proteins.
- Both are synthesized and secreted by young and mature odontoblasts and also by preameloblasts and are found at the mineralization front where they are believed to be involved in the nucleation of hydroxyapatite.
- DPP and DSP are coded by the same gene localized to chromosome 4q21, a site known to be linked to dentinogenesis imperfecta type II.

Dentinogenesis imperfecta



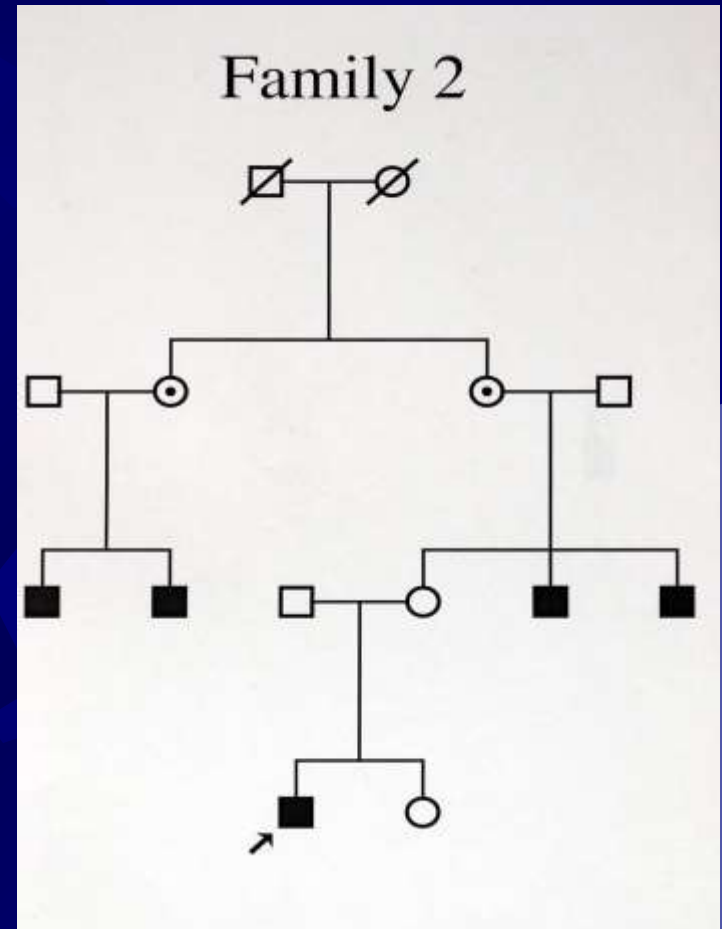
- ✦ Dentinogenesis imperfecta (hereditary opalescent dentine) the most common dental genetic disorder with an incidence of 1:8000, affects both dentitions and transmitted as an autosomal dominant trait. Enamel is normal but fractures off the dentine; root canals and pulp chambers become obliterated by continuous production of dentine. There are 3 subgroups.
- ✦ DGI type I associated with mutations in the type I collagen gene and osteogenesis imperfecta; DGI type II the classically inherited disease affecting dentine only; DGI type II and DGI type III (known as the Brandywine isolate) have been linked to the loci for DPP and DSP on chromosome 4q21. Photographs courtesy of M Harrison.

Tooth agenesis

- ☀ Tooth agenesis or hypodontia of one or more teeth can occur in association with numerous genetic syndromes or as an isolated sporadic or family trait. Oligodontia refers to the congenital absence of more than 6 teeth. *Online Medical Inheritance in Man (OMIM)* lists more than 150 syndromes featuring tooth agenesis; ectodermal dysplasias are the most widely recognized conditions in which severe hypodontia is found.
- ☀ Excluding third molar agenesis which occurs in 20 % of the population, the teeth most affected are the lateral incisors (2.2 %) and second premolars (3.4 %). Patterns of hypodontia suggest that the last tooth of a certain class is the least stable. Affected family members often exhibit differences in the location, symmetry and number of teeth involved, demonstrating variability in the expression of the trait.
- ☀ Non-syndromic familial hypodontia can be transmitted as an autosomal dominant, autosomal recessive or X-linked condition.

Establishing family pedigrees

- ★ Pedigree of a family from the Guy's Dental Hospital database with X-linked tooth agenesis.
- ★ The second generation females in this family demonstrated peg-shaped or diminutive maxillary lateral incisors; the assumption is that these females are obligate carriers of an X-linked trait which gives rise to hypodontia in males.
- ★ The dental phenotype in the third generation of the pedigree is mild involving only maxillary lateral incisors and second premolars. The fourth generation male (arrow) has one peg and one absent lateral incisor in addition to agenesis of all second premolars.

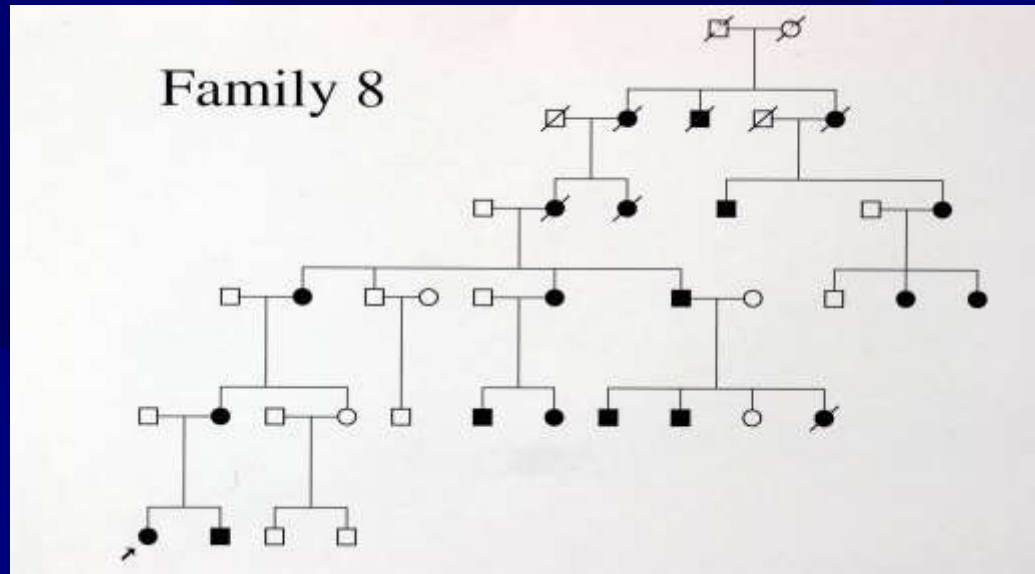


Courtesy of M Harrison.

MSX-1 mutations in hypodontia

- To date *MSX-1* and *PAX-9* are the only two genes shown to be linked to non-syndromic hypodontia.
- Vastardis *et al.* (1996) were the first to identify a mutation in a human gene causing familial hypodontia. They identified a locus on chromosome 4p that includes the *MSX-1* gene, in a family with autosomal dominant hypodontia of all second premolar and third molar teeth; sequence analysis demonstrated an Arg31Pro missense mutation in the homeodomain of *MSX-1* in all affected family members.
- A Dutch family with hypodontia co-segregating with various combinations of cleft lip and palate has also been mapped to the *MSX-1* gene (van den Boogaard *et al.* 2001). The dental phenotype was similar to that reported by Vastardis *et al.*

PAX-9 and molar hypodontia



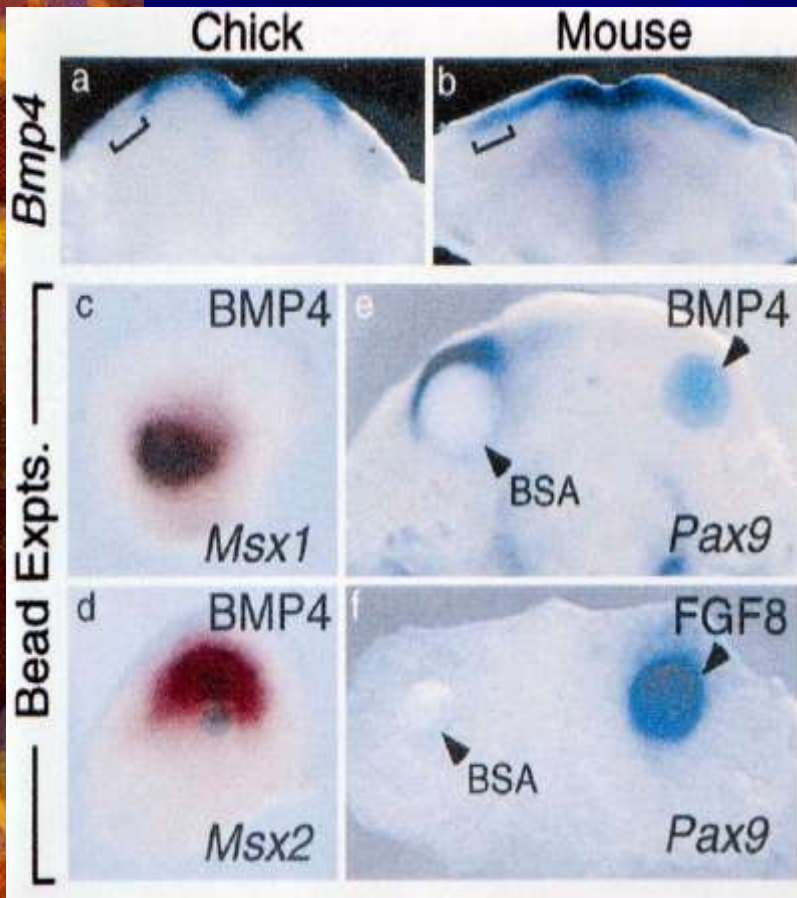
- ✿ A large kindred demonstrating autosomal dominant molar agenesis has been mapped to the *PAX-9* locus on chromosome 14 (Stockton *et al.* 2000).
- ✿ Family from Guy's includes 14 living members in which tooth agenesis includes two or more missing permanent molars; at least 2 first permanent molars are absent in all affected individuals. Absence of one or both lower central incisors occurred in 8 of the 9 individuals examined dentally. Linkage to the *PAX-9* locus has been established for this family. Courtesy of M Harrison.

Can birds form teeth?



- Although modern birds lack a dentition, they are derived from ancestors that were once toothed. Birds such as the Jurassic *Archaeopteryx* shown here and the late Cretaceous *Hesperornis* possessed teeth. However, teeth have been missing from birds (*Aves*) for at least 60 Myr.
- Classical tissue recombination experiments and more recent genetic analyses have identified the oral epithelium as providing the instructive information for the initiation of tooth development in the mouse.
- Several *in vitro* studies have suggested that a number of genes involved in tooth formation which remain silent in birds, can be reactivated upon receiving an appropriate signal.

BMP-4 can induce *Msx* in chick mesenchyme



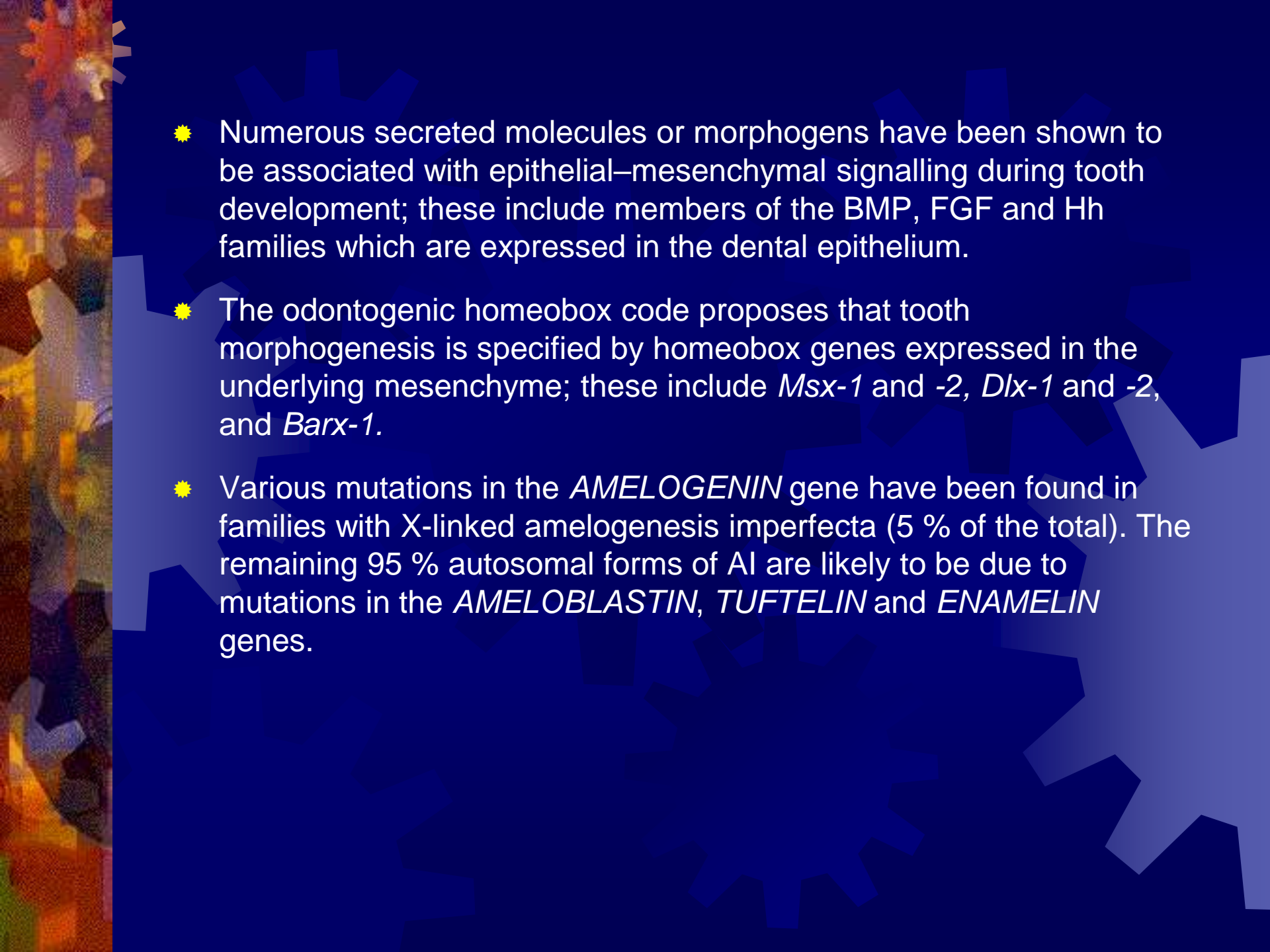
- In Stage 27 chick mandible, *Bmp-4* is expressed in mesial oral epithelium but not distally (a), in contrast to the mouse (b).
- (c, d). BMP-4-soaked beads can induce *Msx-1* and *Msx-2* expression in stage 27 chick mandibular mesenchyme.
- (e). BMP-4 bead represses endogenous *Pax-9* in 5 day chick mandibular explants whereas BSA bead does not.
- (f). FGF-8 bead activates *Pax-9* expression in de-epithelialised day 4.5 chick mandibular explants; BSA does not.
- These data show that although latent, the early signalling pathways involved in odontogenesis remain inducible in *Aves* and suggest that loss of odontogenic *Bmp-4* expression in avian dental epithelium may have been responsible for the arrest of tooth development in living birds.

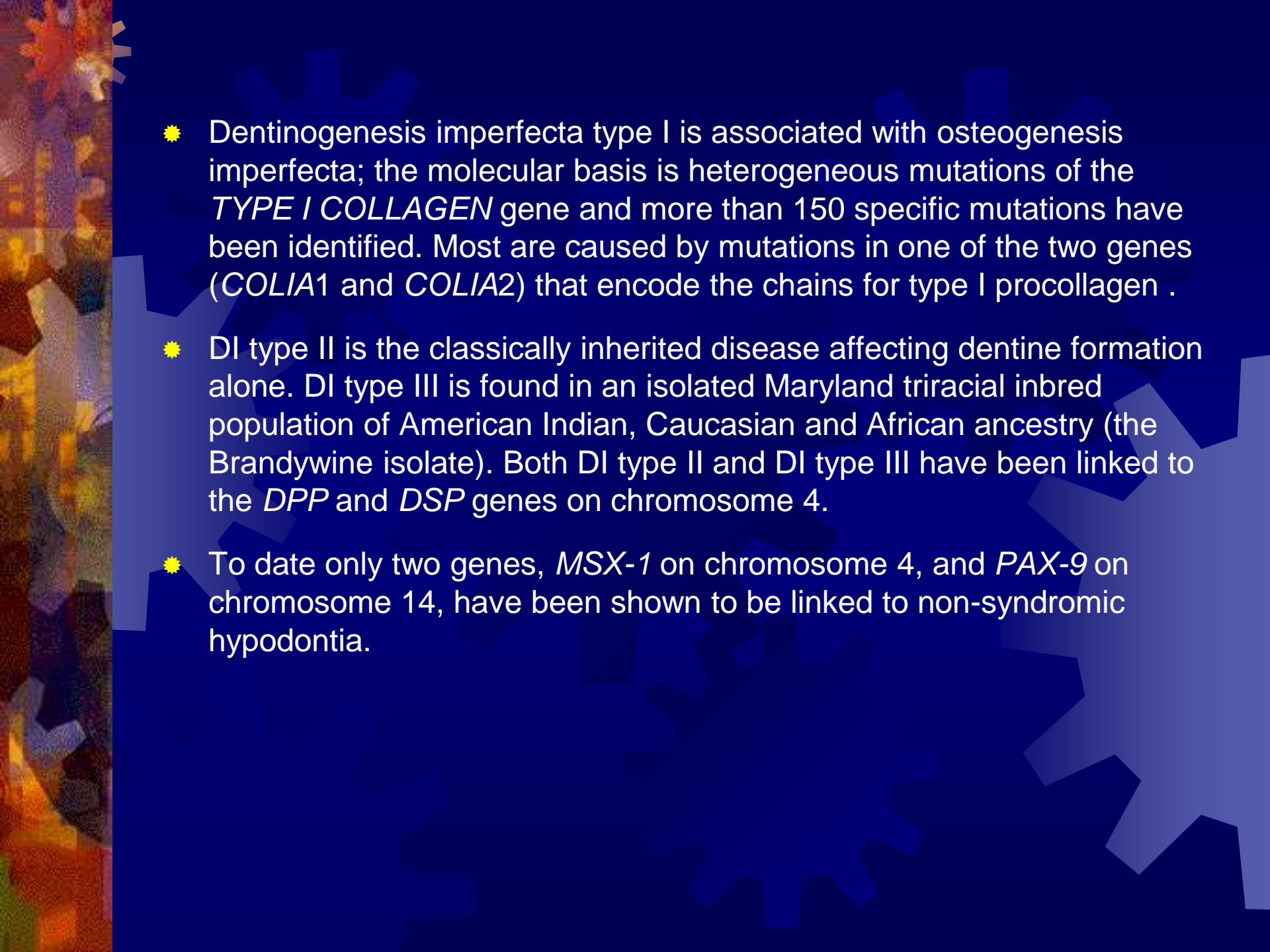
Evidence from mouse/chick chimeras

- More recently , however, Mitsiadis *et al.* (2003; *Proceedings of the National Academy of Sciences of the USA*) carried out neural tube transplantations from mice to chick embryos to replace the chick neural crest cell populations with mouse neural crest. The resulting mouse/chick chimeras obtained showed evidence of tooth formation.
- To test if mouse cranial neural crest contains specific signals that can induce localized *Bmp-4* and *Shh* expression in chick oral epithelium, *in situ* hybridization was performed on chimeras 2–4 days after surgery.
- Their findings indicated that neural crest cells may play a role in the activation of *Bmp-4* and *Shh* expression in tooth forming sites.
- Loss of teeth in *Aves* is thus probably due to the lack of appropriate neural crest-derived signalling molecules involved in the initiation of epithelial–mesenchymal interactions.

Summary

- During gnathostome evolution 450 Myr ago, the fossil record suggests that odontodes in the oral cavity along the margin of the jaws became modified for feeding and gave rise to teeth.
- Recent evidence however, suggests that odontodes may have first appeared in the feeding apparatus of conodonts prior to the appearance of a dermal exoskeleton.
- Teeth are epithelial appendages derived from a series of epithelial-mesenchymal interactions (a characteristic they share with hair, feathers, scales and dermal bones) between the oral epithelium and underlying neural crest-derived mesenchyme.
- In rodents the dental follicle theory for teeth of limited eruption (molars) and the periodontal ligament for continuously erupting teeth (incisors) have been shown to be critical for tooth eruption.

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- Numerous secreted molecules or morphogens have been shown to be associated with epithelial–mesenchymal signalling during tooth development; these include members of the BMP, FGF and Hh families which are expressed in the dental epithelium.
 - The odontogenic homeobox code proposes that tooth morphogenesis is specified by homeobox genes expressed in the underlying mesenchyme; these include *Msx-1* and *-2*, *Dlx-1* and *-2*, and *Barx-1*.
 - Various mutations in the *AMELOGENIN* gene have been found in families with X-linked amelogenesis imperfecta (5 % of the total). The remaining 95 % autosomal forms of AI are likely to be due to mutations in the *AMELOBLASTIN*, *TUFTELIN* and *ENAMELIN* genes.

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- Dentinogenesis imperfecta type I is associated with osteogenesis imperfecta; the molecular basis is heterogeneous mutations of the *TYPE I COLLAGEN* gene and more than 150 specific mutations have been identified. Most are caused by mutations in one of the two genes (*COL1A1* and *COL1A2*) that encode the chains for type I procollagen .
 - DI type II is the classically inherited disease affecting dentine formation alone. DI type III is found in an isolated Maryland triracial inbred population of American Indian, Caucasian and African ancestry (the Brandywine isolate). Both DI type II and DI type III have been linked to the *DPP* and *DSP* genes on chromosome 4.
 - To date only two genes, *MSX-1* on chromosome 4, and *PAX-9* on chromosome 14, have been shown to be linked to non-syndromic hypodontia.