Obecná a srovnávací odontologie

And now something completely different

Vývojové souvislosti 2:

plakody jako signalizační centrum odontogeneze,

evoluční ztráty a případné znovuzískání zubů

Plakody představují klíčová (semi-nezávislá) signální centra pro základ dentální morfogeneze

aktivátory vs. inhibitory

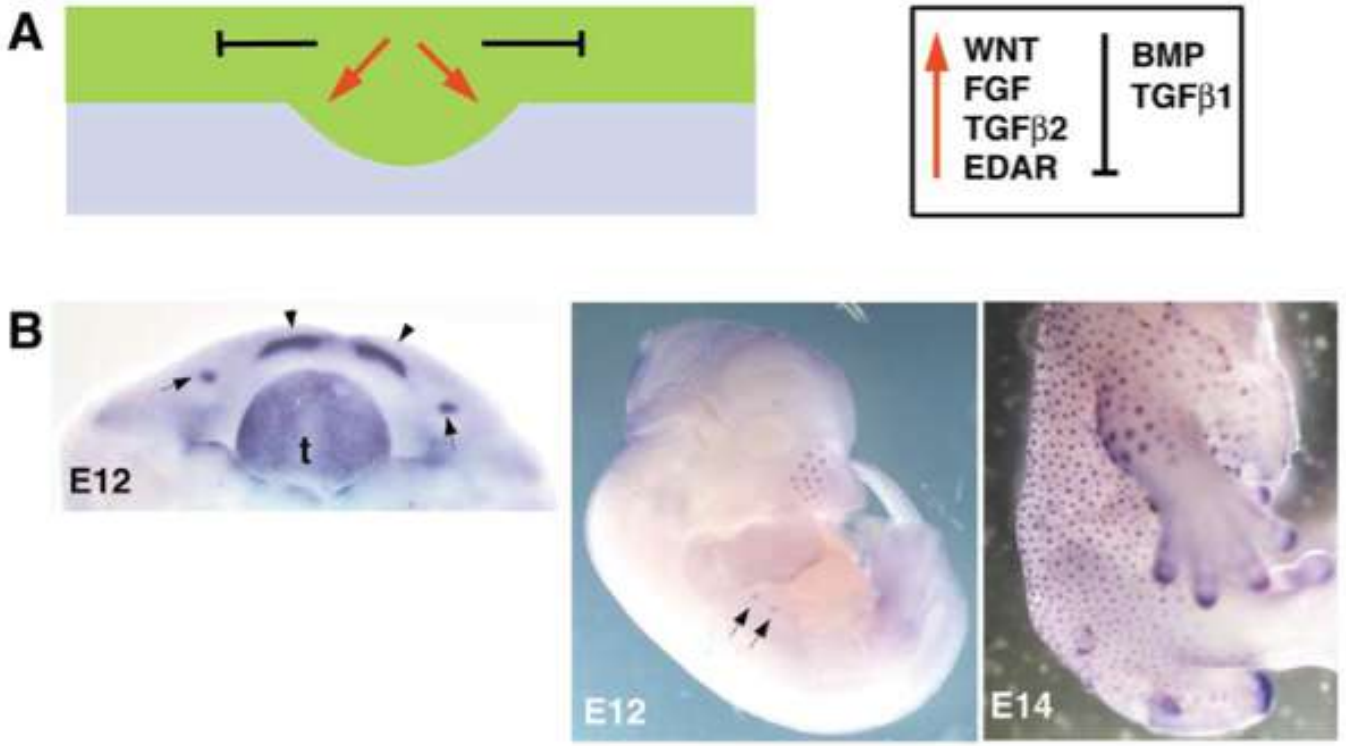

Fig. 2. Placodes as signaling centers. (A) Signaling at the hair and feather placode. Positive signaling (activators, red) promotes placode development, whereas negative signaling (inhibitors, black) represses it. The activity of the inhibitors is believed to be prevented inside the developing placode, whereas they can diffuse outside the placode to mediate lateral inhibition. (B) Placodes can be visualized with whole mount in situ hybridization detecting the restricting expression of many signaling molecules. Molar (arrows) and incisor (arrowheads) tooth placodes express *Shh* (E12 mouse mandible; t, tongue). Vibrissa and mammary gland placodes (arrows) are positive for *Edar* mRNA (E12 mouse embryo). Hair placodes express *Patched* (E14 mouse embryo, expression can also be seen at nails and joints).

Zubní patrnost (velikost & počet zubů) záleží na velikosti zakládající plakody jakožto modulu signalizace (**Inhibitory cascade model**)

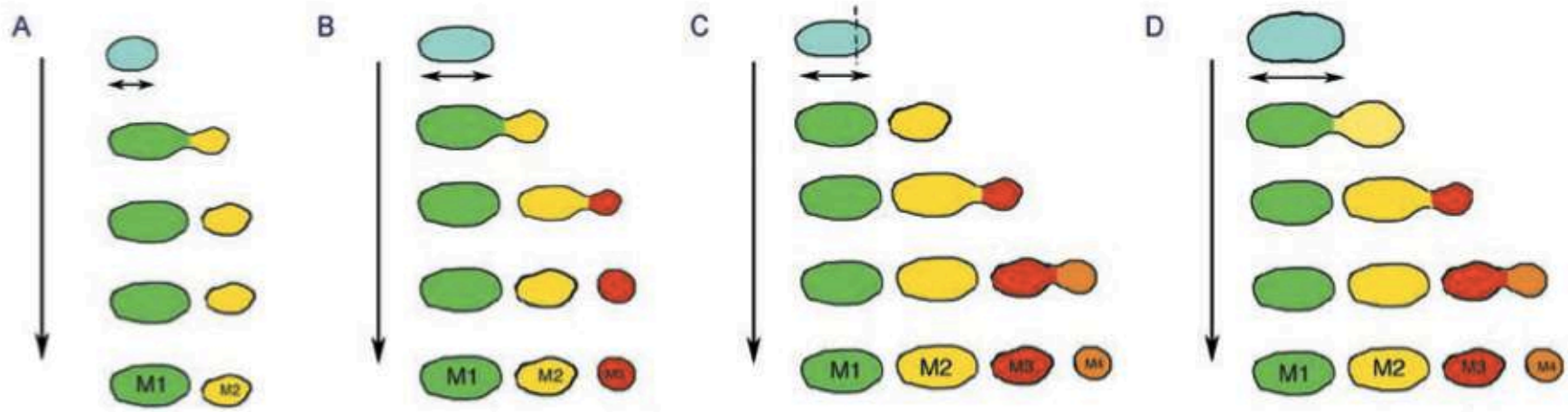

Fig. 4 Changing molar tooth number. The size of the molar field affects the number of teeth that form. (A) A small molar field at E13.5, as generated by recombination or in an *Eda* mutant, leads to the formation of a reduced number of teeth. (B) A wild-type molar field at E13.5. Inhibitory signals from the intermolar region lead to the formation of three molars of diminishing size. (C) If the anterior part of the molar field is cut off at E13.5, the posterior part is released from inhibition by M1 and up to four molars can form, with M2 reaching the normal size of M1. M2 and M3 when isolated from M1 have an accelerated initiation compared to that of whole cultured explants or *in vivo* (compare to B). (D) If the molar field is large in size, as after recombination with large numbers of mesenchymal cells, four molars can form, with M2 reaching the normal size of M1. Downward arrows indicate development of the molar field from E13.5 to formation of distinct teeth.

Patterning by heritage in mouse molar row development

Jan Prochazka[a,b,1,2], Sophie Pantalacci[c,1], Svatava Churava[a,d], Michaela Rothova[a,b], Anne Lambert[c], Hervé Lesot[e], Ophir Klein[f], Miroslav Peterka[a,d], Vincent Laudet[c], and Renata Peterkova[a]

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It is known from paleontology studies that two premolars have been lost during mouse evolution. During mouse mandible development, two bud-like structures transiently form that may represent rudimentary precursors of the lost premolars. However, the interpretation of these structures and their significance for mouse molar development are highly controversial because of a lack of molecular data. Here, we searched for typical tooth signaling centers in these two bud-like structures, and followed their fate using molecular markers, 3D reconstructions, and lineage tracing in vitro. Transient signaling centers were indeed found to be located at the tips of both the anterior and posterior rudimentary buds. These centers expressed a similar set of molecular markers as the "primary enamel knot" (pEK), the signaling center of the first molar (M1). These two transient signaling centers were sequentially patterned before and anterior to the M1 pEK. We also determined the dynamics of the M1 pEK, which, slightly later during development, spread up to the field formerly occupied by the posterior transient signaling center. It can be concluded that two rudimentary tooth buds initiate the sequential development of the mouse molars and these have previously been mistaken for early stages of M1 development. Although neither rudiment progresses to form an adult tooth, the posterior one merges with the adjacent M1, which may explain the anterior enlargement of the M1 during mouse family evolution. This study highlights how rudiments of lost structures can stay integrated and participate in morphogenesis of functional organs and help in understanding their evolution, as Darwin suspected long ago.

Patterning by heritage in mouse molar row development

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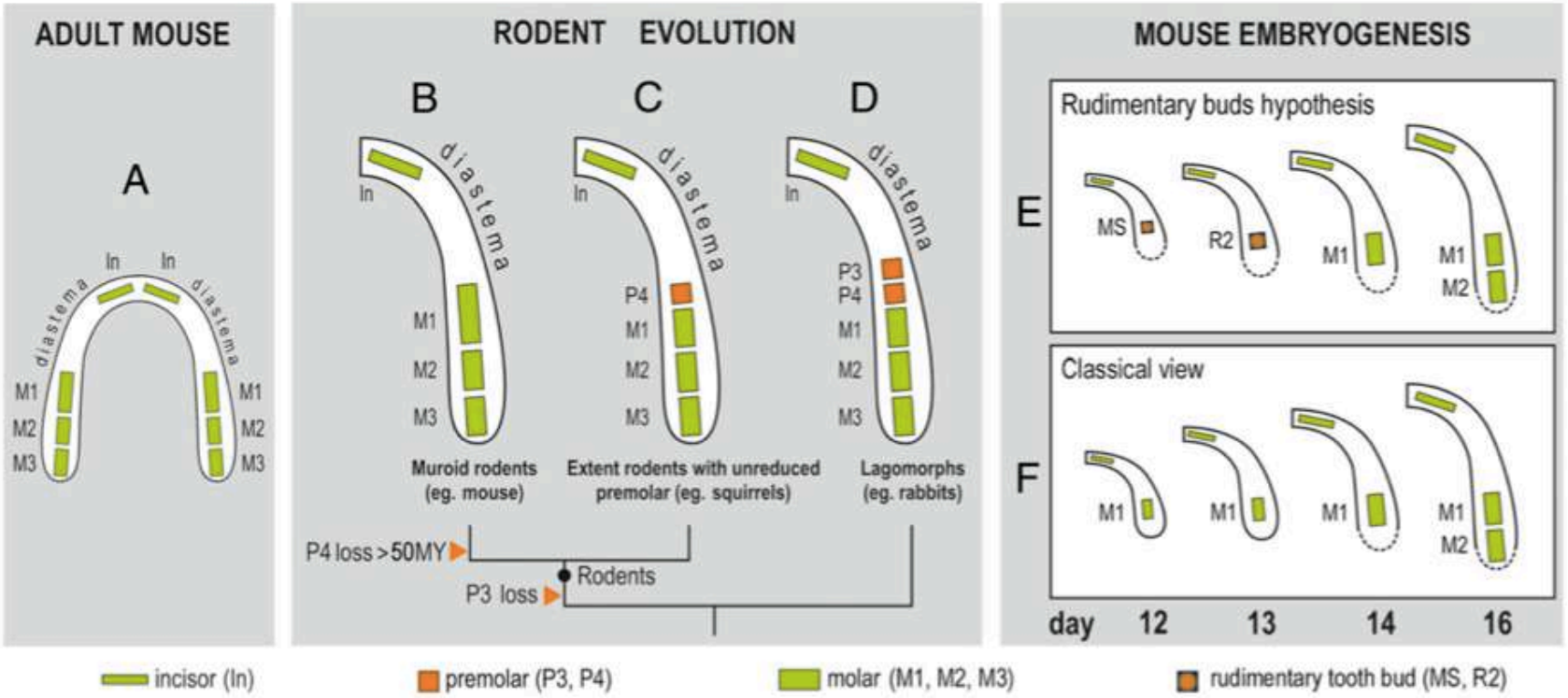
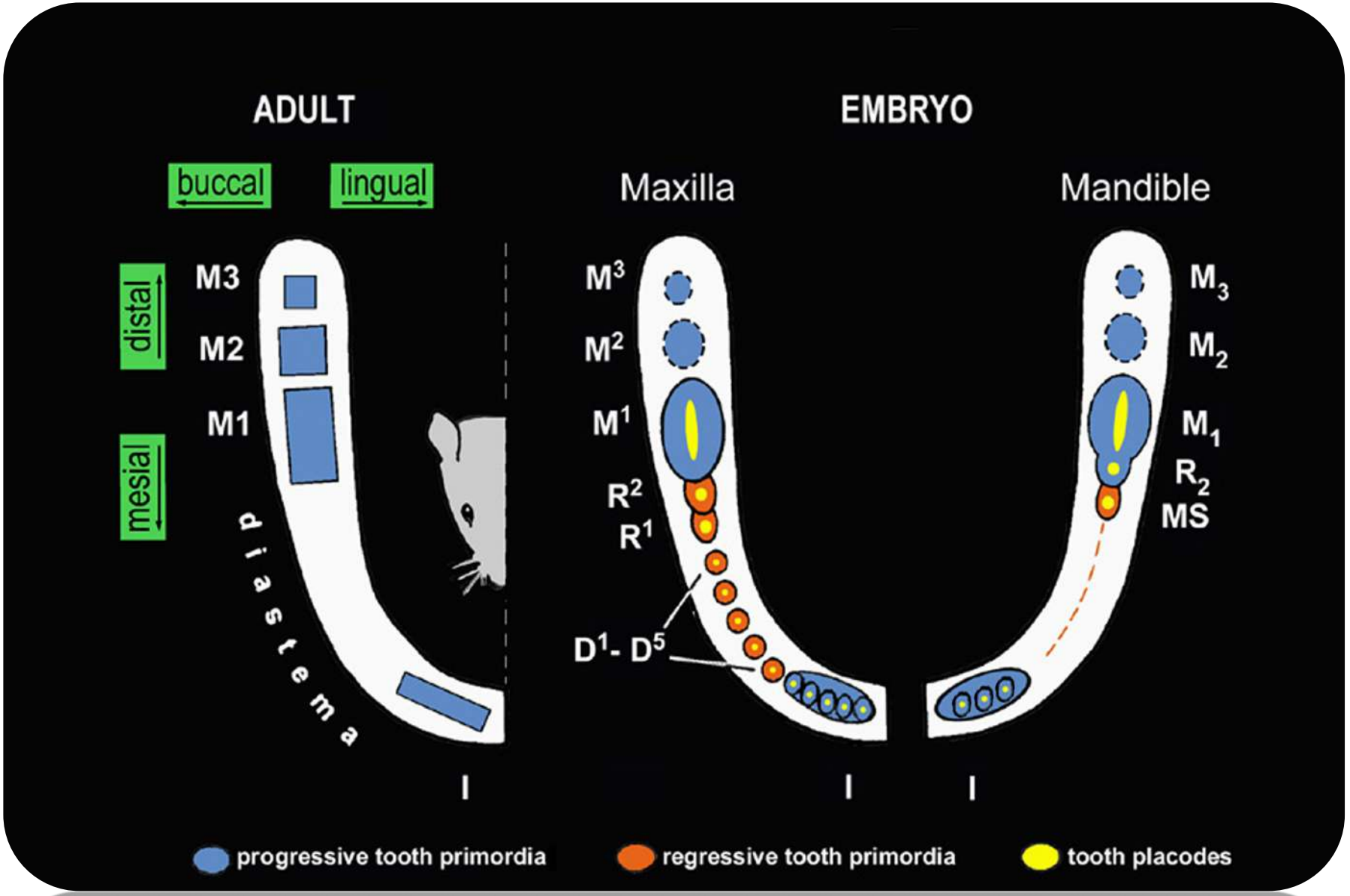


Fig. 1. Reduction of the lower cheek teeth during mouse evolution and their pattern during mouse ontogeny. (*A*) Dentition in the adult mouse is considerably reduced. Each jaw quadrant comprises only one incisor (In) and three molars (M1, M2, M3); a large toothless diastema occurs at the place of missing canine and premolar teeth. (*B–D*) Evolution of the mouse lower molars from a common ancestor of lagomorphs and rodents. (*D*) Two premolars (called P3 and P4) were lost in the mouse lineage (*B*). (*E* and *F*) The two current interpretations of mouse lower molar development. The "rudimentary buds hypothesis" of mouse lower molar row development (*E*) has a basis in descriptive morphological studies and evolutionary data: Two rudimentary premolar buds (MS and R2) are the first tooth primordia, which sequentially develop in the cheek region of mandible, before and in front of molars. These buds' progression is stopped by apoptosis. (*F*) In the classic view, the first molar (M1) is the first tooth primordium that appears in the cheek region of the mandible. Afterward, the other molars (M2, M3) are sequentially added.


ADULT
EMBRYO
buccal
lingual
distal
mesial
M3
M2
M1
diastema
Maxilla
Mandible
M3
M2
M1
R2
R1
D1- D5
M3
M2
M1
R2
MS
progressive tooth primordia
regressive tooth primordia
tooth placodes

JOURNAL OF EXPERIMENTAL ZOOLOGY (MOL DEV EVOL) 306B (2006)

Phylogenetic Memory of Developing Mammalian Dentition

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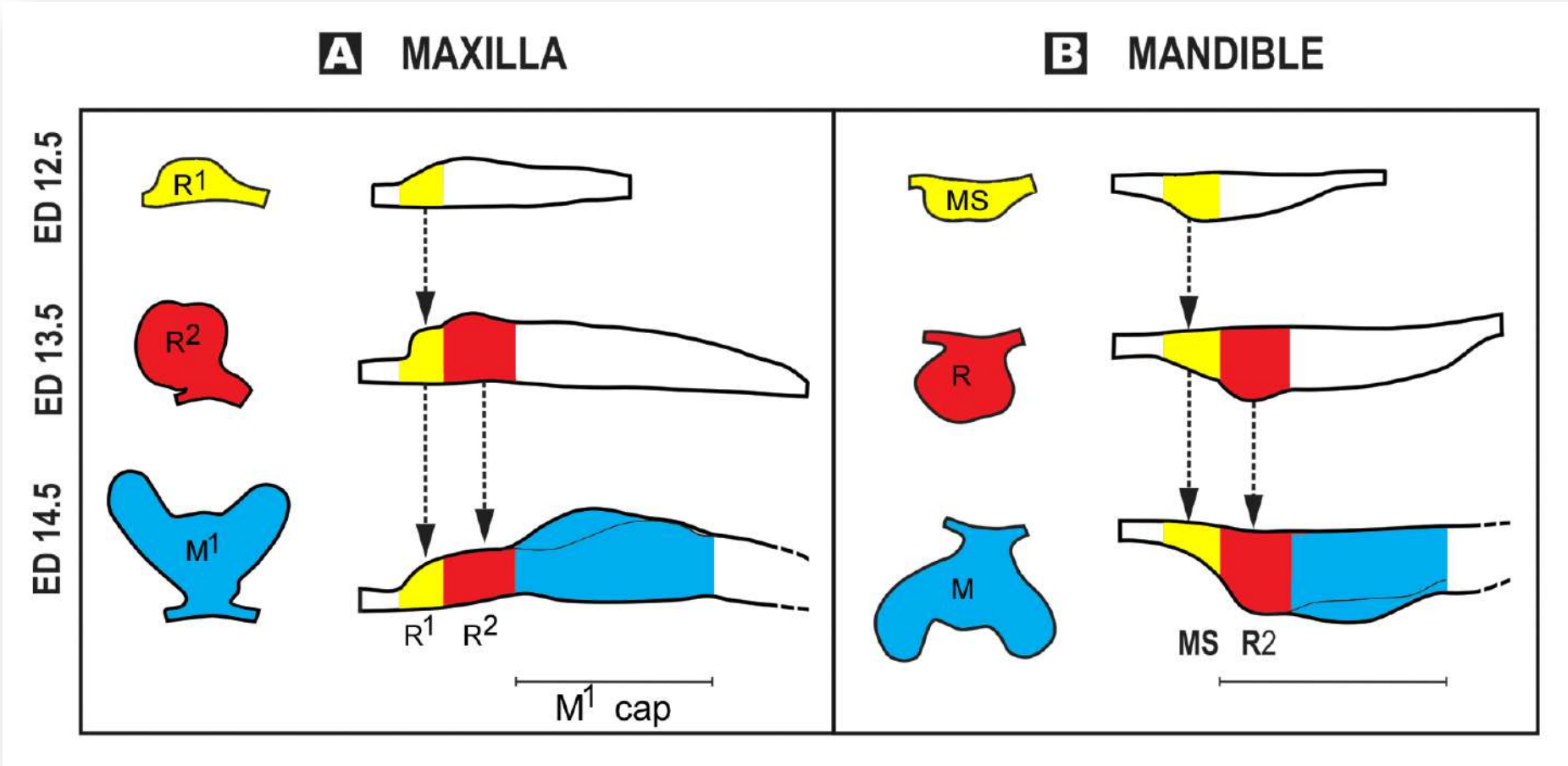
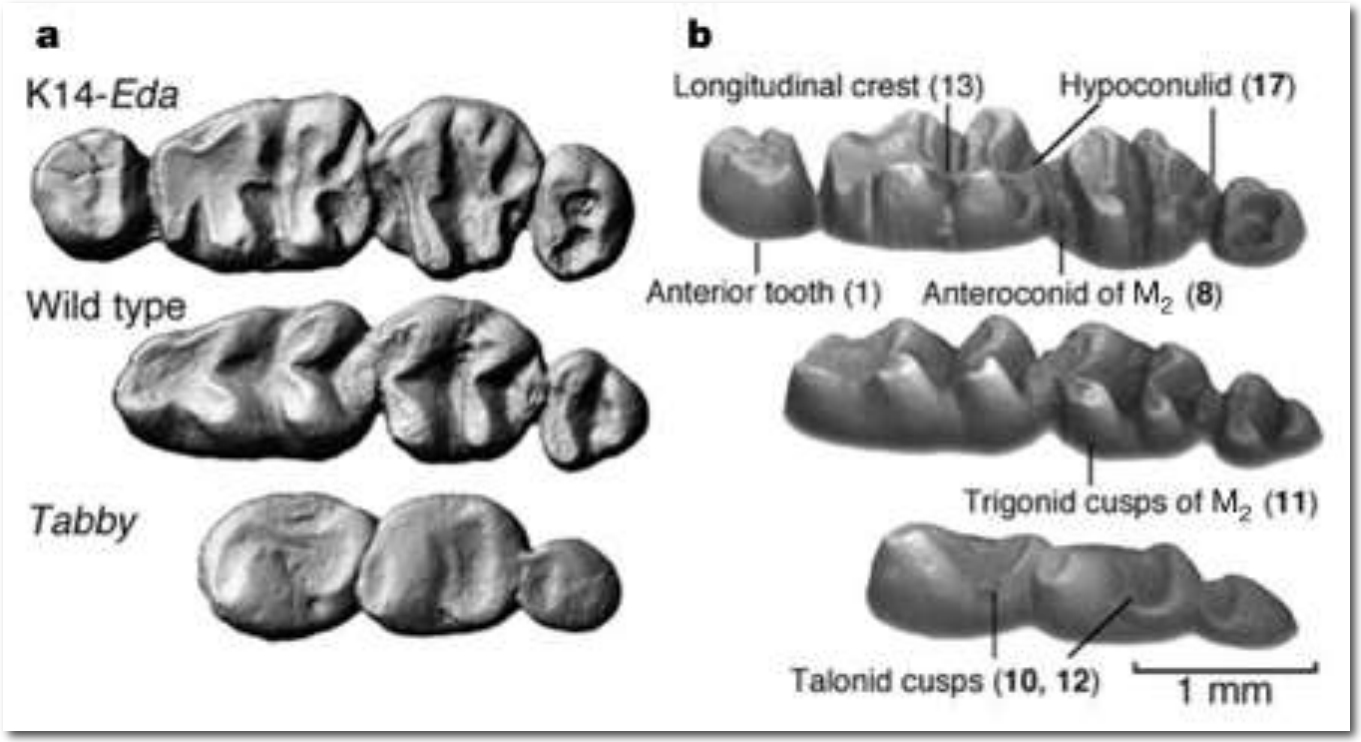


Morfologie zubu (přítomnost zubních hrbolů) závisí na činnosti signalizačních center, tzv. sklovinotvorných hrbolů

- Zubní povrch je generován záhyby epitelo-mesenchymového rozhraní
- Jednotlivé hrboly jsou generovány skolovinovými uzly
- Hrboly nemají pražádný topograficky "specifický" genetický kod - selektován je celkovostní tvar



Zubní povrch je generován záhyby epitelo-mesenchymového rozhraní

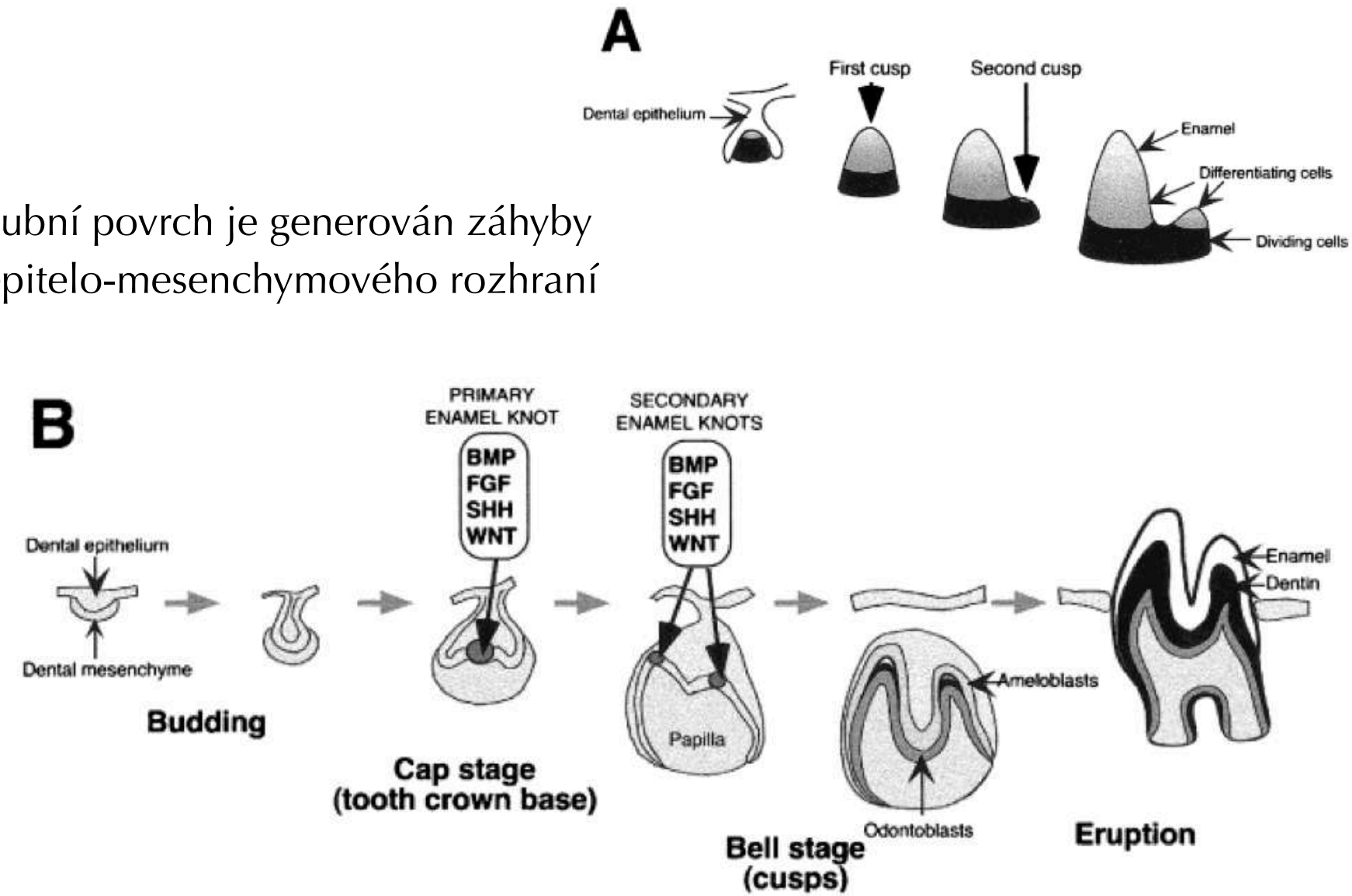


Fig. 3. Schematic representations of tooth development. **A:** Beginning in the cap stage, the tooth crown forms by unequal growth of the inner enamel epithelium (shaded). **B:** Molecular signals considered to be important for particular developmental stages are indicated above the morphology. Note how the same signaling pathways are used repeatedly during advancing tooth development. BMP, bone morphogenetic protein; FGF, fibroblast growth factor; SHH, sonic hedgehog; WNT, wingless-integrated.

Sklovinotvorný hrbol, enamel knot

Enamel knot:
Jednotlivé hrboly zubu jsou generovány skolovinovými uzly, které přímo řídí tvar a pozici zubních hrbolů (primární, sekund., i terciální)

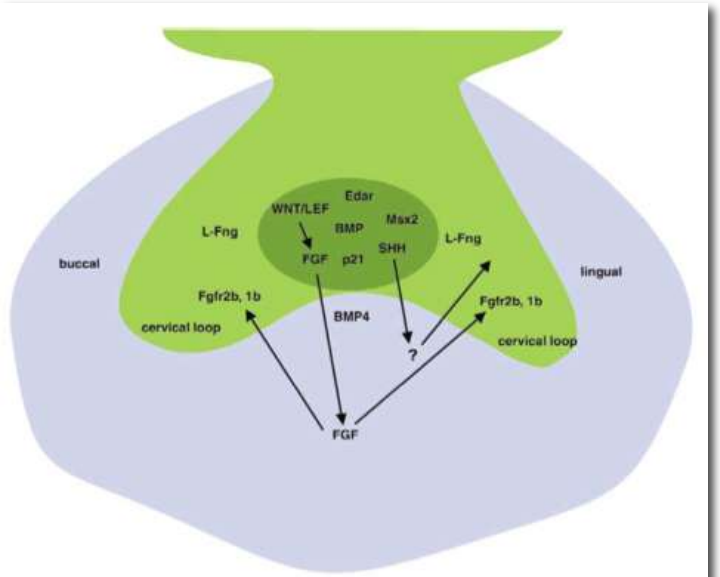
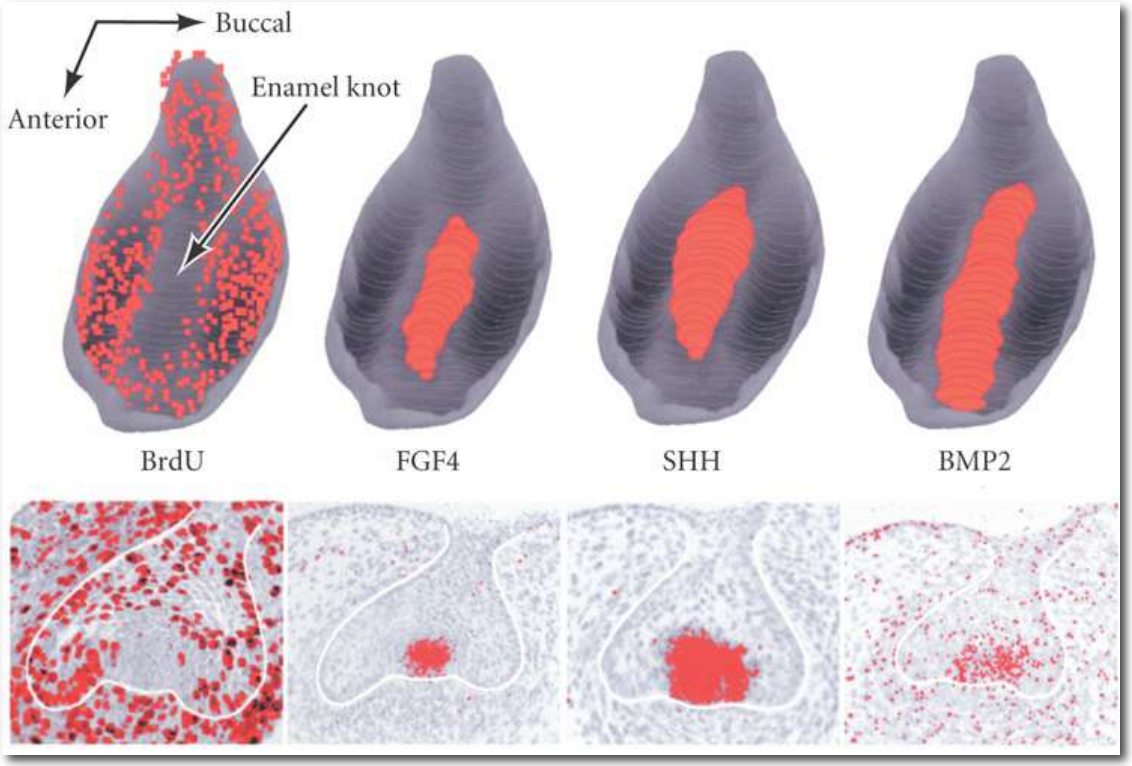


Fig. 3. Regulation of tooth morphogenesis by the signaling center, the enamel knot. More than 10 signaling molecules are locally expressed in the enamel knot (dark green), and regulate the growth and morphogenesis of tooth crown. The function of the enamel knot is regulated by at least Edar and LEF1. Wnt signaling mediated by LEF1 in the enamel knot upregulates FGF, which then induces mesenchymal FGFs, promoting proliferation in the cervical loops. SHH from the enamel knot acts via the mesenchyme to regulate epithelial growth specifically on the lingual side of the tooth germ. Lunatic fringe (L-fng) presumably contributes to the modulation of enamel knot signaling.



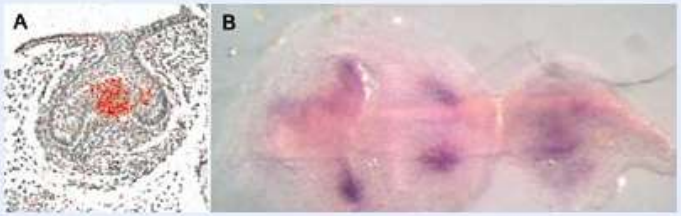
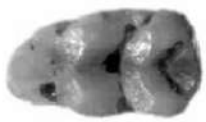
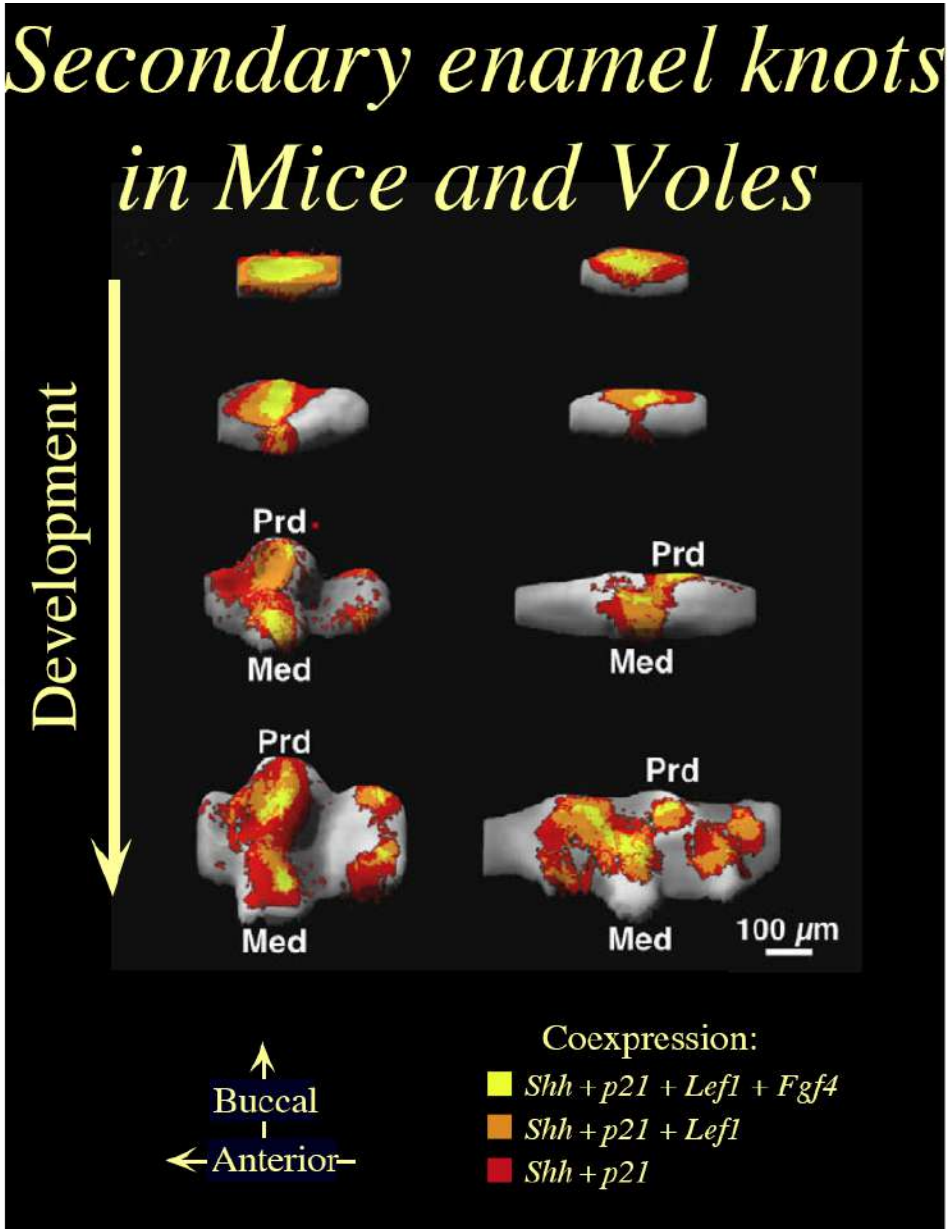


Fig 6 *A. The primary enamel knot is visualized in a frontal section through the cap stage tooth germ (expression of Edar, the receptor for ectodysplasin). B. Secondary enamel knots of the bell stage first molar (left) prefigure cusps. The second molar (right) is at cap stage and the primary enamel knot is seen. (p21 expression, occlusal view of whole mount in situ hybridization).*

Zubní hrboly (cusps) nemají žádný topograficky "specifický" genetický kod - selektován je celkovostní tvar



1st lower molar



1st lower molar

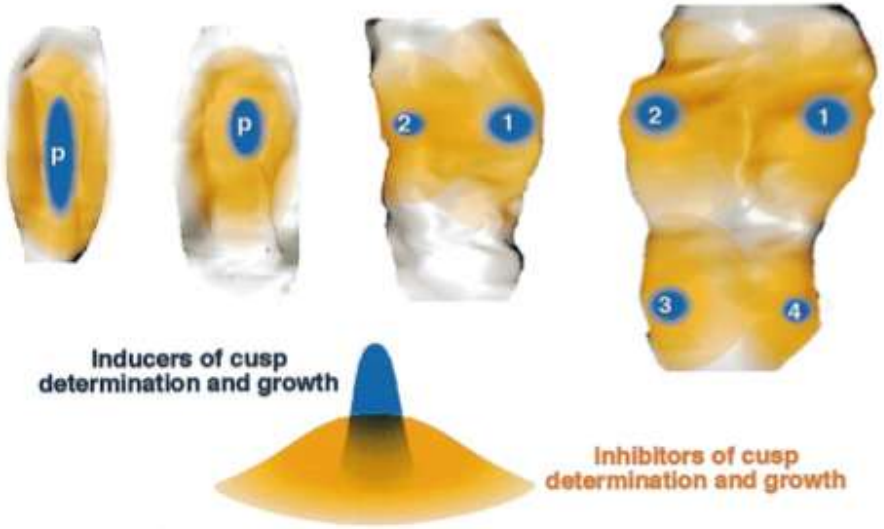
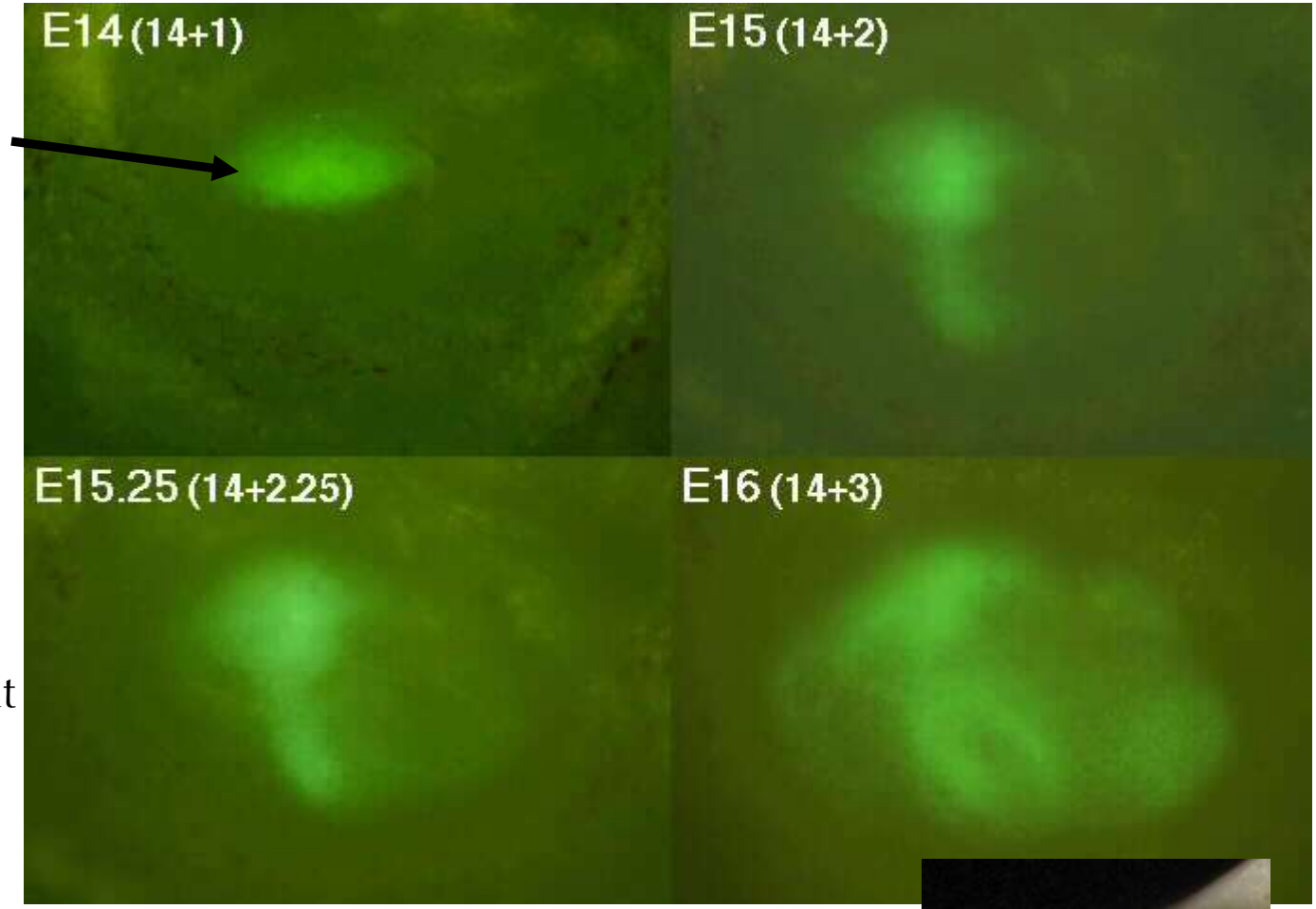


Fig. 5. Sequence of cusp formation in mouse first lower molar (viewed from above). The primary enamel knot (p) establishes the tooth crown base and is removed apoptotically except for the anterior portion in the area forming the first secondary enamel knot (1). The initial folding of the epithelium is longitudinal around the primary knot and subsequent folding happens around each individual secondary enamel knot. The second secondary enamel knot (2) forms in the widest portion of the tooth crown base directly lingual to the first secondary knot. As a model to regulate tooth cusp patterns, FGF4, the expression of which is restricted to the enamel knots (in blue), functions as an activator promoting cusp initiation and growth. Inhibitors, such as BMP4 which has more diffuse expression domain in the mesenchyme, control the minimal distance between adjacent secondary enamel knots and could also negatively regulate cusp growth (in orange). The total number of cusps on a tooth would be limited by the size of the tooth crown base. If the primary knot is small, a small crown base is formed altering the positions and numbers of cusps. In the case of *Tabby* molars (Fig. 4), the primary enamel knot is small and only one large secondary enamel knot forms in the place of two separate ones (Pispa et al., 1999). It is not known if the sequential cusp patterning is controlled by autocrine signaling within epithelium or if it involves continuous paracrine signaling from the mesenchyme. Anterior is toward the top, and buccal toward the right.

Zubní hrboly (cusps) nemají žádný topograficky "specifický" genetický kod - selektován je celkovostní tvar

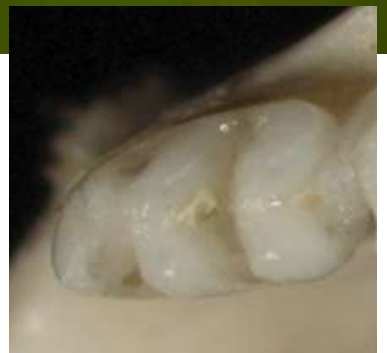
Enamel knot, exprese Shh (GFP-SHH-cre mouse)

Enamel Knot signaling center



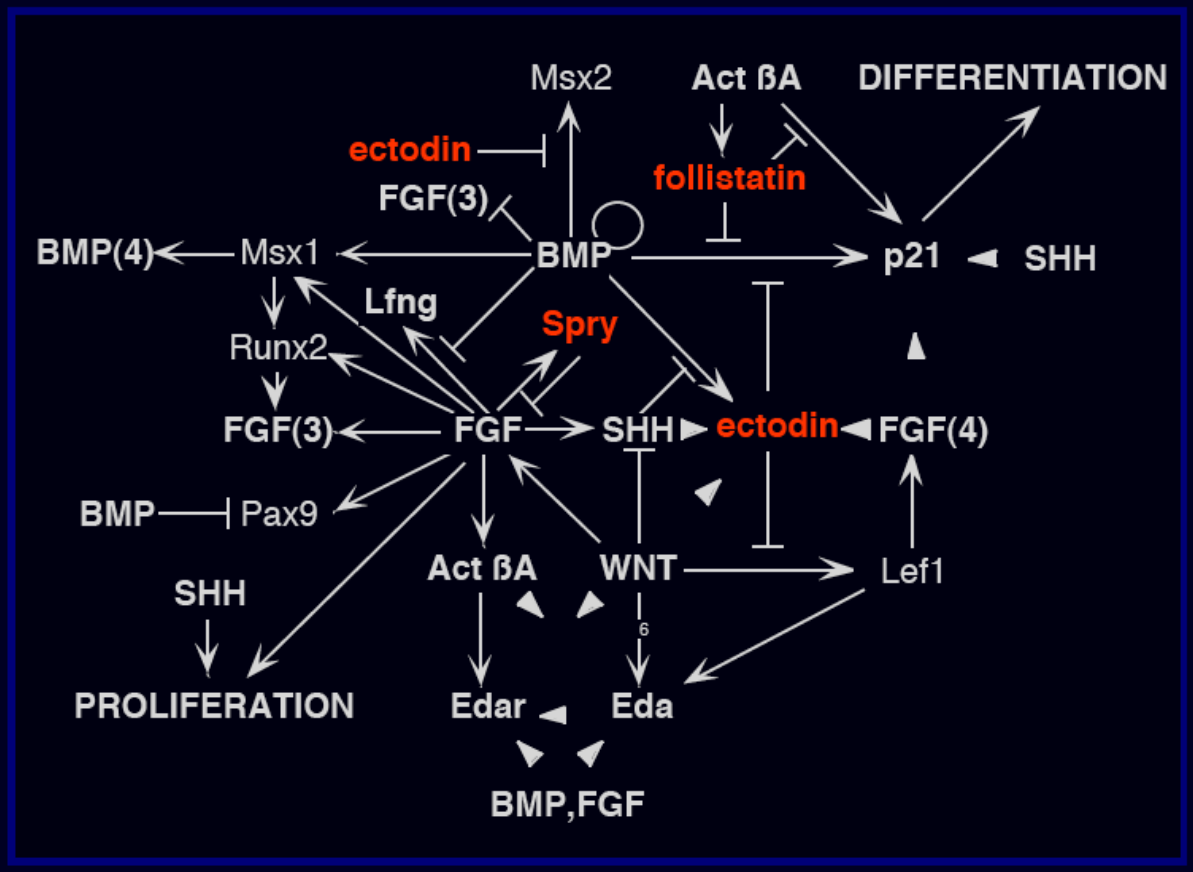
Sonic Hedgehog, SHH, is a developmental regulatory gene that marks the position of the tooth and prospective cusps.

Adult M1



Activator-Inhibitor gene regulatory network of the developing tooth

An emerging pattern from experiments and mathematical modeling: inhibition of the enamel knots



Identification of a secreted BMP antagonist, ectodin, integrating BMP, FGF, and SHH signals from the tooth enamel knot

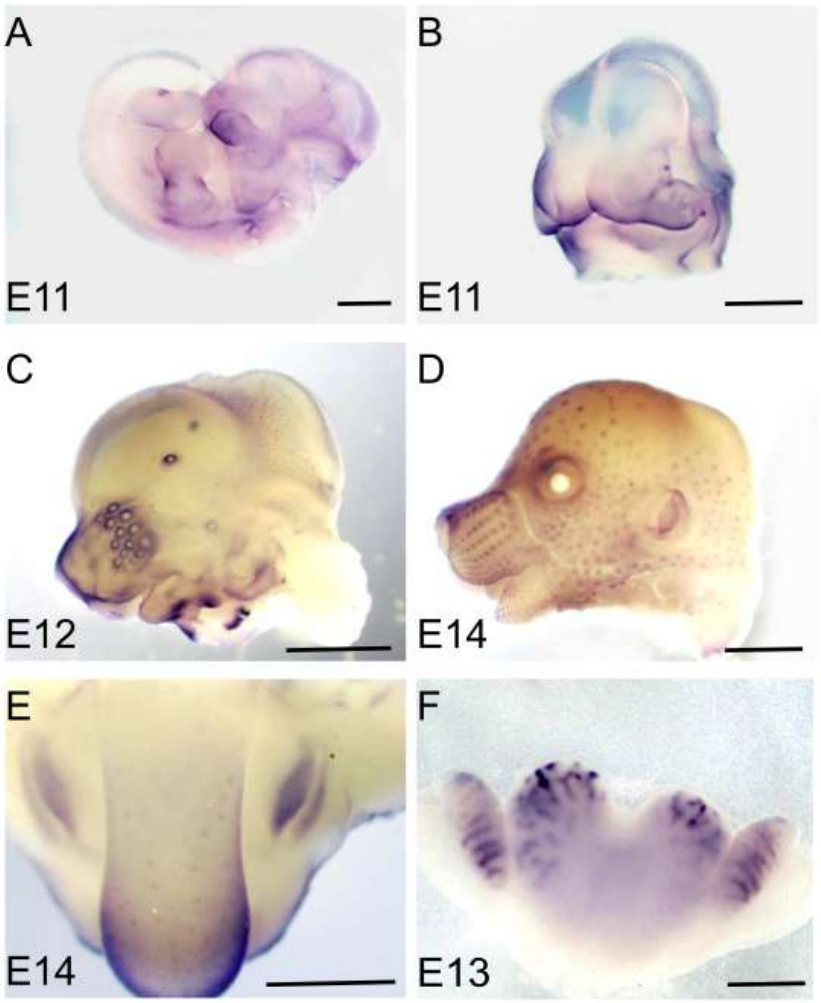
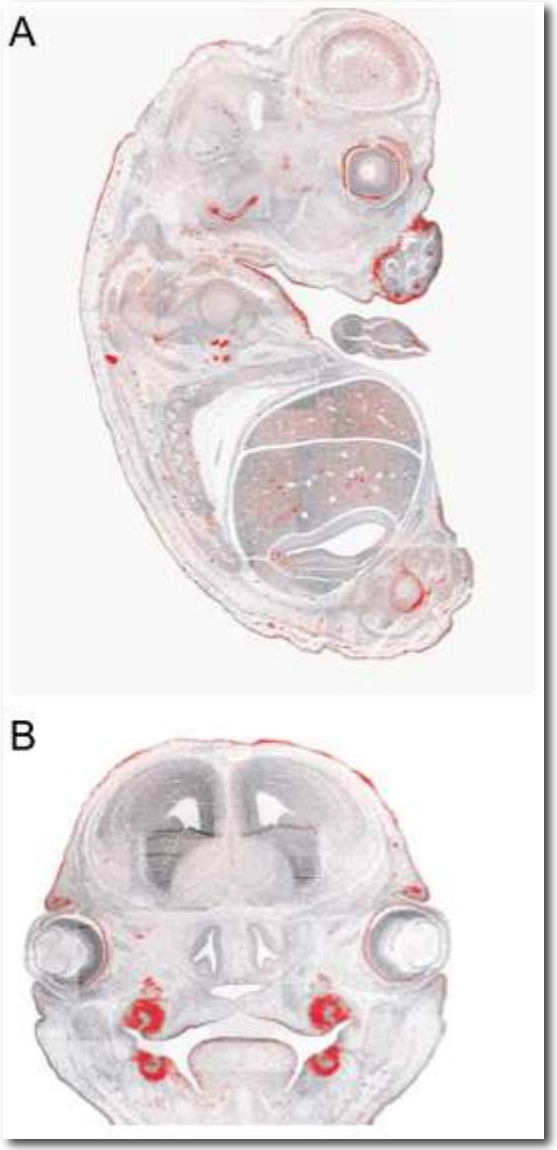


Fig. 5. Whole-mount in situ hybridization analysis of ectodin expression (A) E11 mouse embryo showing expression on the surface of the branchial arches and in limb buds. (B) Frontal view of E11 head shows staining at the surface of facial processes. (C) At E12, staining in vibrissae is seen as circles. (D) At E14, the vibrissae, hair follicles, and ear auricle show *ectodin* expression. (E) In the dissected E14 mandible molar tooth germs, tongue papillae and surface ectoderm express *ectodin* intensely. (F) In the dissected urogenital block of E13 embryo, *ectodin* expression is seen in the stalk and tips of ureter in the kidneys. In the testes, expression is intense in the spermatic ducts. Bar, 100 mm.



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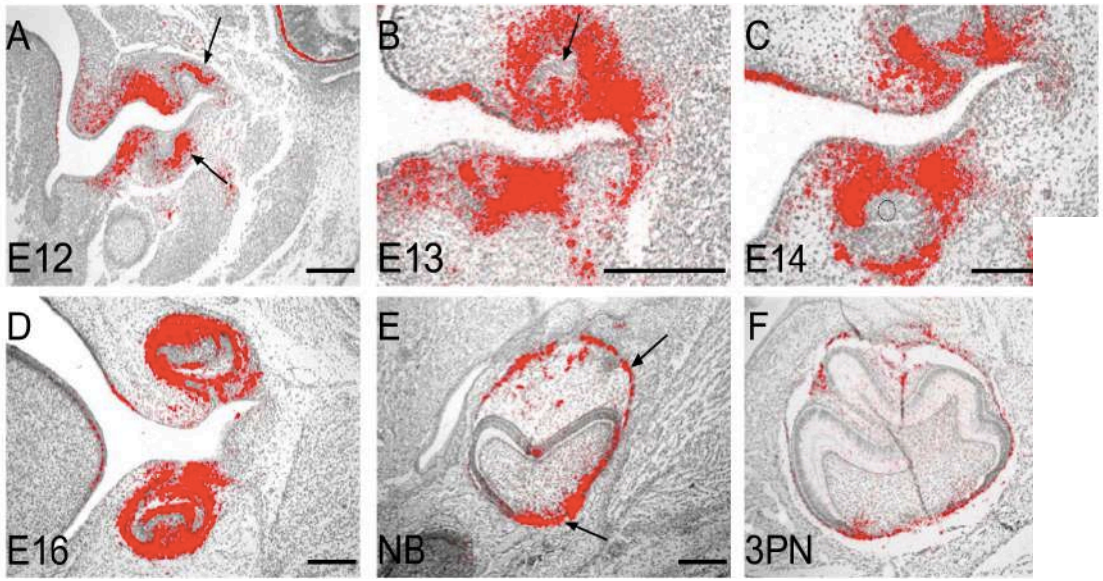


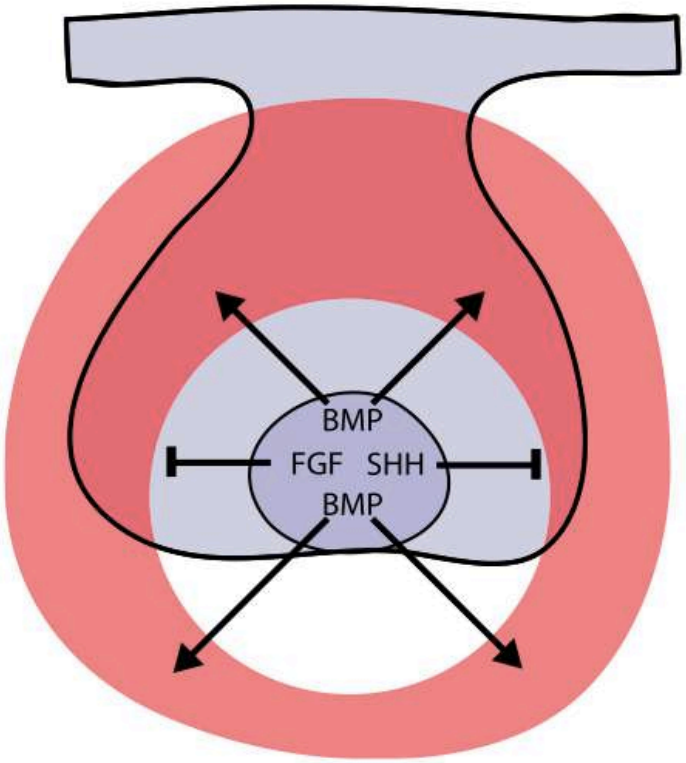
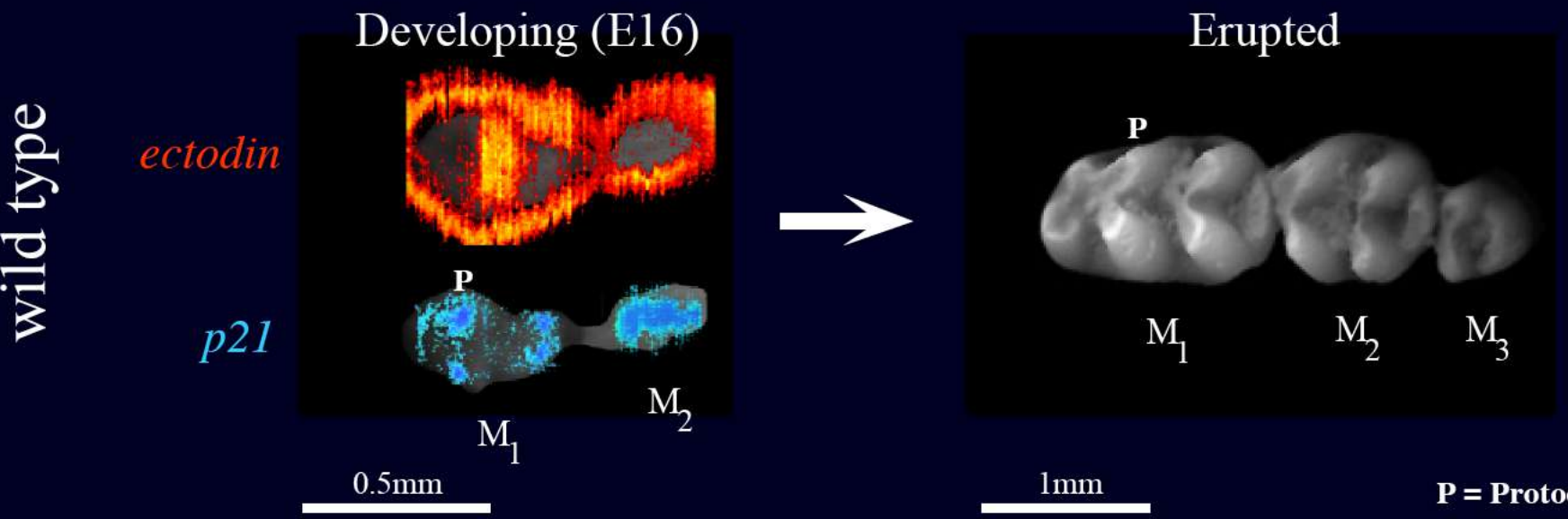


Fig. 10. Schematic picture showing how ectodin integrates BMP, SHH, and FGF signals from the enamel knot signalling center. BMPs (BMP2, −4, −7) stimulate the expression of their inhibitor ectodin around the enamel knot. The stimulation is counteracted by SHH and FGFs, also expressed in the enamel knot, and this results in an ectodin-negative area around the enamel knot determining the target field of BMP signaling. Red, area of ectodin expression; circled area, enamel knot (compare with Figs. 6B and 7C).

Inhibiting enamel knots

ectodin (a.k.a.: *Sostdc1, USAG1, wise*), a BMP antagonist, member of DAN/Cerberus family



Science 309, 2067 (2005)

Inhibiting enamel knots

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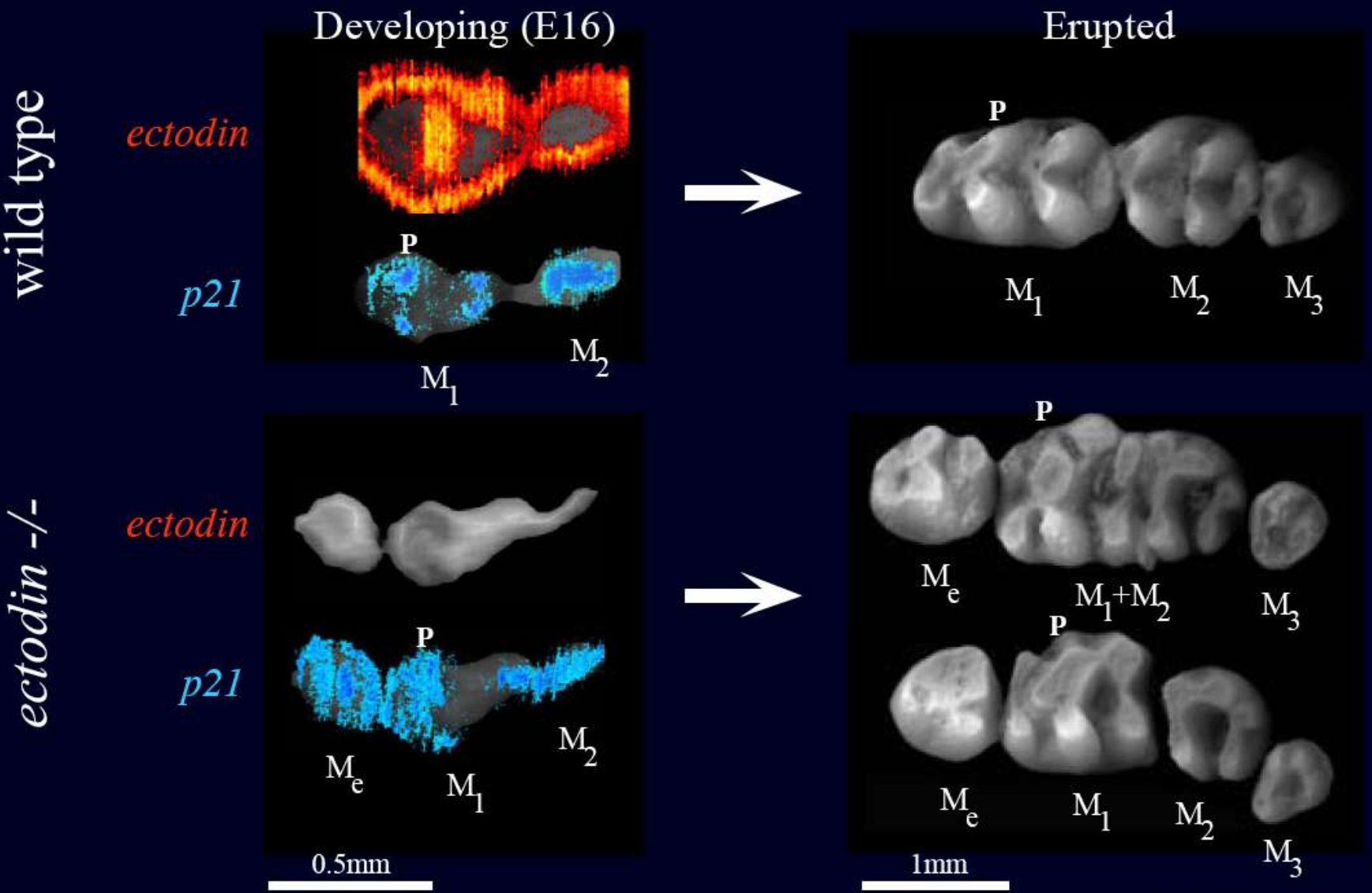

Science 309, 2067 (2005)

Morfologie zubních hrbolů je odvislá od činnosti sklovinotvorných hrbolů - enamel knots

- Zubní povrch je generován záhyby epitelo-mesenchymového rozhraní
- Jednotlivé hrboly jsou generovány skolovinovými uzly
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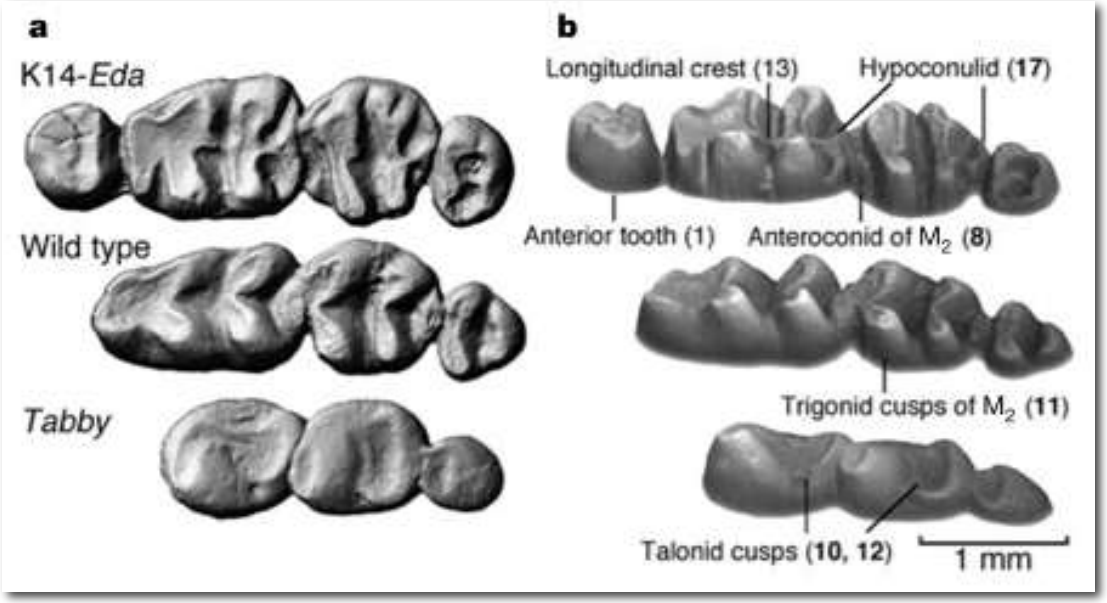
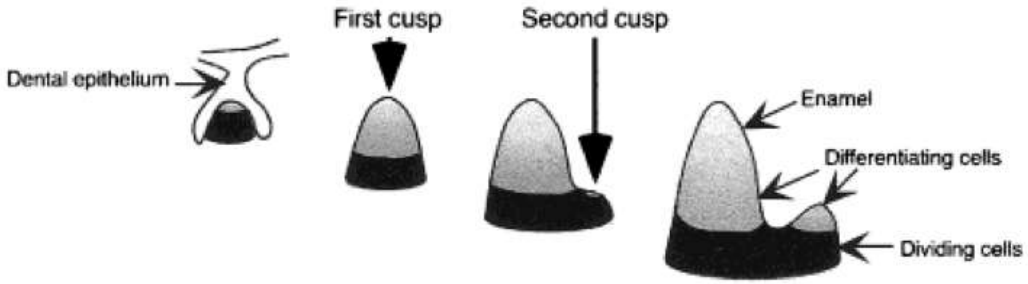
Ectodysplasin ++

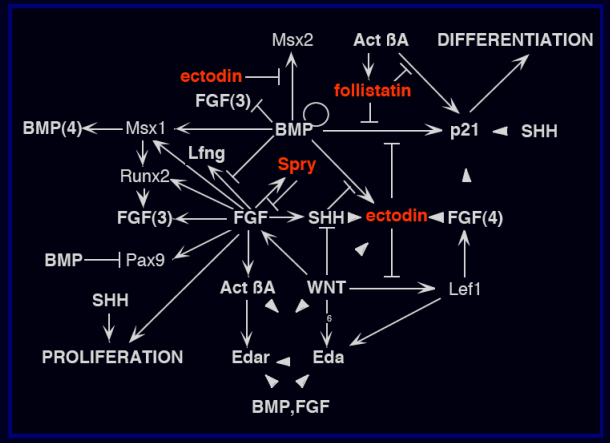


Ectodysplasin --

- Identický modul genetické signalizace (GRN), díky kterému vzniká ZUB, je repetitivně používán pro vznik mnoha dalších struktur...
  - Platí též v rámci zubu pro jednotlivé zubní vrcholy (cusps)
    - Platí též pro ZUB – DENTICE!



An emerging pattern from experiments and mathematical modeling: inhibition of the enamel knots



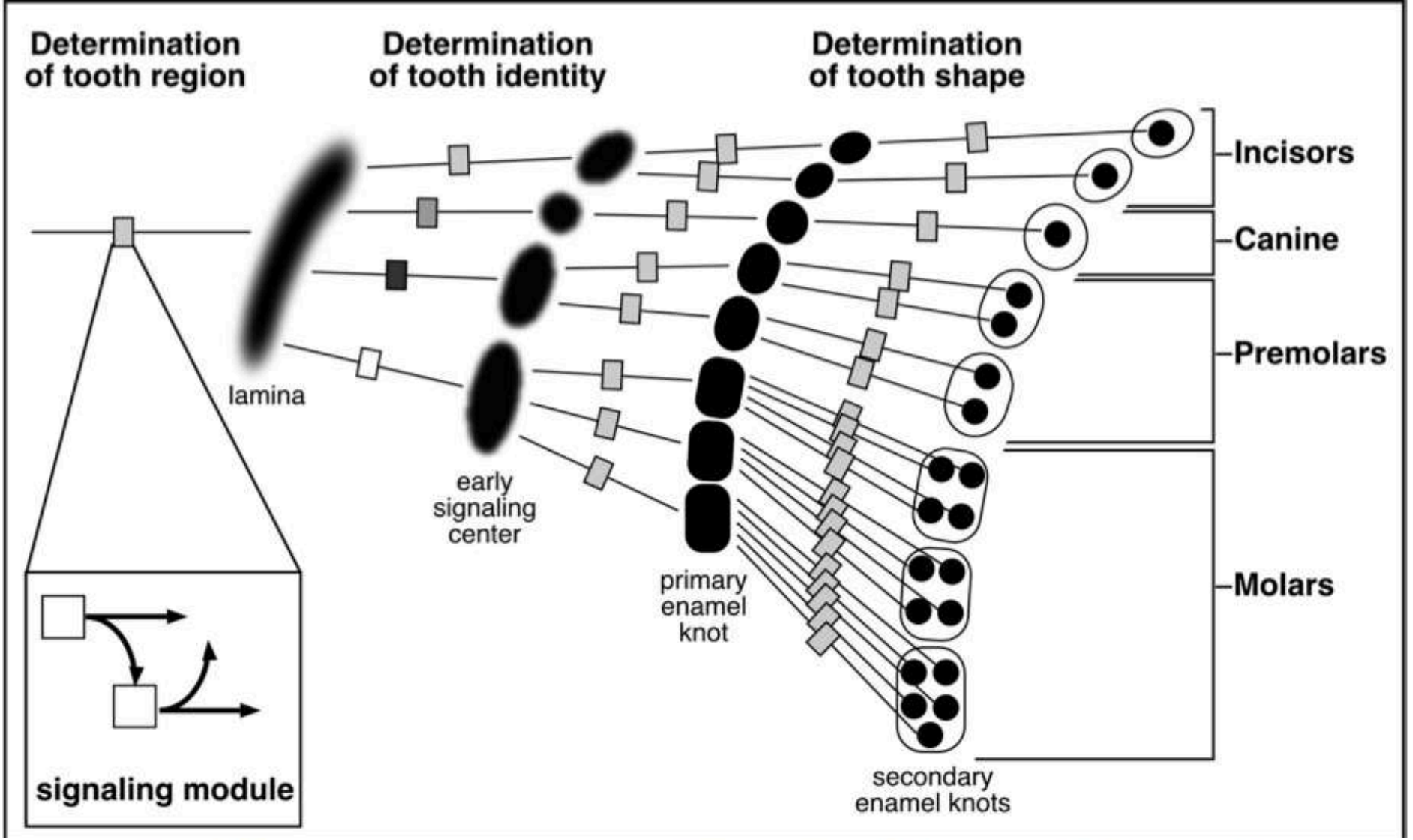


Fig. 6. Largely the same signaling modules (see Fig. 2) are reiterated from the tooth initiation to the formation of cusps. In each iteration, the dental region is partitioned into new compartments and a progressively larger number of the signaling domains are induced. The first partitioning involves the formation of tooth identity (incisor, canine, premolar, or molar identity) and may be regulated by differences in signaling (represented as different shadings in the signaling boxes) after the determination of dental lamina or already prior to lamina formation (see for discussion Weiss et al., 1998a,b). Generally, the premolar and molar teeth have several cusps but they can also be unicusped (e.g. many seals); incisors can also have many cusps (e.g. flying lemurs, Dermoptera). Deciduous teeth (milk teeth) are generally equal or more complex in morphology than their replacement teeth (teeth not shown). As the same genes are repeatedly used in tooth development, knockout experiments affecting signaling will mostly result in early disruption of tooth development and also affect other organs sharing the same signaling pathways (e.g. Kratochwil et al., 1996; Hardcastle et al., 1998; De Moerlooze et al., 2000).

- Identický modul signalizace funguje pro zubní vznik (ontogenezi) i následné dorůstání zubů (replacement, renewal)
- U savců neschopnost kontinuálního obnovování zubů (díky značné specializaci) kompenzována trvalým růstem některých zubů
- **Vzájemně propojený systém jehož modulací vzniká diverzita zubních patternů**

Development 139, 3487-3497 (2012) doi:10.1242/dev.085084

Tooth shape formation and tooth renewal: evolving with the same signals

Jukka Jernvall* and Irma Thesleff*

Summary

Teeth are found in almost all vertebrates, and they therefore provide a general paradigm for the study of epithelial organ development and evolution. Here, we review the developmental mechanisms underlying changes in tooth complexity and tooth renewal during evolution, focusing on recent studies of fish, reptiles and mammals. Mammals differ from other living vertebrates in that they have the most complex teeth with restricted capacity for tooth renewal. As we discuss, however, limited tooth replacement in mammals has been compensated for in some taxa by the evolution of continuously growing teeth, the development of which appears to reuse the regulatory pathways of tooth replacement.

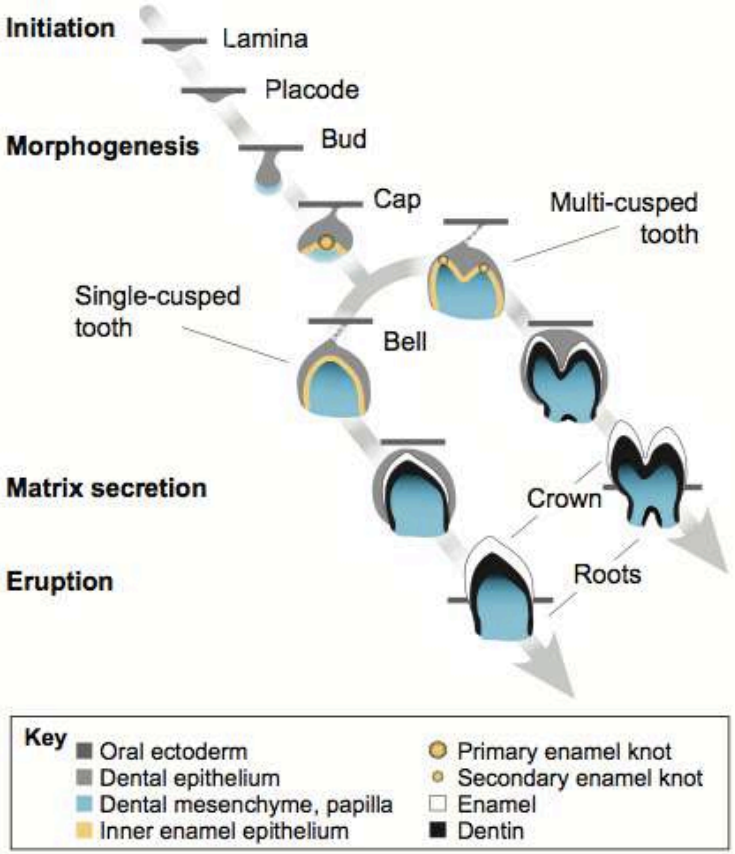


Fig. 1. The principal stages of tooth formation. Prior to the initiation of tooth development, the tooth-forming region (the dental lamina) appears within the dental epithelium. The development of individual teeth is then initiated within specific domains of the lamina, referred to as placodes. During the bud stage, the dental epithelium invaginates into the dental mesenchyme, which condenses around the epithelium to form a bud. Then, during the cap stage, the epithelium extends further into the mesenchymal tissue and wraps itself around the condensing mesenchyme. The cap stage is followed by the bell stage, during which species-specific cusp patterns emerge: in a single-cusped tooth, a primary enamel knot, which first appears at the cap stage, gives rise to the tip of the crown; in multicusped mammalian teeth, secondary enamel knots form at the places of future cusps. This stage is then followed by final growth and matrix secretion, during which time the inner enamel epithelium differentiates into ameloblasts, which produce enamel, and the adjacent mesenchymal cells differentiate into odontoblasts that secrete dentin. Roots continue to develop during eruption.

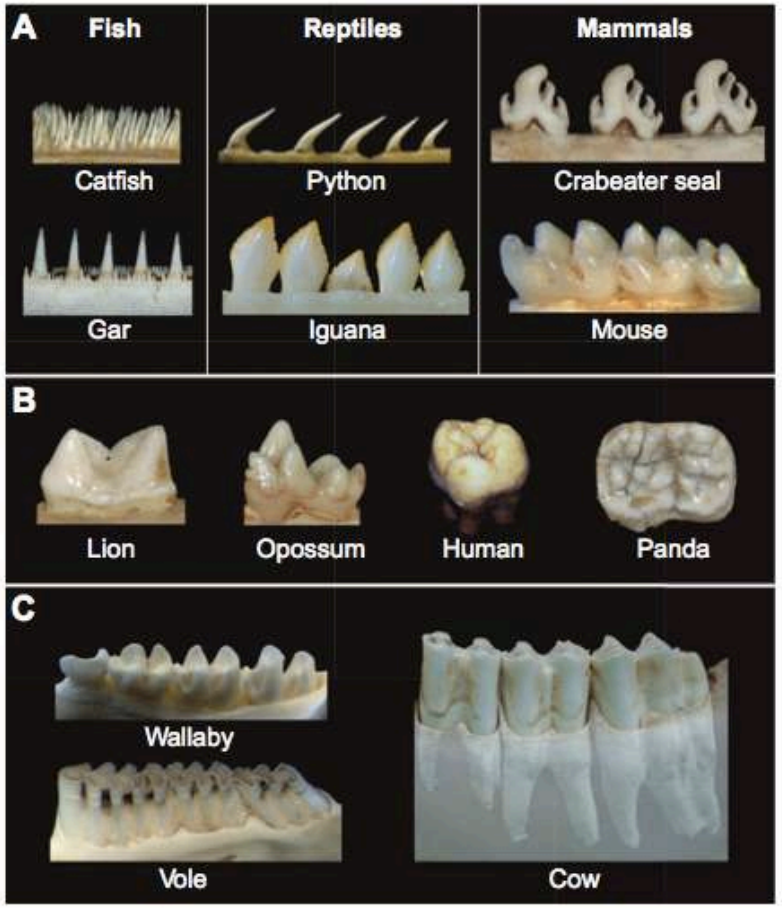


Fig. 2. Evolutionary diversity within the teeth of living species. (**A**) Teeth are found across the vertebrates but mammalian teeth tend to have more cusps and are generally more complex, whereas fish and reptiles tend to have larger numbers of simple teeth. (**B**) Among mammals, molars are generally simpler in animal-eating species (e.g. lions), whereas plant-eating species (e.g. pandas) have complex crown topography. (**C**) Mammals have limited tooth renewal capacity and only a few groups of mammals (e.g. wallabies) have species that can develop new molars posteriorly. A more common solution is tall (hyposdont) teeth, where only part of the crown is visible outside the jaw (note that the parts inside the jaw are made visible in the image of cow molars). Some mammalian species (e.g. the vole) have ever-growing (hypselodont) molars. By contrast, fish and reptiles have continuously replaced teeth (e.g. see tooth being replaced in iguana, in A). Anterior is towards the left. Images are not to the same scale.

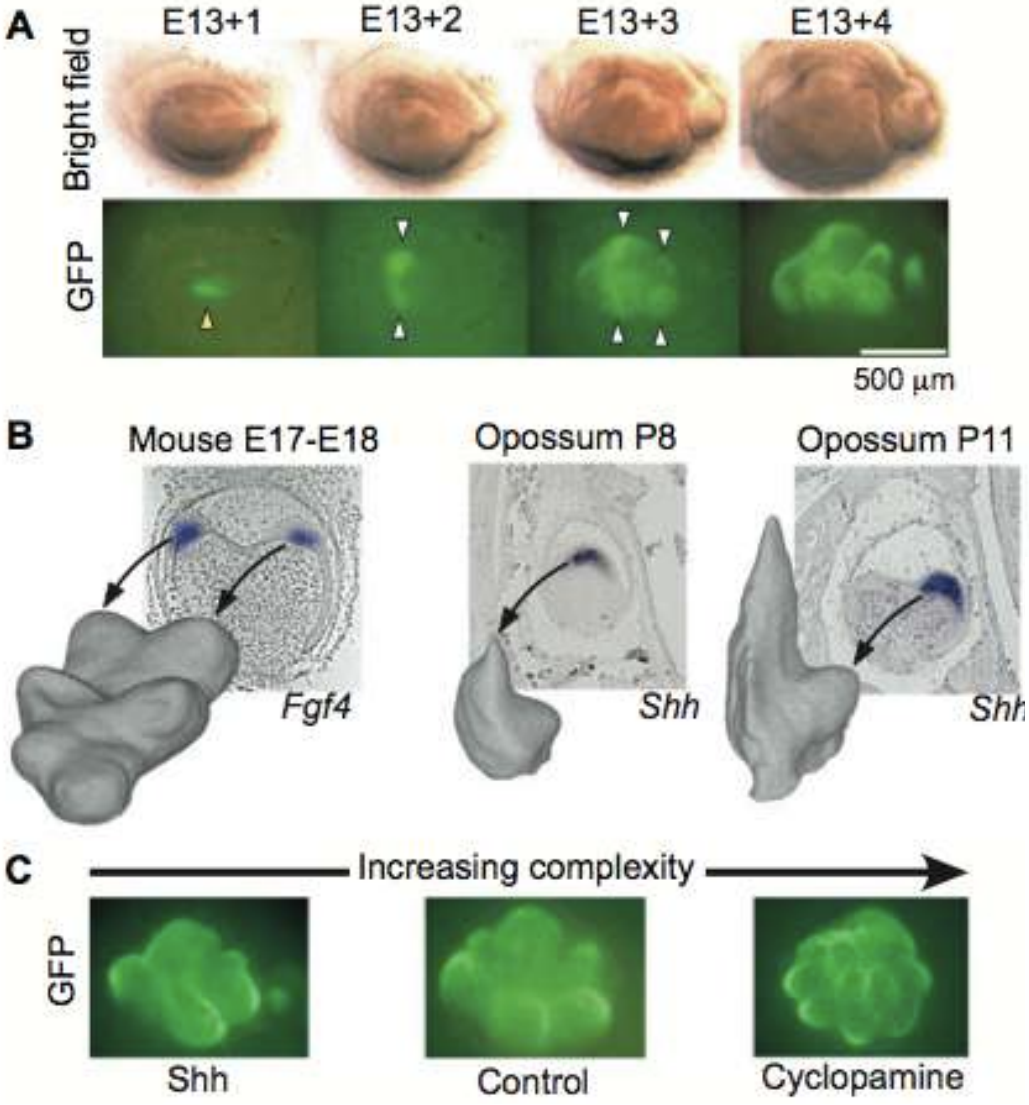


Fig. 3. Cusp formation in mammals is regulated by secondary enamel knots. (**A**) A molar from a heterozygous *Shh*-GFP transgenic mouse, which expresses green fluorescent protein (GFP) under a *Shh* promoter. Bright-field and fluorescence images show the primary enamel knot (yellow arrowhead) at E13+1 and secondary enamel knots of the main cusps (white arrowheads) at E13+2 and E13+3. Later (at E13+4), GFP, and hence Shh, is expressed in the differentiating ameloblasts throughout the crown. (**B**) Histological sections (top) stained for *Fgf4* and *Shh* expression and three-dimensional renderings of the epithelial-mesenchymal interface (bottom) of developing mouse and opossum teeth show the appearance of cusps. Mouse cusps are initiated very close in time to each other and the cusps become close to equal in height. By contrast, opossum cusps are initiated several days apart resulting in cusps that are unequally tall (as also seen in the opossum tooth shown in Fig. 2B). (**C**) Mouse molars cultured with Shh (left) exhibit a delay in cusp formation and a reduction in cusps (compared with control molars; centre), whereas culture with the Shh inhibitor cyclopamine (right) increases cusp formation, allowing them to form close to each other. Molars were treated for 4 days and the images are at 8 days. The sections of opossum teeth are modified, with permission, from Moustakas et al. (Moustakas et al., 2011); the section of mouse teeth is modified, with permission, from Jernvall et al. (Jernvall et al., 1994); and Shh treatments in C are modified, with permission, from Harjunmaa et al. (Harjunmaa et al., 2012).

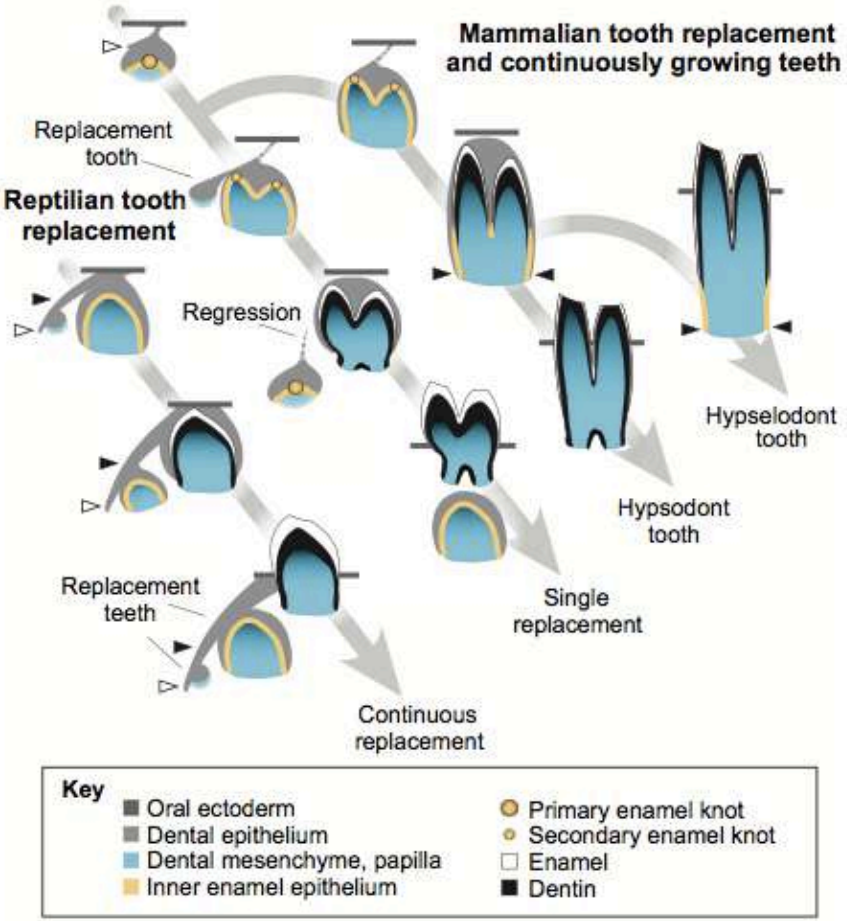


Fig. 4. Tooth renewal differs among species. The reptilian mode of continuous tooth replacement (shown on the left) involves a dental lamina that extends to form a successional lamina (white arrowheads). A stem cell niche is retained in the dental lamina (black arrowheads). This form of replacement is also likely to be present in many fish. In mammals, the replacement tooth bud develops from the successional lamina as in reptiles, but the lamina regresses and continuous tooth replacement does not occur. Instead, in many mammalian lineages teeth have become tall (hypsodont) by delaying root formation; hence, the teeth can wear more (also see Fig. 2C). The most-derived stage of tooth regeneration is hypselodonty, which is found, for example, in rodent incisors and vole molars, where the tooth retains stem cells at its base and continues to grow throughout the life of the individual.

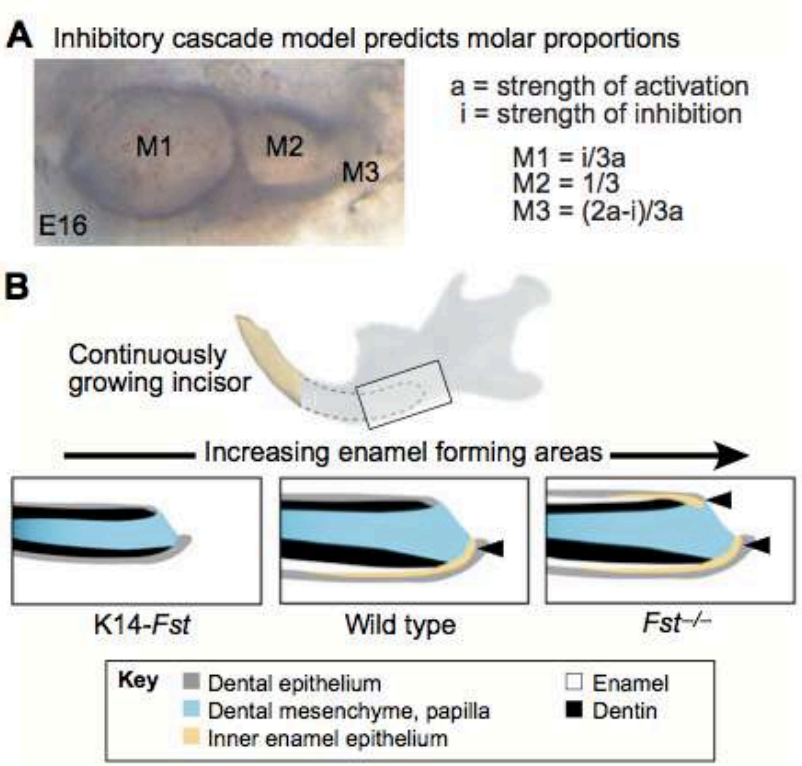


Fig. 6. Sequential initiation of molars and continuous growth of rodent incisors. (**A**) Molar teeth (M1, M2 and M3) develop sequentially. Many species have the potential to develop a fourth molar after the formation of the third molar and a few species have continuous generation of new molars. An inhibitory cascade model, derived from experimental manipulation of mouse molars, predicts molar proportions and number. When supernumerary molars form, the molars should be roughly the same size. In this model, 'a' denotes strength of the activator and 'i' denotes strength of the inhibitor. Candidate activators include BMPs and activin A; candidate inhibitors include Shh. (**B**) Continuously growing incisors of a wild-type mouse retain epithelial stem cells (black arrowheads) on their labial (outer) side. The lingual side lacks enamel. Deletion of follistatin (*Fst*$^{-/-}$) results in ectopic lingual enamel, whereas the expression of follistatin throughout the epithelium under the control of the keratin 14 promoter (K14-*Fst*) causes a loss of enamel and of the stem cell niche. Follistatin is not normally expressed in the labial inner enamel epithelium. Molar culture image in A is modified, with permission, from Kavanagh et al. (Kavanagh et al., 2007).

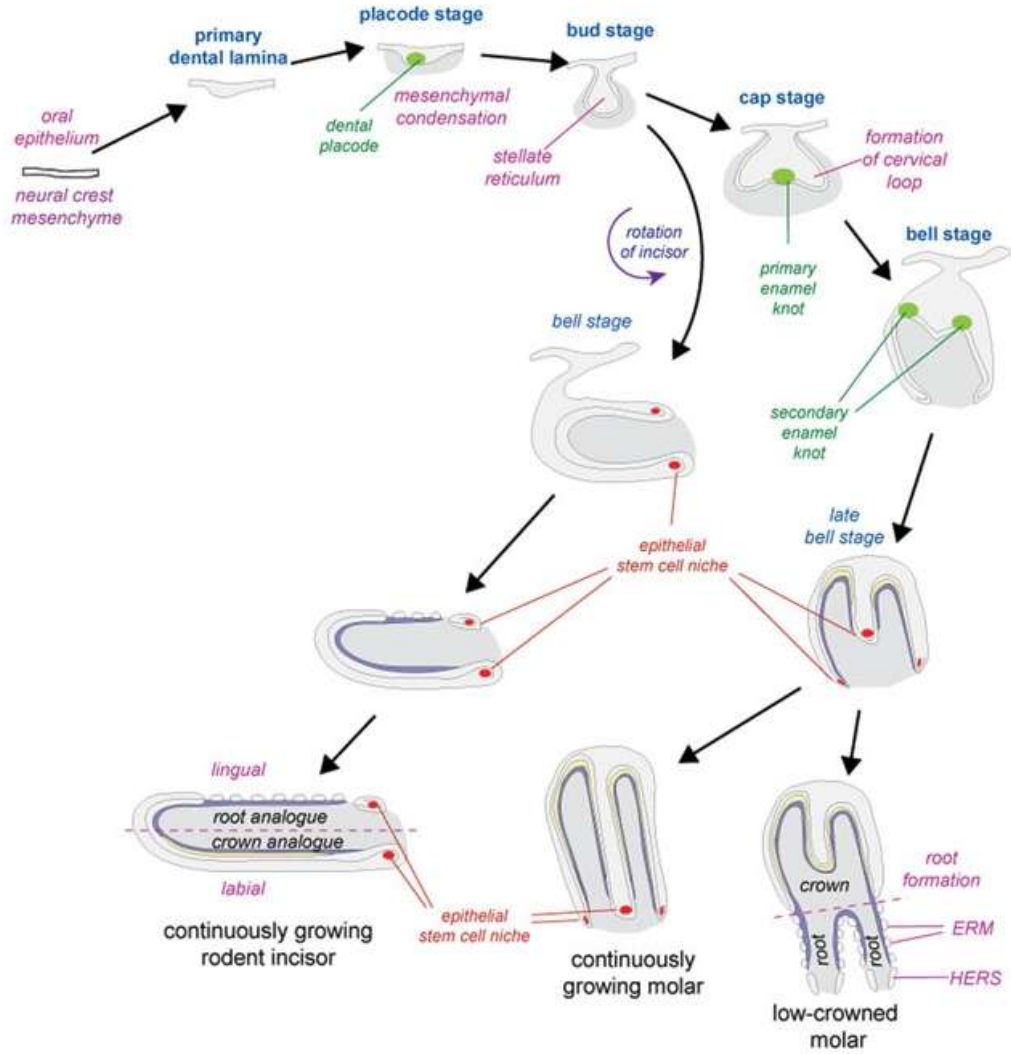
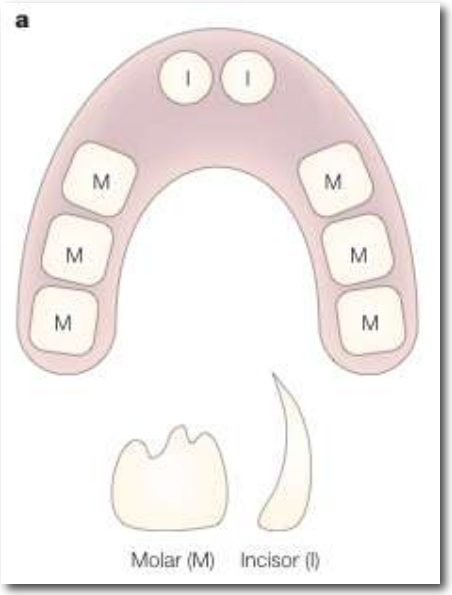


Figure 1.

The developmental anatomy of early tooth morphogenesis and the formation of different tooth types: low-crowned molar, continuously growing molar with a complex cusp pattern, and continuously growing incisor lacking a complex cusp pattern.



Fgf-signalizace jako příčina ztráty orální dentice u kaprovitých ryb

Developmental genetic mechanisms of evolutionary tooth loss in cypriniform fishes

David W. Stock*, William R. Jackman and Josh Trapani†

The fossil record indicates that cypriniform fishes, a group including the zebrafish, lost oral teeth over 50 million years ago. Despite subsequent diversification of feeding modes, no cypriniform has regained oral teeth, suggesting the zebrafish as a model for studying the developmental genetic basis of evolutionary constraint. To investigate the mechanism of cypriniform tooth loss, we compared the oral expression of seven genes whose mammalian orthologs are involved in tooth initiation in the zebrafish and the Mexican tetra, *Astyanax mexicanus*, a related species retaining oral teeth. The most significant difference we found was an absence in zebrafish oral epithelium of expression of *dlx2a* and *dlx2b*, transcription factors that are expressed in early *Astyanax* odontogenic epithelium. Analysis of orthologous genes in the Japanese medaka (*Oryzias latipes*) and a catfish (*Synodontis multipunctatus*) suggests that expression was lost in cypriniforms, rather than gained in *Astyanax*. Treatment of *Astyanax* with an inhibitor of Fibroblast growth factor (Fgf) signaling produced a partial phenocopy of the zebrafish oral region, in that oral teeth, and expression of *dlx2a* and *dlx2b*, were lost, whereas *shh* and *pitx2*, genes whose expression is present in zebrafish oral epithelium, were unaffected. We hypothesize that a loss of Fgf signaling to oral epithelium was associated with cypriniform tooth loss.

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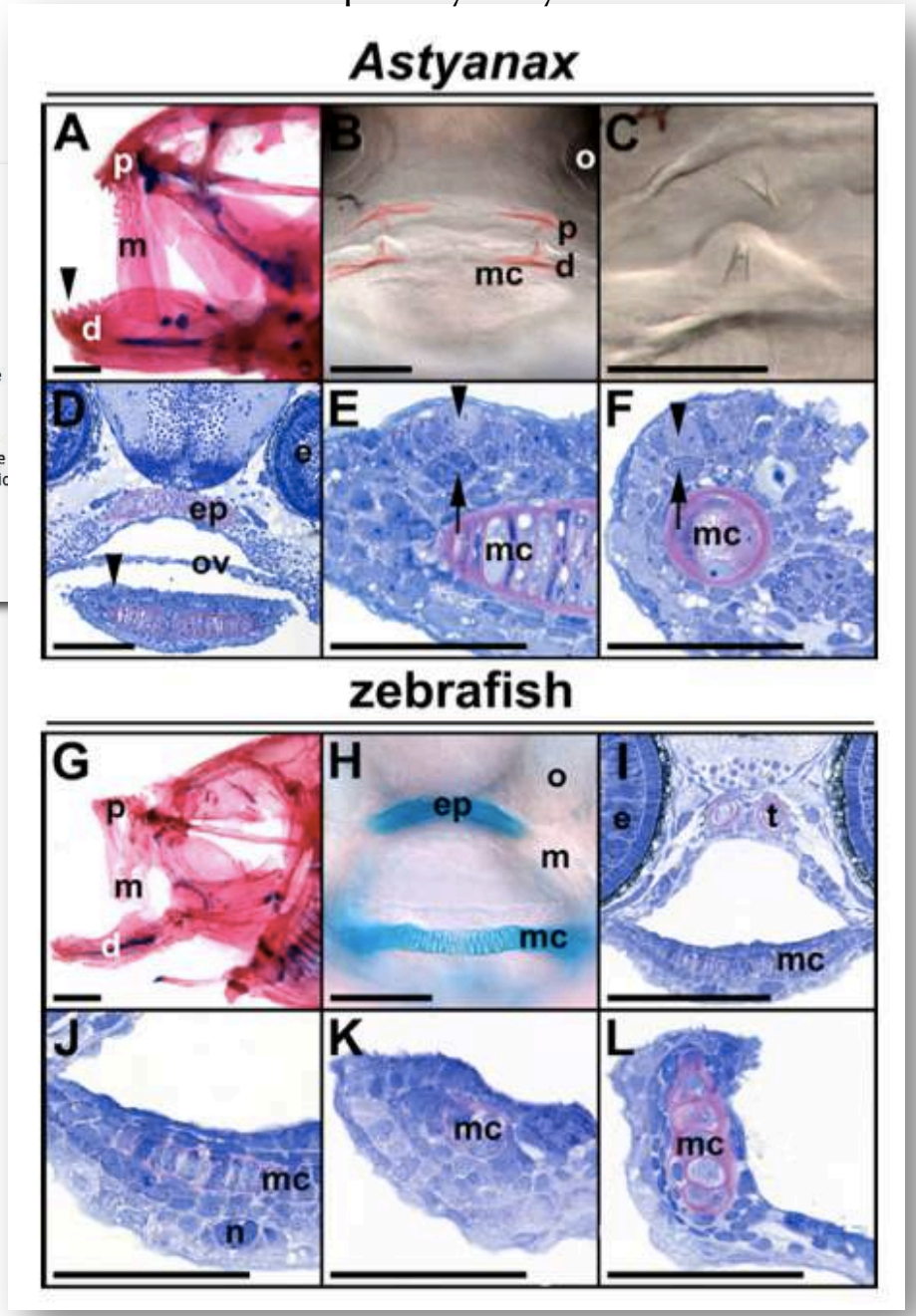
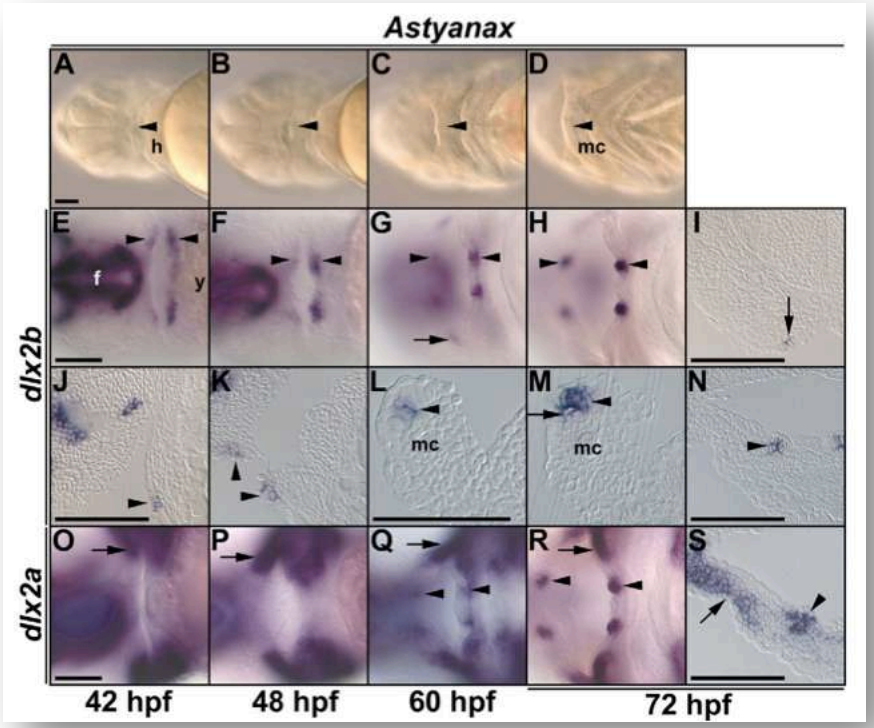


Fig. 1. Oral morphology in *Astyanax* and zebrafish. (**A**) Teeth are present on premaxillary (p), maxillary (m) and dentary bones (d, arrowhead) of adult *Astyanax* (lateral view, cleared and stained with Alcian blue and Alizarin red). (**B**,**C**) A single tooth is present on each premaxillary and dentary bone in 120 hpf *Astyanax* (frontal views; teeth and bones digitally colored red in B). (**D-F**) Bell-shaped tooth germs in 72 hpf *Astyanax* lower jaw. Dental epithelium indicated by arrowhead, darkly-stained dental mesenchyme by arrow. Transverse sections in D,E; sagittal in F. (**G**) Toothless oral cavity of adult zebrafish. (**H**) Toothless oral cavity of 124 hpf zebrafish larva cleared and stained with Alcian green. (**I-L**) No tooth germs are visible in sectioned, Toluidine blue-stained zebrafish larvae. (I,J) Identical transverse sections of a 72 hpf specimen. (K,L) Sagittal views of the lower jaw of 72 hpf and 120 hpf specimens, respectively. d, dentary; e, eye; ep, ethmoid plate; m, maxillary; mc, Meckel's cartilage; n, neuromast; o, olfactory organ; ov, oral valve; p, premaxillary; t, trabecula. Scale bars: 1 mm in A,G; 100 μm in B,D,H,I; 50 μm in C,E,F,J-L.



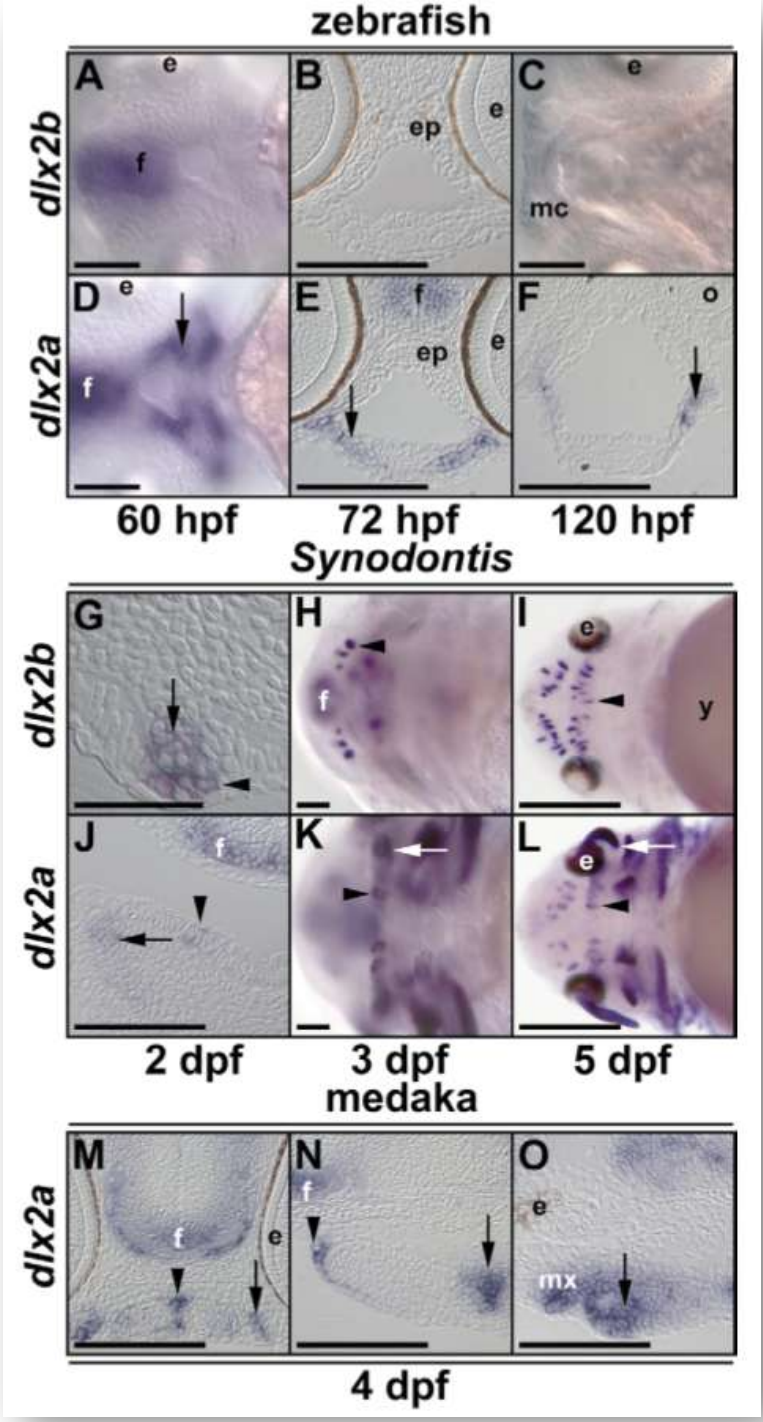


Fig. 4. Expression of Dlx2 orthologs in zebrafish, *Synodontis* and medaka. (**A-C**) *dlx2b* expression is absent at all stages from the zebrafish oral region, but present in forebrain (f). (**D-F**) *dlx2a* expression is absent from zebrafish oral epithelium at all stages, but present laterally in jaw mesenchyme (arrows) and forebrain. (**G-L**) *dlx2a* and *dlx2b* are expressed in tooth germs of *Synodontis*, while only *dlx2a* is expressed laterally in the jaw mesenchyme (arrows in J,K) and barbels (arrow in L). Both genes are expressed (arrowheads) in tooth germ epithelium (cytodifferentiation stage premaxillary germ in G; initiation stage dentary germ in J), and *dlx2b* was additionally detected in tooth germ mesenchyme (arrow in G). Arrowheads indicate one of three premaxillary germs per side in H, one of two dentary germs per side in K, and one of numerous germs visible in upper and lower jaws of I,L. (**M-O**) Medaka *dlx2a* is expressed in oral epithelium (arrowheads in M,N) and mesenchyme (arrows in M-O). Transverse section (M) indicates epithelium expression is in a medial band and sagittal sections (N,O) reveal that mesenchymal expression is lateral to this. Indicated stage is before visible signs of tooth initiation. All whole mounts in ventral view; transverse sections in B,E-G,J,M; sagittal sections in N,O. Abbreviations as in Fig. 1. f, forebrain; mx, maxillary process; y, yolk. Scale bars: 100 μm in A-H,J-K,M-O; 500 μm in I,L.

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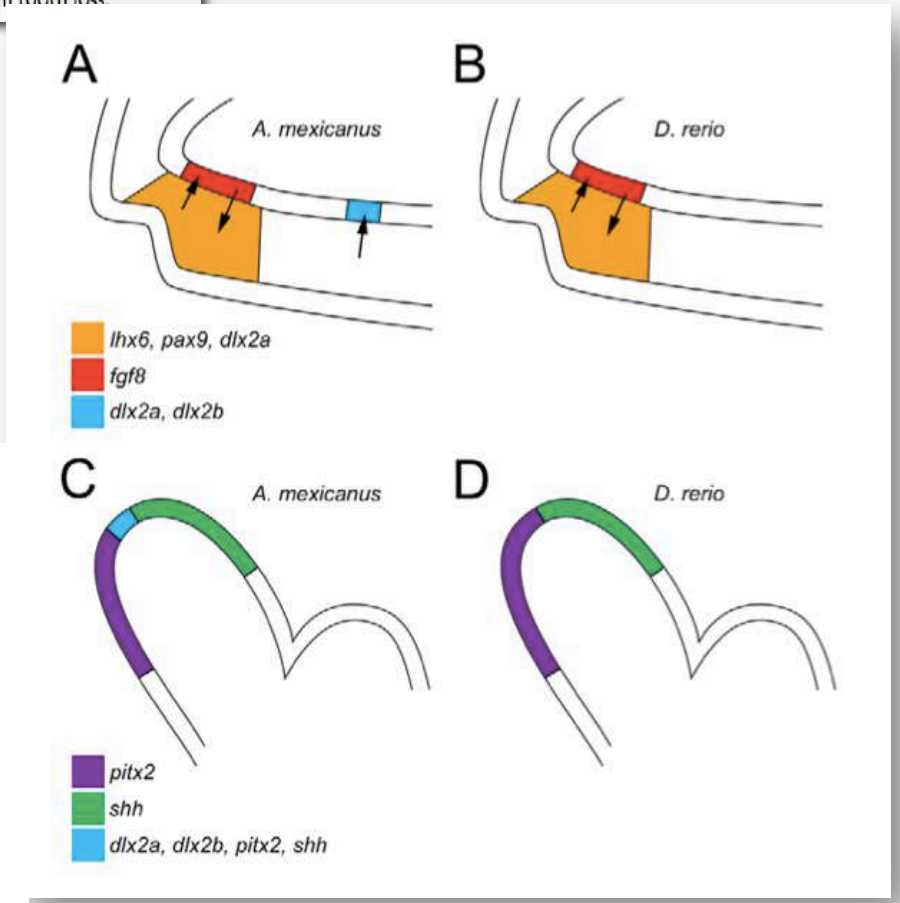

Fig. 8. Comparison of gene expression and hypothesized Fgf signals (arrows) between *Astyanax* and the zebrafish.
(**A**,**B**) Transverse views of the left side of the mandible. Lateral epithelial and mesenchymal gene expression common to both species is Fgf dependent. Loss of a medial Fgf signal to the epithelium is hypothesized to have caused cypriniform tooth loss. See text for basis of hypothesized ligand sources. (**C**,**D**) Lateral views of selected features of mandibular epithelial expression. *pitx2* and *shh* expression common to both species is Fgf independent. The zebrafish may lack a domain of overlapping *pitx2* and *shh* expression corresponding to a tooth germ (marked by *dlx2a* and *dlx2b* expression in *Astyanax*).

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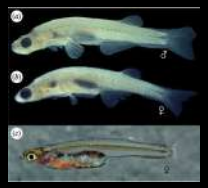

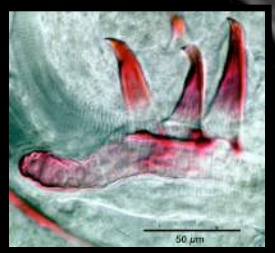

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'Dracula' fish shows baby teeth

By Richard Black
Environment correspondent, BBC News website

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Spectacular morphological novelty in a miniature cyprinid fish, *Danionella dracula* n. sp.

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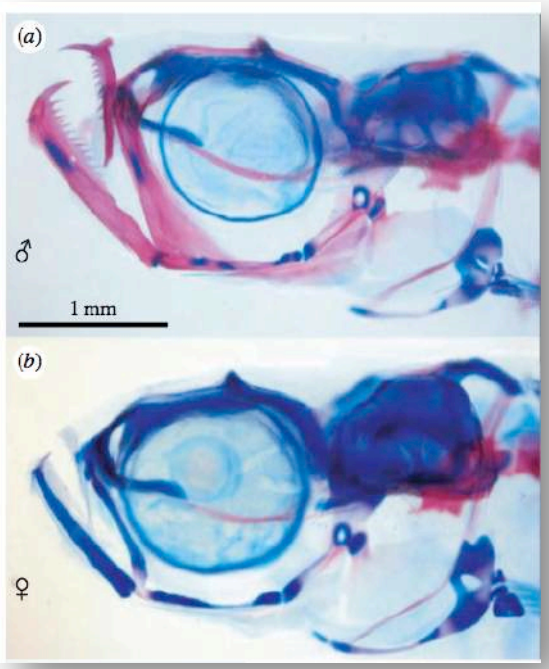
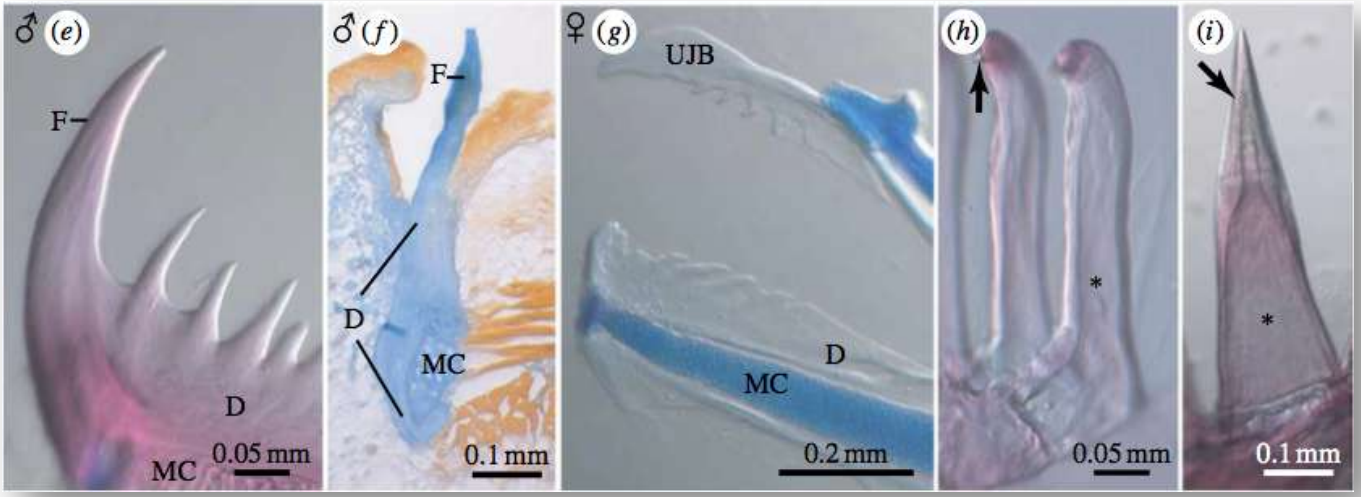
Danionella dracula is a new species of sexually dimorphic, miniature and highly developmentally truncated cyprinid fish. Compared with its close relative, the zebrafish *Danio rerio*, it lacks 44 bones or parts thereof and represents one of the most developmentally truncated vertebrates. Absence of the majority of bones appears to be due to developmental truncation via terminal deletion. In contrast to these larval-like features, *D. dracula* also shows several hyperossifications. Uniquely, among carp-like fishes, male *D. dracula* have a series of long, pointed odontoid processes on the jaws greatly resembling the jaw dentition of teleosts with true teeth. The anterior-most process in each jaw is extended as a canine-like fang projecting through the epithelium. True jaw teeth are absent from all 3700 species of cypriniforms and were lost at least in the Upper Eocene. It remains to be investigated, however, whether the conserved pathways to regulate tooth development in cypriniforms have been used in *D. dracula* to form and pattern the odontoid processes. This new species represents a remarkable example linking progenetic paedomorphosis via heterochronic change in developmental timing to the evolution of morphological novelties.

Keywords: *Danionella*; Cypriniformes; jaw teeth; miniaturization; developmental truncation; evolutionary novelty





Conservation of early odontogenic signaling pathways in *Aves*

YiPing Chen*†, Yanding Zhang*†, Ting-Xing Jiang‡, Amanda J. Barlow§, Tara R. St. Amand†, Yueping Hu†, Shaun Heaney*, Philippa Francis-West§, Cheng-Ming Chuong‡, and Richard Maas*¶

Teeth have been missing from birds (*Aves*) for at least 60 million years. However, in the chick oral cavity a rudiment forms that resembles the lamina stage of the mammalian molar tooth germ. We have addressed the molecular basis for this secondary loss of tooth formation in *Aves* by analyzing in chick embryos the status of molecular pathways known to regulate mouse tooth development. Similar to the mouse dental lamina, expression of *Fgf8*, *Pitx2*, *Barx1*, and *Pax9* defines a potential chick odontogenic region. However, the expression of three molecules involved in tooth initiation, *Bmp4*, *Msx1*, and *Msx2*, are absent from the presumptive chick dental lamina. In chick mandibles, exogenous bone morphogenetic protein (BMP) induces *Msx* expression and together with fibroblast growth factor promotes the development of *Sonic hedgehog* expressing epithelial structures. Distinct epithelial appendages also were induced when chick mandibular epithelium was recombined with a tissue source of BMPs and fibroblast growth factors, chick skin mesenchyme. These results show that, although latent, the early signaling pathways involved in odontogenesis remain inducible in *Aves* and suggest that loss of odontogenic *Bmp4* expression may be responsible for the early arrest of tooth development in living birds.

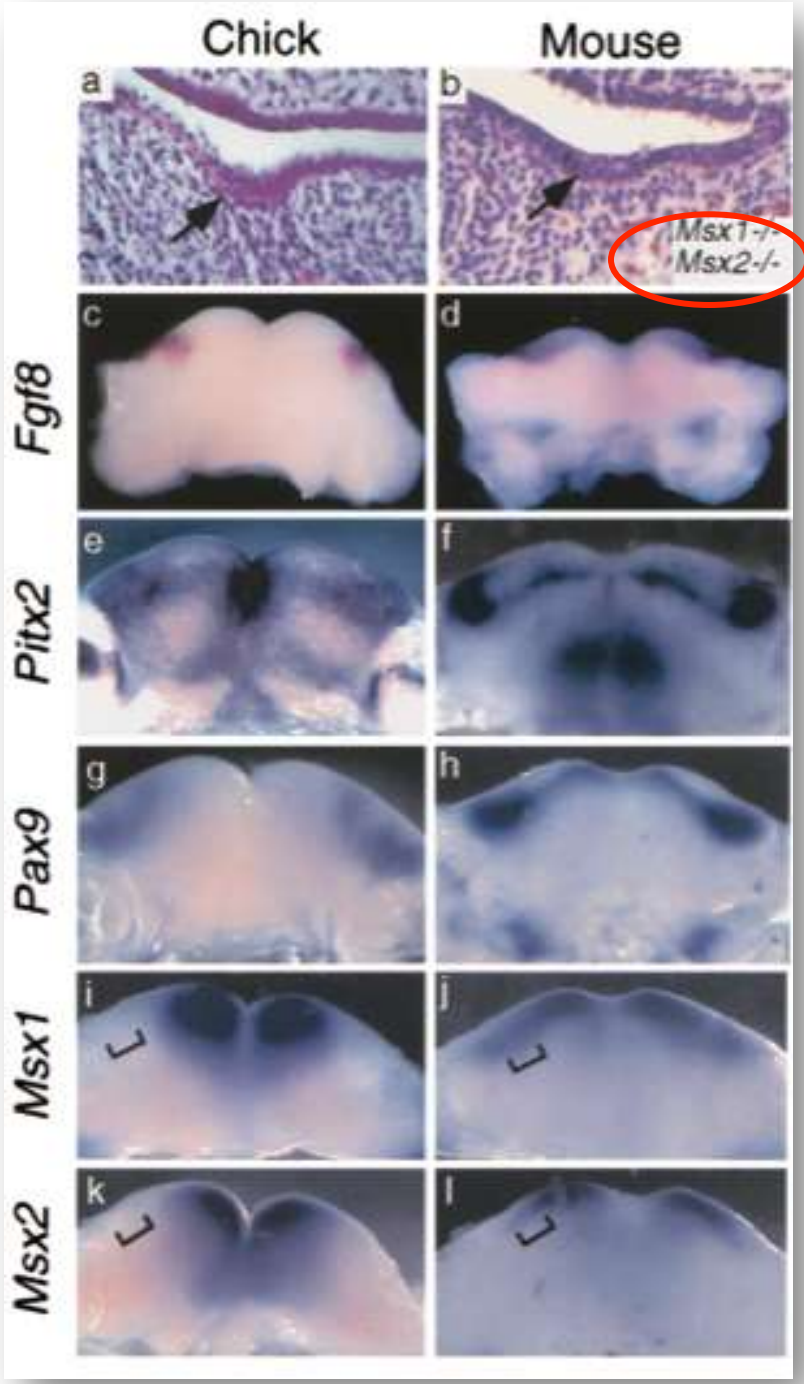
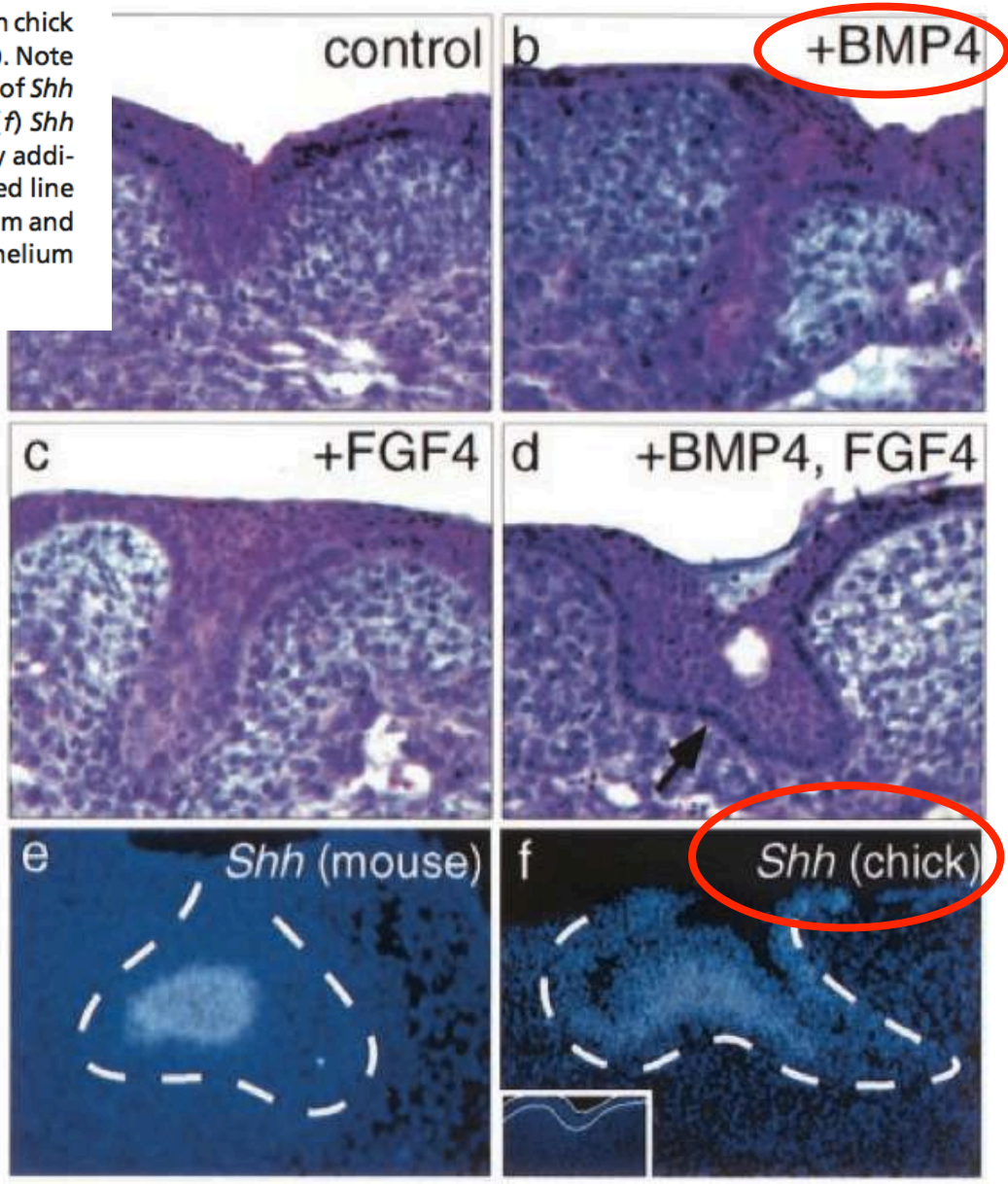


Fig. 3. Induction of chick oral epithelial appendages by BMP and/or FGF. (*a*) Section through a control, untreated chick mandible after 6 days of culture showing region of thickened epithelium. (*b* and *c*) Bud-like structures induced in chick mandibles after 6 days of culture with 100 ng/ml of exogenous BMP4 (*b*) or FGF4 (*c*). (*d*) More advanced epithelial structure induced to form in chick mandibles after 6 days of culture with BMP4 and FGF4 (100 ng/ml each). Note convoluted epithelium (arrow). The clear space is a cyst. (*e*) Localization of *Shh* transcripts in the enamel knot of an E14.5 mouse molar tooth germ. (*f*) *Shh* expression induced in the central portion of the epithelial structure by addition of BMP4 and FGF4 to chick mandibles in explant culture. The dotted line in *e* and *f* indicates the location of the basal lamina separating epithelium and mesenchyme. (*Inset*) *Shh* is not expressed in control explants; the epithelium resides between the white lines.



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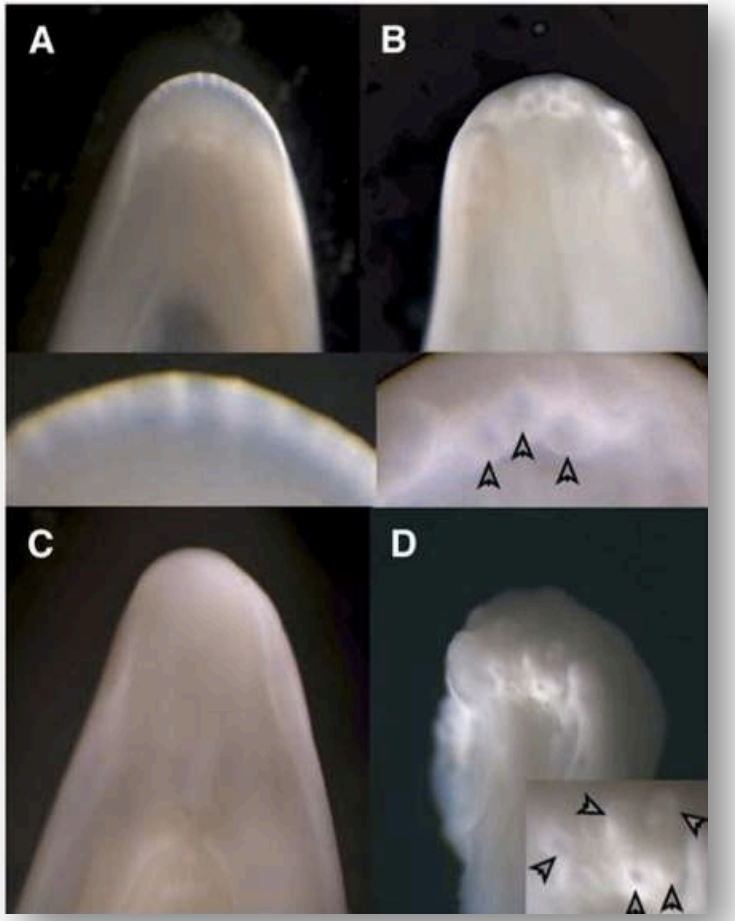
Report

The Development of Archosaurian First-Generation Teeth in a Chicken Mutant

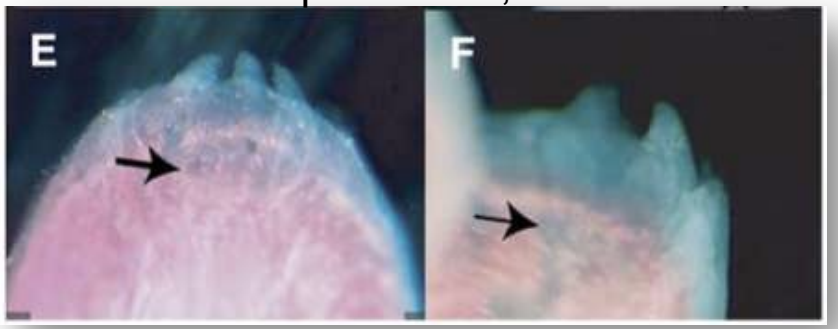
Summary

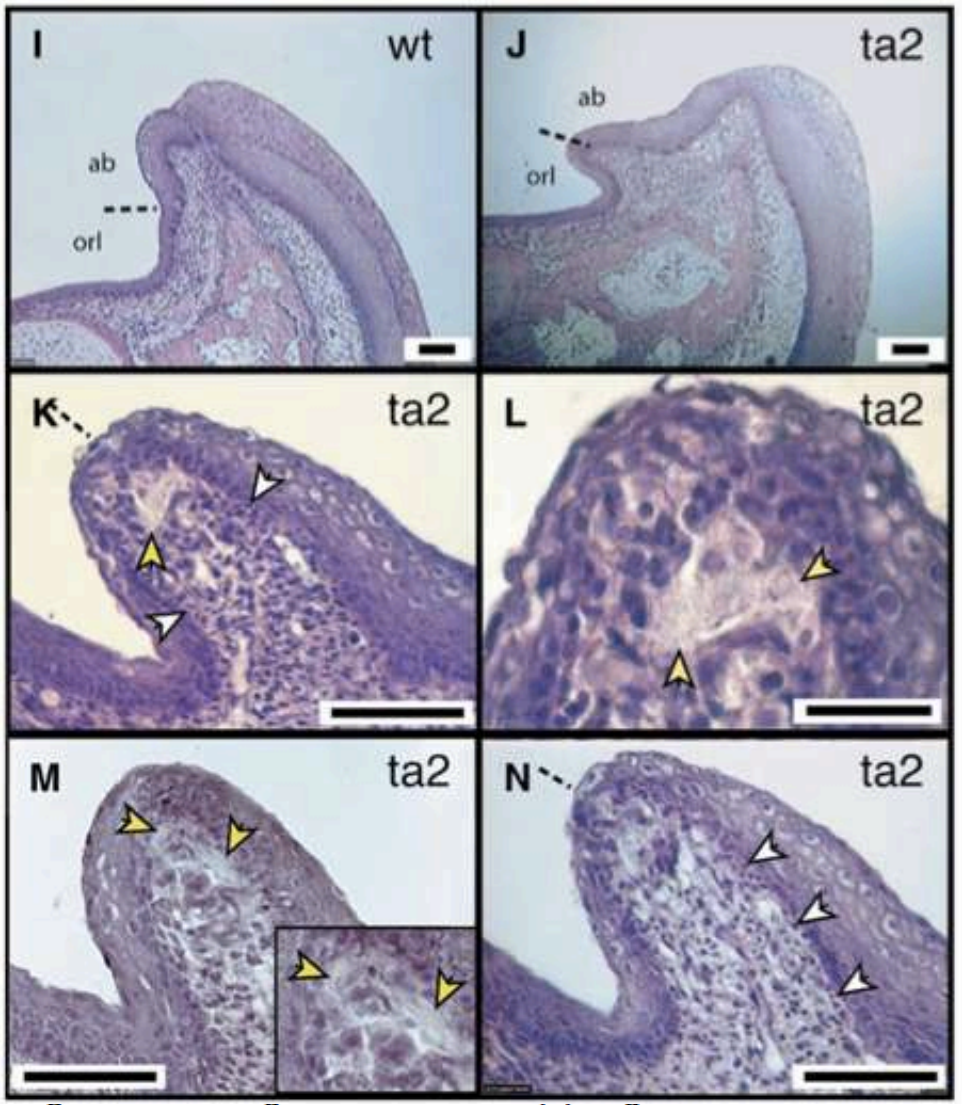
Modern birds do not have teeth. Rather, they develop a specialized keratinized structure, called the rhamphotheca, that covers the mandible, maxillae, and premaxillae. Although recombination studies have shown that the avian epidermis can respond to tooth-inductive cues from mouse or lizard oral mesenchyme and participate in tooth formation [1, 2], attempts to initiate tooth development de novo in birds have failed. Here, we describe the formation of teeth in the *talpid*^{2} chicken mutant, including the developmental processes and early molecular changes associated with the formation of teeth. Additionally, we show recapitulation of the early events seen in *talpid*^{2} after in vivo activation of *β-catenin* in wild-type embryos. We compare the formation of teeth in the *talpid*^{2} mutant with that in the alligator and show the formation of decidedly archosaurian (crocodilian) first-generation teeth in an avian embryo. The formation of teeth in the mutant is coupled with alterations in the specification of the oral/aboral boundary of the jaw. We propose an epigenetic model of the developmental modification of dentition in avian evolution; in this model, changes in the relative position of a lateral signaling center over competent odontogenic mesenchyme led to loss of teeth in avians while maintaining tooth developmental potential.

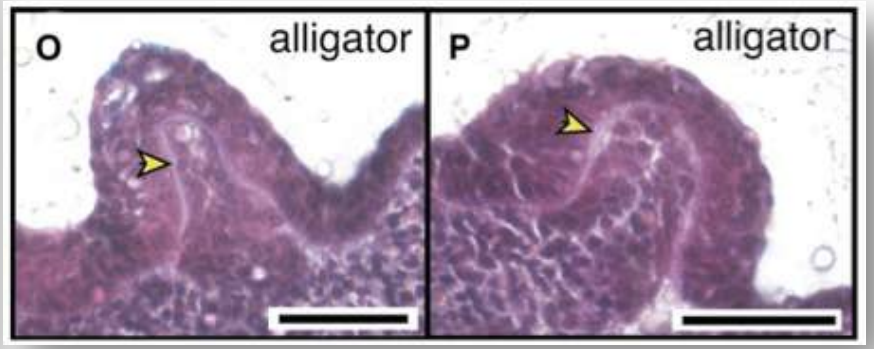
wt talpid mutant, *ta*^{2}



talpid mutant, *ta*^{2}



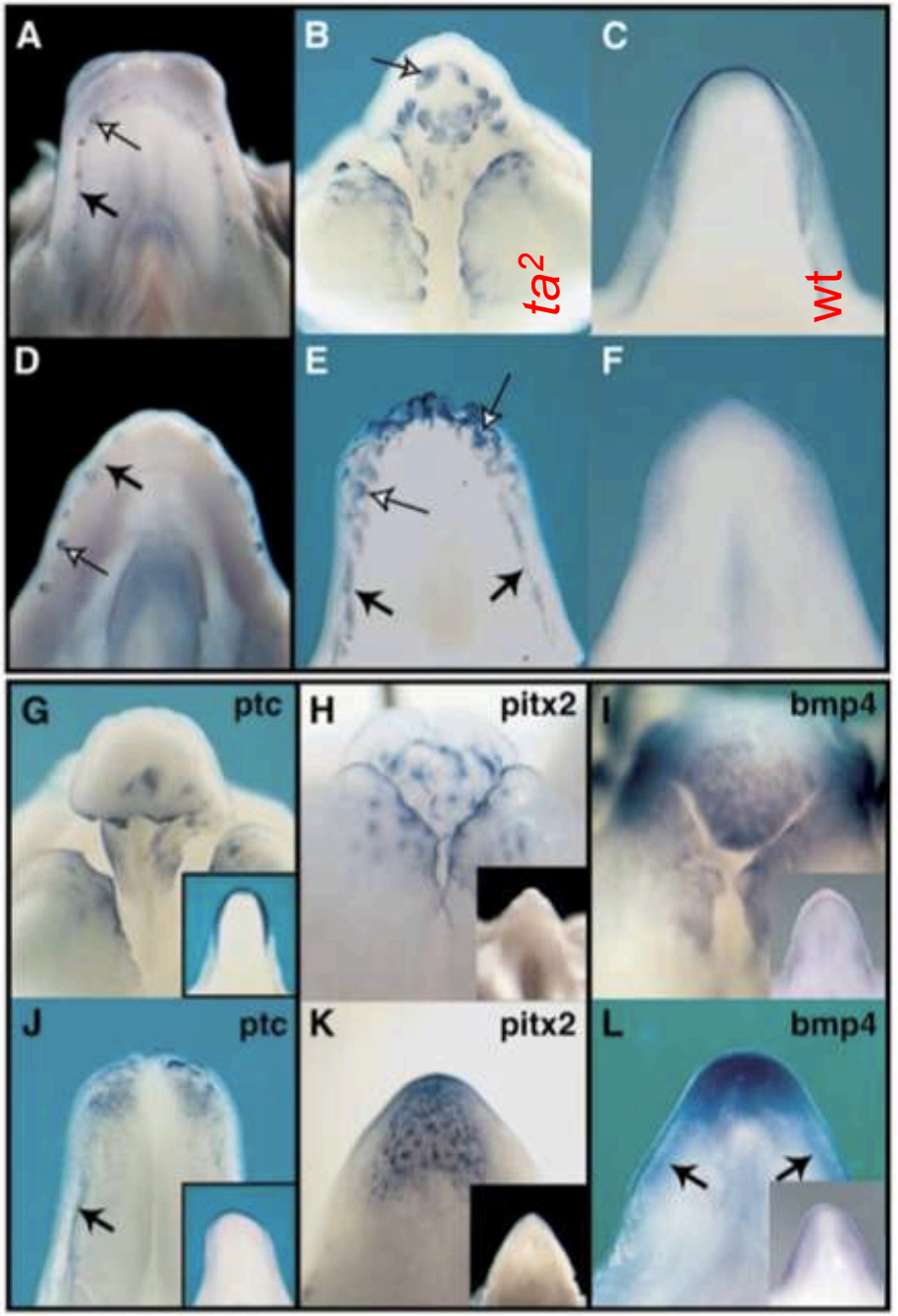




(I–N) Haematoxylin- and eosin-stained histological sections of forming oral appendages of E14 ta^2 embryos. In (I)–(J), the lower jaw of wild-type (wt) sibling and ta^2 embryos (ta2) shows formation of outgrowths in more medial positions of the oral cavity. The oral/aboral boundary, indicated by a shift of epithelial differentiation, is marked with a dotted line (G, I, J, K, and N). In (K), (M), and (N), tooth primordia from ta^2 show specific differentiation of the dental mesenchyme, including central vascularization and circumferential, immature odontoblasts (white arrowheads). (L) shows a close-up of the distal portion of (K).

(O–P) Haematoxylin- and eosin-stained histological sections of rudimentary teeth of stage-17 alligator [40] embryo. Putative dentine matrix is seen at the distal tip of the ta^2 dental structures and in the alligator (yellow arrowheads, [K–P]). The scale bar equals 50 μm in all panels except (L) and (H), in which the scale bar equals 20 μm.

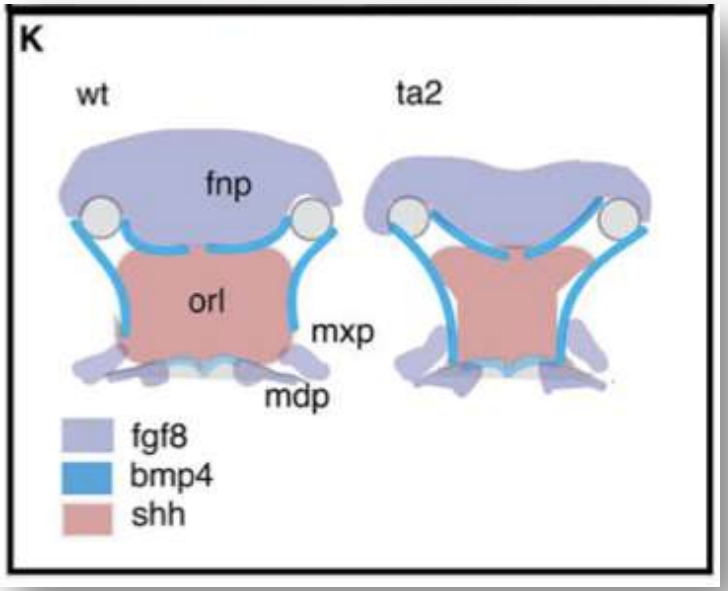
aligator *Shh*

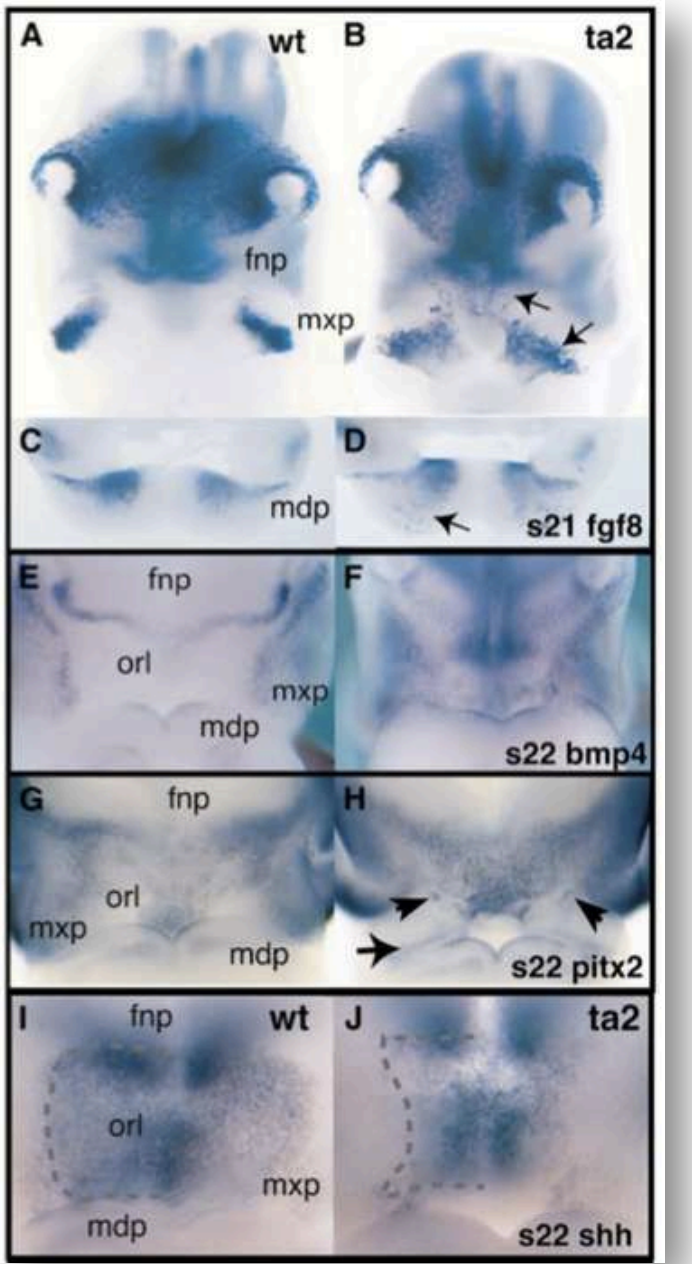


(A–C and G–I) Ventral view of the upper jaw.
(D–F and J–L) Dorsal view of the associated lower jaw.
(A and D) *shh* expression in developing first-generation teeth of a s20 [40] alligator embryo (white arrows). *shh* expression also marks a linear domain between forming tooth primordia thought to be the location of dental lamina formation (black arrows).
(B, C, E, and F) *shh* expression in the oral cavity of E10 ta^2 mutant (B and E) and its absence in wild-type siblings (C and F) are shown. ta^2 mutants show punctate, circular placodes on the maxillae and mandible (white arrows, [B and E]), and a similar linear expression domain along the aboral boundary is seen as in the alligator ([A and D], black arrows).
(G–L) WMISH analysis of *ptc* (E10, [G and J]), *pitx2* (E8, [H and K]), and *bmp4* (E8, [I and L]) in the ta^2 mutant compared with age-matched wild-type siblings (inserts).

Figure 2. Tooth Developmental Pathways Are Initiated in ta^2

Figure 3. Early Developmental Specification of the Oral/Aboral Boundary Is Altered in *ta*^{2}





WMISH analysis of *fgf8*, *bmp4*, *pitx2*, and *shh* expression in developing facial primordia of wild-type (A, C, E, G, and I) and *ta*^{2} embryos (B, D, F, H, and J).

(A–D) *Fgf8* expression in s21 wild-type (A and C) and *ta*^{2} embryos (B and D). Arrows indicate sites of ectopic expression in the mutant (B and D).

(E–H) The expression of *bmp4* (E and F) and *pitx2* (G and H) in s22 embryos show medial expression into the oral cavity. Ectopic expression of *pitx2* is seen along the forming maxillary process of the mutant (arrow) and foci of the frontonasal process (arrowhead).

(I and J) *shh* expression in the epidermis of the oral cavity of wild-type and *ta*^{2} (dotted line outlines expression domain on one side).

(K) Schematic of gene expression seen in early development of the wild-type and *ta*^{2} mutant jaw showing coordinated changes in *fgf8*, *bmp4*, and *shh* expression in outlining the boundary of the oral/aboral boundary. *pitx2* is left out of the schematic for simplicity. The following abbreviations are used: mxp, maxillary; mdp, mandibular; fnp, frontonasal processes; and orl, oral cavity.

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Report

The Development of Archosaurian First-Generation Teeth in a Chicken Mutant

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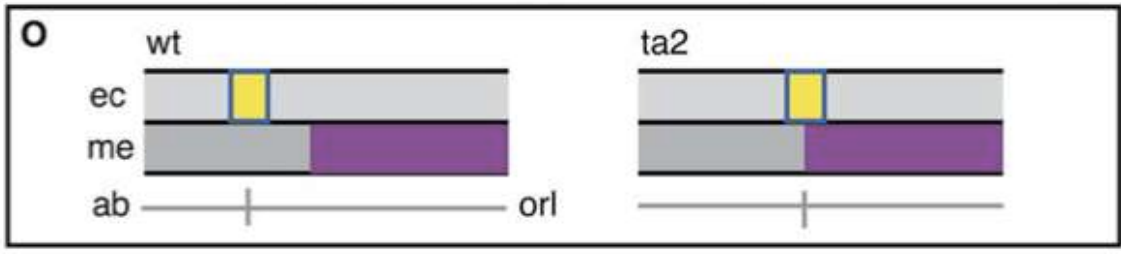
Developmental Potential of the Oral/Aboral Epidermis

As shown in recombination studies, the avian ectoderm and mesenchyme both have potential to participate in tooth development. Given the association of the observed outgrowths and the novel position of the oral/aboral boundary in the mutant, we postulated that initiation of tooth programs in the *ta*^{2} chick was due to the developmental repositioning of an epithelium with signaling potential to overlie mesenchyme competent to form teeth.



(O) Model of alteration in the inductive interactions in wild-type and *ta*^{2} jaw leading to the initiation of teeth in the *ta*^{2} mutant. In the wild-type, a regional signaling center is localized in the epithelium (yellow) by the interaction between *fgf8*, *bmp4*, and *shh* signaling. This signaling center demarcates the boundary between the oral and aboral epithelium (vertical mark on horizontal ab-orl line). This epithelial signaling center does not overlie oral mesenchyme (purple) competent to make appendage structures. In the *ta*^{2} mutant, early changes in *fgf8*, *bmp4*, and *shh* signaling lead to medial positioning of the forming oral/aboral boundary such that the signaling center and underlying competent mesenchyme are juxtaposed, permitting initiation of tooth developmental programs. The following abbreviations are used: ec, ectoderm; me, mesenchyme; ab, aboral; and orl, oral epidermis. The scale bar equals 50 μm.

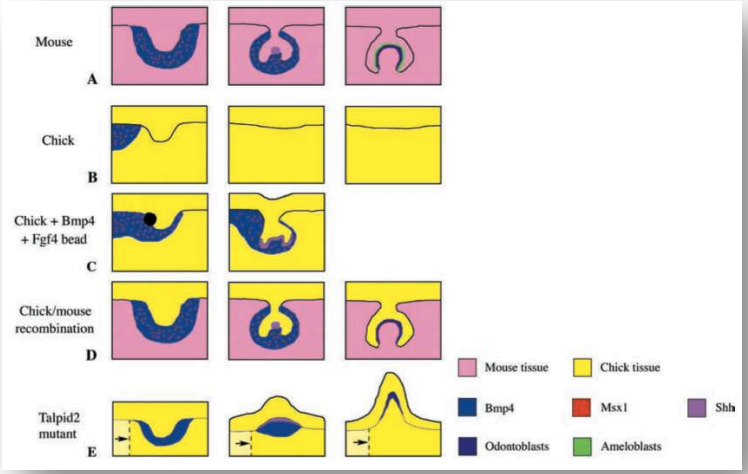
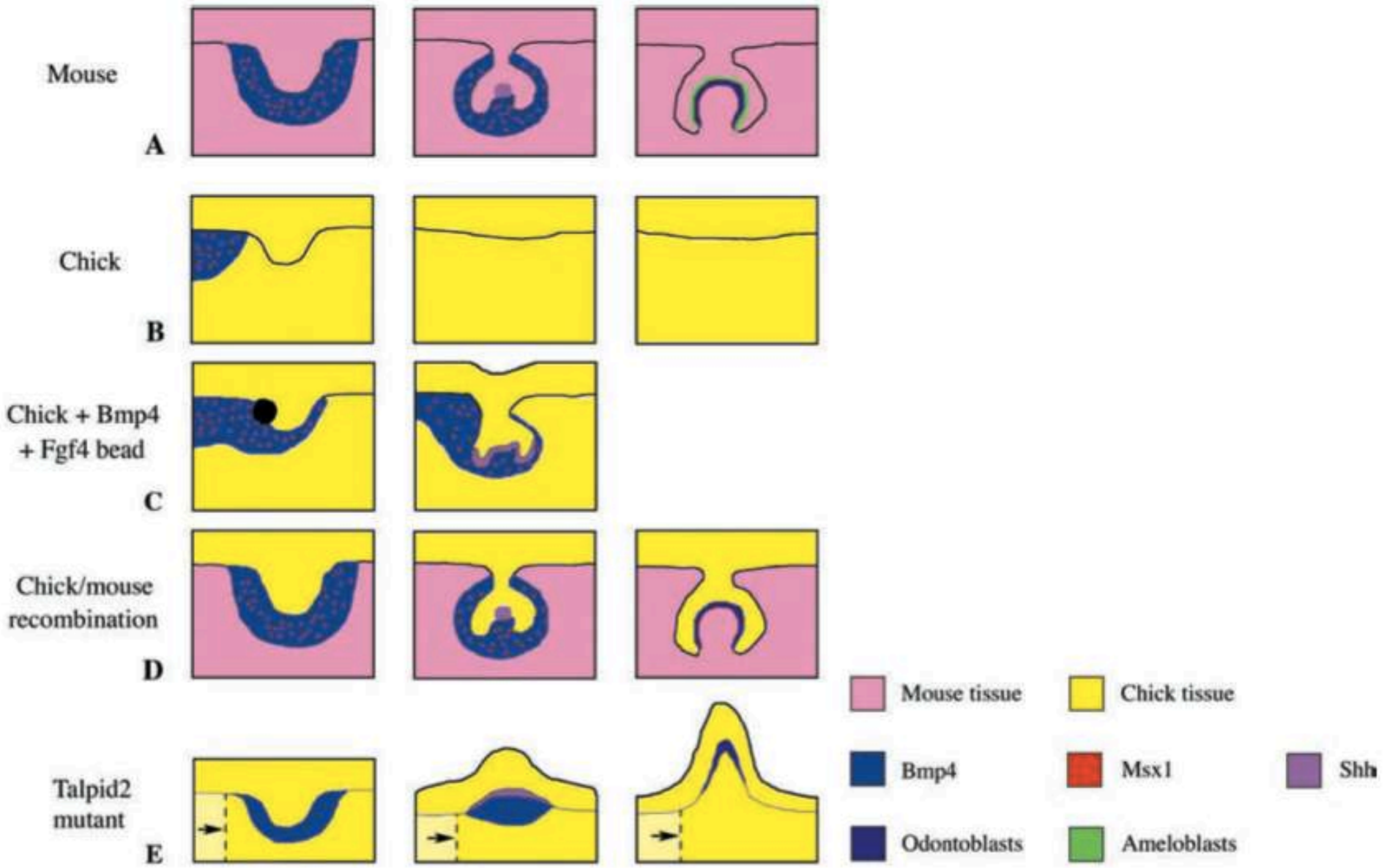


Fig. 3 A shift in the positioning of the odontogenic epithelium relative to the dental competent mesenchyme could explain the loss of the ability to form teeth in the modern bird ancestor. Schematic drawings summarizing the chick tooth experiments. (A) Mouse molar developmental stages, from bud [embryonic day (E)12.5] to cap (E14.5) to bell (E16.5). The condensing mesenchyme around the bud stage tooth germ expresses *Bmp4* and *Msx1* and induces development of the enamel knot at the cap stage, which expresses signalling molecules such as *Shh*. The inner enamel epithelium forms the ameloblasts that form enamel, whereas the adjacent mesenchyme forms the odontoblasts that form dentine (see Caton & Tucker, 2009). (B) Chick development. At Hamburger & Hamilton (HH) stage 28 a bud-like thickening of the oral epithelium is observed. Expression of *Bmp4* and *Msx1* is not, however, associated with this region. No further tooth development is observed at later stages and the thickening regresses. Note that, at an earlier stage (stage 24), *Bmp4* expression is epithelial and shifts into the mesenchyme at stage 28 (Francis-West et al. 1994). (C) When a bead impregnated with *Bmp4* and *Fgf4* is implanted into the chick epithelium, the expression of *Bmp4* and *Msx1* in the mesenchyme extends around the developing tooth bud. This leads to the extension and folding of the bud epithelium, and induction of *Shh*. No further progression of the tooth germs is observed, however (Chen et al. 2000). (D) When mouse mesenchyme is combined with chick epithelium (either by recombination of mandible tissue or by earlier neural crest grafts of mouse neural crest into a chick embryo), the chick epithelium induces *Msx1* and *Bmp4* in the mouse mesenchyme. The tooth germ progresses to the cap stage and forms an enamel knot-like structure expressing *Shh*. The mouse tissue differentiates into odontoblasts and forms a bell stage tooth germ. Tooth differentiation does not proceed beyond this stage and enamel is not deposited (Wang et al. 1998; Mitsiadis et al. 2003). (E) In the chick mutant *talpid*^{2} a shift in the positioning of the epithelium and mesenchyme has been described (indicated by dashed lines and arrows). The chick epithelium is able to induce expression of *Bmp4* in the underlying mesenchyme and expresses *Shh*. The tooth germ develops by evagination, similar to that observed in alligator embryos. At later stages differentiated odontoblasts are identified by histology but no further differentiation occurs (Harris et al. 2006).


Mouse
A
Chick
B
Chick + Bmp4
+ Fgf4 bead
C
Chick/mouse
recombination
D
Talpid2
mutant
E
Mouse tissue
Chick tissue
Bmp4
Msx1
Shh
Odontoblasts
Ameloblasts

Research article

Hen's teeth with enamel cap: from dream to impossibility

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Abstract

Background: The ability to form teeth was lost in an ancestor of all modern birds, approximately 100-80 million years ago. However, experiments in chicken have revealed that the oral epithelium can respond to inductive signals from mouse mesenchyme, leading to reactivation of the odontogenic pathway. Recently, tooth germs similar to crocodile rudimentary teeth were found in a chicken mutant. These "chicken teeth" did not develop further, but the question remains whether functional teeth with enamel cap would have been obtained if the experiments had been carried out over a longer time period or if the chicken mutants had survived. The next odontogenetic step would have been tooth differentiation, involving deposition of dental proteins.

Results: Using bioinformatics, we assessed the fate of the four dental proteins thought to be specific to enamel (amelogenin, AMEL; ameloblastin, AMBN; enamelin, ENAM) and to dentin (dentin sialophosphoprotein, DSPP) in the chicken genome. Conservation of gene synteny in amniotes allowed definition of target DNA regions in which we searched for sequence similarity. We found the full-length chicken AMEL and the only N-terminal region of DSPP, and both are invalidated genes. AMBN and ENAM disappeared after chromosomal rearrangements occurred in the candidate region in a bird ancestor.

Conclusion: These findings not only imply that functional teeth with enamel covering, as present in ancestral Aves, will never be obtained in birds, but they also indicate that these four protein genes were dental specific, at least in the last toothed ancestor of modern birds, a specificity which has been questioned in recent years.