The odontode explosion: The origin of tooth-like structures in vertebrates

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Essentially we show recent data to shed new light on the thorny controversy of how teeth arose in evolution. Essentially we show (a) how teeth can form equally from any epithelium, be it endoderm, ectoderm or a combination of the two and (b) that the gene expression programs of oral *versus* pharyngeal teeth are remarkably similar. Classic theories suggest that (i) skin denticles evolved first and odontode-inductive surface ectoderm merged inside the oral cavity to form teeth (the 'outside-in' hypothesis) or that (ii) patterned odontodes evolved first from endoderm deep inside the pharyngeal cavity (the 'inside-out' hypothesis). We propose a new perspective that views odontodes as structures sharing a deep molecular homology, united by sets of co-expressed genes defining a competent thickened epithelium and a collaborative neural crest-derived ectomesenchyme. Simply put, odontodes develop 'inside and out', wherever and whenever these co-expressed gene sets signal to one another. Our perspective complements the classic theories and highlights an agenda for specific experimental manipulations in model and non-model organisms.

Keywords:
dentition; gene network; neural crest; odontode; taste bud

Introduction

Evolving odontodes: on the origins of vertebrate dentition

How and when tooth-like units (odontodes) originated during vertebrate evolution continues to cause a stir among paleontologists and evolutionary developmental biologists [1]. Odontodes are classified here from Ørvig's description [2, 3] as simply all structures that comprise a mineralised hard tissue unit consisting of attachment bone, dentine (with great histological diversity early in the fossil record [2]) sometimes with a superficial layer of enamel/enameloid, formed from a single papilla (see also [1, 4, 5]). Odontodes are by far the most readily fossilised vertebrate structures and are heavily used for early phylogenetic reconstructions [5]. Despite the rich record of fossil tooth-like structures, the actual sequence of events accounting for the evolution of oral *versus* dermal odontodes continues to evoke controversy. Uncertainty arises with conflicting, although not coincidental, fossil evidence of (i) jawless vertebrates with an oro-pharyngeal tooth apparatus (conodonts [5–8]), (ii) jawless vertebrates with both skin denticles and patterned pharyngeal tooth whorls (thelodonts [9]) and (iii) still other jawless vertebrates with an extensive dermal skeleton ornamented with odontodes, lacking any oropharyngeal denticles (*e.g.* ostracoderms [4, 5, 10]). Some evidence suggests that the first jawed vertebrates (albeit derived, placoderms [11–13]) possessed teeth on their jaws (gnathal bones), ordered denticles on the bone of the posterior pharyngeal wall, as well as dermal tubercules.

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Abbreviations:
BMP, bone morphogenetic protein family; **CEG,** gene co-expression group; **cLL**, cephalic lateral line; **dGRN,** dental GRN; **epCEG,** epithelial placode CEG; **FGF,** fibroblast growth factor family; **GRN,** gene regulatory network; **mesCEG,** mesenchymal CEG; **ncGRN,** neural crest GRN; **oGRN,** odontode GRN; **tbCEG,** taste bud CEG.

Box

Glossary of terms

Tooth
A mineralised hard tissue unit consisting of attachment bony basal pad, dentine or similar dentinous tissue sometimes with a superficial layer of enamel/enameloid formed from a single papilla present only in the oro-pharyngeal cavity, with a distinctive patterned oro-pharyngeal distribution with associated/connected replacements developing in advance of their requirement [4].

Denticle
A mineralised structure, present on the dermal surface and within the oro-pharyngeal cavity of basal vertebrates (*e.g.* elasmobranchs), consisting of attachment bone, dentine sometimes with a superficial layer of enameloid ormed from a single papilla [4, 14, 15], with a random (non-patterned) distribution and unrelated replacements.

Odontode
All structures that comprise a mineralised hard tissue unit consisting of an attachment bony basal pad, dentine or similar dentinous tissue sometimes with a superficial layer of enamel/enameloid formed from a single papilla; odontodes include both teeth and denticles [2, 3].

Neural crest
A migratory multipotent embryonic progenitor cell population that emerges from the dorsal neural tube to invade diverse regions of the embryo, giving rise to numerous derivatives in vertebrates including neurons, glia, pigment cells, bone, cartilage and dentine.

Gene regulatory network (GRN)
A coordinated collection of genes that govern the time, position and rates at which other genes in the network are transcribed. A GRN is usually derived from data obtained through experimental manipulation in model organisms, and is often portrayed as a logical wiring diagram [16, 17].

Gene co-expression group (CEG)
Genes expressed with spatial and temporal similarity, only suggestive of GRN function; similar to synexpression groups [18].

Agnathan
Jawless vertebrates, likely a paraphyletic group to the gnathostomes.

Gnathostomes
Vertebrates with opposable oral jaws.

Oro-pharyngeal cavity
The anterior opening of the mouth and the cavity leading to the gut.

Debate about the appearance and evolution of odontodes during early vertebrate evolution is firmly rooted in the classic problem of anatomical homology [1, 19, 20]. Odontodes can be found in multiple locations around the body of lower vertebrates, whether covering the dermal surface as in extant sharks and rays, as a dentition in oral and pharyngeal locations, or lining the oro-pharyngeal cavity associated with gill arches. The perceived homology of all odontodes is based on palaeontological evidence, structural and developmental similarities [4, 14, 21, 22]. According to Reif [4, 21], the highly specialised mode of formation makes it unlikely that odontodes convergently evolved in various vertebrate groups. Notably and without contention, teeth and tooth-like structures evolved in vertebrates ahead, and independently, of the oral jaws [23, 24]. Thus, odontodes had their origins in ancient jawless (agnathan) vertebrates [1]. However, did the first odontode appear within the evolving oro-pharyngeal cavity for food breakdown during the transition to more predatory behaviour – or did the first tooth-like structures appear as external dermal armour in a predator-rich environment?

Two main theories polarise the field: the traditional view, that skin denticle competent ectodermal-epithelium folded and integrated into the mouth to provide the inductive capacity for teeth – the 'outside-in theory' – is contested by the 'inside-out theory' that teeth, born from endoderm, originated in the posterior pharynx of jawless vertebrates with dental potential co-opted anteriorly to oral jaws during gnathostome evolution [23]. In this article, we discuss the main theories of tooth and general odontode origin, and develop a new perspective, based on recent data from evolutionary developmental biology, that pushes beyond contemporary debate. We propose that odontodes evolved as the gene regulatory networks (GRNs) of basic epithelial (ectoderm or endoderm) structures combined with those of migrated neural crest cells. We hope to galvanise a research effort that seeks to understand the development of placode-like elements in extant organisms as a means to infer deep molecular homology uniting all odontodes, whatever their location.

Current hypotheses that explain the evolution of teeth

Outside-in model of tooth evolution

According to the classical theories [4, 25–27], collectively the 'outside-in' model, teeth came to reside within the oral cavity of jawed vertebrates when the odontode-competent tissue layer moved there from the body surface. This view is largely based on the anatomical resemblance of shark skin denticles to teeth, although they do not grade into each other [28, 29] and no continuous transition exists between the two structures [21]. The 'outside-in' theory posits the following principles: (1) odontodes originated from ectoderm, on the external surface of an organism in the form of skin denticles;

(2) for oral teeth to have evolved, ectodermal cells that form denticles on the surface must have mixed and incorporated into the oro-pharyngeal cavity during development and (3) only ectodermal cells have the capacity to form odontodes and thus all derivatives must have originated *via* ectodermal organogenesis. Teeth are, under this theory, modified skin denticles [30–32] (see Fig. 1A1). According to this model, the major difference between a skin denticle and a tooth is their locus of formation. Denticles form superficially on the skin surface, arising directly at the epithelium/mesenchyme interface, whereas teeth form inside the oro-pharyngeal cavity, at positions of future tooth-bearing bones and develop from a specialised epithelial invagination called the dental lamina [4] or from a similar structure called the odontogenic band [33–36].

To substantiate the 'outside-in' scenario, fossil evidence would be required to show that the earliest odontodes were located externally in organisms without oro-pharyngeal counterparts. Secondly, odontodes in fossil and extant creatures should form primarily on regions derived from ectoderm. Classically, palaeontological support for this theory comes from extinct jawless vertebrates, for example ostracoderms [5, 10] and thelodonts [37], and from both fossil and recent elasmobranchs (sharks and rays) (see 'evolving odontodes' section and citations therein). The caveat here is that, in both thelodonts and elasmobranchs, odontodes were/are concurrently located on the skin and inside the pharynx [28, 29]. With respect to ectodermal origins, it is nearly impossible to infer such detail from fossils – and is surprisingly difficult to ascertain in extant organisms (see below, this applies equally to the 'inside-out' model).

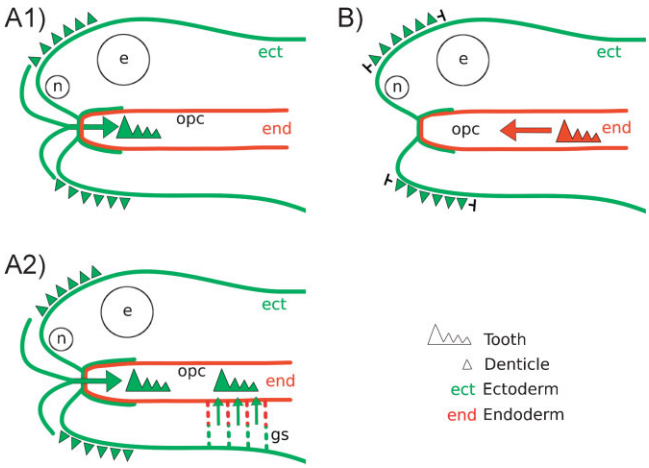

Figure 1. Theories of odontode evolution. Schematic diagrams represent a generalised (hypothetical) early vertebrate/fish in lateral/sagittal view: **A1**: Outside-in theory; ectodermal tissue is hypothesised to have integrated (green arrow) into the oro-pharyngeal cavity (opc), leading to the evolution of oral odontodes and subsequently oral and pharyngeal teeth. **A2**: Modified outside-in theory; ectodermal tissue integrated (green arrow) into the endodermal oral cavity *via* the mouth opening (the anterior boundary of the endoderm and ectoderm) and the gill slits (gs) in early vertebrates to initiate/transfer dental competence (arrow) to the endoderm of the oro-pharyngeal cavity. The point is made that ectoderm must be in regional contact with endoderm for teeth to form. **B**: Inside-out theory; skin denticles and teeth are structures forming independently from ectoderm and endoderm, respectively. This theory states that teeth originated in the posterior pharyngeal endoderm of jawless vertebrates; a dental competence that was co-opted anteriorly (red arrow) in concert with the evolution of oral jaws. This theory states that skin denticles did not grade into teeth. e, eye; n, nasal placode; opc, oro-pharyngeal cavity.

Inside-out model of tooth evolution

The 'inside-out' model states that teeth first appeared in the endoderm-dominated oro-pharyngeal cavity of jawless vertebrates [28]. This hypothesis was initially proposed by Smith and Coates [9, 23, 29] who argued that sets of patterned odontodes (prototype tooth sets) first evolved in the pharyngeal cavity. It was from the pharyngeal cavity that tooth competence and pattern was co-opted anteriorly to the oral jaw margins of gnathostomes [9, 23]. However, neither the mechanism of pattern co-option, nor the role of neural crest and their derived cells (ectomesenchyme) was discussed.

The 'inside-out' theory of tooth evolution poses the following: (1) tooth sets originated inside the endoderm-dominated posterior pharynx of now extinct jawless vertebrates; (2) subsequently, the molecular controls for competence were co-opted anteriorly to the oral jaws; (3) teeth and skin denticles appeared independently from alternative tissue layers, endoderm and ectoderm, respectively and (4) the unique patterning mechanism for a dentition lies specifically within the endoderm of the oro-pharynx (this includes the boundary zone where endoderm is juxtaposed with ectoderm in the oral cavity) [28], that is given the distinction that teeth are well organised or 'patterned', whereas denticles are more randomly distributed. According to the 'inside-out' hypothesis, denticles are not teeth and *vice versa* (see Fig. 1B). To substantiate this scenario, fossil evidence would be required to show that the earliest odontodes were located internally in early vertebrates without any superficial counterparts. Furthermore, odontodes in fossil and extant vertebrates should form primarily from endoderm.

A 'modified' outside-in theory

Recently, a 'modified outside-in hypothesis' has been suggested [38]. In concordance with the classic 'outside-in' theory, evolutionary precursors of teeth are believed to be epidermal (ectodermal) denticles, where teeth evolved only after an odontogenic competent ectodermal tissue had mixed inside *via* the mouth and each of the gill slits [38]. In contrast to the original 'outside-in' scenario where teeth develop solely from ectoderm [4], this 'modified' hypothesis allows that the initial odontogenic potential of the ectoderm may have been subsequently transferred to endoderm upon contact and cell mixing. Direct contact of both epithelial germ layers is thus a prerequisite for teeth to form. The arguments for this are as follows: (1) experimental and *in vitro* studies indicate that both ectoderm and endoderm cells are needed for teeth to develop; however, until now these studies were undertaken mostly on salamanders [39–41]; (2) classical observations on the ecto-endoderm boundaries within the pharyngeal cavity of

cypriniform fishes revealed complex epithelial morphodynamics with pharyngeal teeth developing from the endoderm epithelia, with the ectoderm lining in close proximity [42, 43] (see Fig. 1A2). Further support for this 'modified' hypothesis is offered by the fact that pharyngeal teeth are only found in species with gill slits and that, accordingly, pharyngeal teeth are absent in tetrapods, coincident with the retained closure of the pharyngeal cavity [38]. However, in some tetrapods-like salamanders or frog tadpoles, gill slits remain open, but no pharyngeal teeth develop [44, 45].

Evidence of odontode evolution from the fossil record

The earliest mineralised skeleton known from the fossil record was probably present in a group of animals known as the conodonts [8, 46]. Conodonts are extinct eel-like jawless vertebrates that did not possess a dermal armour but did contain an intricately patterned series of odontodes throughout the oro-pharyngeal cavity [6]. The conodonts appeared at the dawn of vertebrates and it is possible that they brought with them the first vertebrate dentition. Although conodont relationships remain contentious, the true conodonts (Euconodonta) are thought to have possessed a dentition putatively homologous to the vertebrate oro-pharyngeal dentition, composed of dentine and enamel-like tissues [1, 5, 7].

Conodonts may provide evidence for an oro-pharyngeal location of the earliest odontode, but their uncertain phylogenetic relationships do not close the argument. Further fossil evidence has been discussed in this context. A subset of another jawless vertebrate group, the Thelodonts, exemplified by the species *Loganellia scotica*, possess both skin denticles, similar in structure to extant elasmobranch skin denticles, and oro-pharyngeal tooth whorls that are uniquely patterned [9, 23, 37]. This diverse array of odontodes within one species suggests that there may be a distinction between these 'inside' and 'outside' populations; a specific oro-pharyngeal pattern links internal odontodes [28]. *Loganellia* fossils further support the notion that this unique 'dental' pattern for pharyngeal odontodes originated prior to functional jaws [9, 23, 28], both oral and pharyngeal [47]. Thus, whatever their ancestral homology, odontodes of the oro-pharyngeal cavity and the dermis might develop under alternative patterning mechanisms. This is the main evidence used by Smith and Coates [9] to suggest that pharyngeal odontodes developed independently from epidermal odontodes (Fig. 1B).

The closest fossil relatives to the gnathostomes among jawless vertebrates are all covered by large dermal plates often covered by odontodes [4, 5, 10]. Thus, for example the early Ordovician fossils-like *Arandaspis* or *Anatolepis* might be seen as excellent support for the 'outside-in' hypothesis, since these animals undoubtedly possessed odontodes on their outer surfaces with none situated internally. Moreover, in some heterostracans, another group of fossil jawless vertebrates, external headshield structures have been found to intergrade with some internally situated oro-pharyngeal denticles [48]. These latter fossil data thus do not support a distinction between oral and skin odontodes.

To sum up, current hypotheses about odontode origins have pushed the fossil record as far as it will go. Phylogenetic uncertainty, issues of anatomical homology and dubious inference of germ layer (ectoderm *vs*. endoderm) from extinct organisms make it difficult to differentiate between even the well-delineated predictions of the 'outside-in' *versus* 'inside-out' theories. We thus turn our attention to a synthesis of recent and sometimes surprising data, collected from extant model and non-model organisms, to address the common ingredients for seemingly diverse odontodes.

New perspectives

Complex ectoderm-endoderm boundary and the epithelial ingredients for teeth

Definitive evidence of teeth originating from a single germ layer in extant vertebrates would be ideal to differentiate between 'outside-in' *versus* 'inside-out' scenarios; however, the reality is less straightforward than once suspected. In the vertebrate head, ectoderm and endoderm meet at the prospective mouth region and between each pharyngeal arch, where ectoderm always forms the outer and endoderm the inner epithelial lining [49, 50]. The morphodynamics of these epithelial linings during embryogenesis is complex and not fully understood. In the mouth, the natural ectoderm-endoderm boundary is formed by the juxtaposition of the ectoderm layer of the stomodeal invagination and the endoderm layer of the anterior alimentary canal, called the oro-pharyngeal membrane [44, 51]. During the course of development the oro-pharyngeal membrane thins, cell number is reduced, the basement membrane adjoining the ectoderm and endoderm disappears and eventually breaks through [52]. Strikingly similar conditions also hold for the ectoderm-endoderm contact areas between individual pharyngeal pouches and corresponding pharyngeal clefts where these epithelia together form so-called branchial membranes (*e.g.* [49, 50, 53–56]). As in the case of oral development, perforation of the branchial membranes probably causes a loss of definitive ectoderm-endoderm boundaries. Subsequent cell rearrangements potentially occur to produce a cryptic distribution of these epithelia [38, 43].

Recent observations challenge the classic view (*e.g.* [45]) of a static and definitive ectoderm-endoderm boundary after the perforation of oro-pharyngeal membranes. In *Xenopus*, for example intercalation of ectoderm and endoderm occurs prior to the perforation of the oro-pharyngeal membrane and such mixing probably reflects a loss of the ascribable germ layer identity in these cells [52]. In urodele amphibians, contrary to the common vertebrate scheme, oral ectoderm does not invade to form a stomodeum, but further ectoderm ingrowth still occurs leading to epithelia of double germ layer origin [42]. In the Mexican axolotl, the basal layer of the oral ectoderm moves internally to cover the surface of the mouth endoderm, whereas the apical layer remains outside/at place. The breaking of the oro-pharyngeal membrane and the opening of the mouth leads to the formation of epithelia, which consist of an ectodermal basal layer and an endodermal apical layer [42]. Interestingly, it was suggested long ago that during the

formation of gill slits in carp, pharyngeal ectoderm and endoderm undergo comparable morphodynamics, but result in the formation of double germ layer epithelia with an apical ectoderm layer and a basal endoderm layer (*i.e.* reciprocal situation to axolotl oral epithelia) [43].

Likewise, the ecto-endoderm boundary is not easily recognised in dental epithelia. In the Mexican axolotl, the only animal in which the germ layer origin of teeth has been analysed by means of lineage tracing [42, 57], tooth epithelia are derived from both ectoderm and endoderm. In fact, some teeth are found to be of a dual origin. In the case of the mouse dentition, commonly considered ectodermal [58, 59], endoderm cells have been fate-mapped adjacent to tooth buds [60]. In zebrafish, an animal with no oral but only pharyngeal teeth that are considered to develop from endoderm epithelia (*e.g.* [61, 62]), some have speculated that the dentition might be derived from endoderm with some pharyngeal ectoderm epithelial cells in close proximity [38]. According to available data, vertebrate teeth can be derived equally from ectoderm as well as endoderm cells. Moreover, the case of the Mexican axolotl, where a single tooth bud can be of dual epithelial origin [42], implies that 'ectodermal' and 'endodermal' teeth might not differ at any substantial level.

Do all odontodes require neural crest-derived ectomesenchyme?

It has long been supposed that vertebrate odontodes, whether oro-pharyngeal teeth or skin denticles, require reciprocal cell signalling between an epithelium (see last section) and underlying mesenchymal cells (ectomesenchyme) that originate from migrated neural crest [39, 40, 63]. Interestingly, according to Reif's initial ideas [4], odontodes evolved only after neural crest cells were able to migrate and when an inductive interaction occurred between ectodermal epithelia and mesenchyme (although we now know that endodermal epithelium should be included in this statement). It is generally agreed that cells with the properties of neural crest were present prior to the origin of vertebrates [64–67], and that during the early evolution of vertebrates the neural crest cell lineage was expanding its repertoire of migratory derivatives [64–66].

We now understand the molecular interactions that control the earliest stages of neural crest development. A neural crest gene regulatory network (ncGRN), thought to be conserved among vertebrates [68], guides neural crest development from induction at the neural plate border to migration to ultimate differentiation of distinct cell populations including neurons, glia, pigment, bone, cartilage and dentine (this last step is poorly understood). This ncGRN involves interacting signals including Wnts, bone morphogenetic protein families (BMPs) and fibroblast growth factor families (FGFs), and transcriptional regulators including Msx1/2, Zic and Pax3/7, in sequential modules (*e.g.* the neural crest specifier module, which includes the transcription factors Snail2, Sox9/10, FoxD3 and N-Myc [67, 69–72]). These modules co-regulate each other, as well as downstream effector genes that confer properties such as multipotency, the ability to undergo an epithelial to mesenchymal transition, extensive migratory capacity and, subsequently, the ability to differentiate into numerous and distinct derivatives depending upon their final location.

Despite our understanding of gene networks in early neural crest development, the signals and factors governing differentiation of cranial (or any other) neural crest cells to competent ectomesenchyme are relatively unknown [73]. It is clear that FGFs are required for the ectomesenchymal cell lineage within the pharyngeal arches at least [64, 73]. Teeth require ectomesenchyme derived from cranial neural crest [74, 75], but the molecular basis of the neural crest to ectomesenchyme transition is not fully known. Although the neural crest-derived nature of dental ectomesenchyme is now well documented in the mouse, the exact origin of the mesenchymal component for osteichthyan (*i.e.* actinopterygian and basal sarcopterygian fish) dermal scales and chondrichthyan skin denticles is not. Studies suggest that the formation of the dermal skeleton, at least in osteichthyan and chondrichthyan fishes, uses trunk neural crest-derived mesenchyme [2, 22, 30, 76, 77]; however, definitive data are needed to address whether trunk and/or cranial neural crest are necessary and/or sufficient, or whether mesodermal mesenchymal cells and their associated GRN(s) are involved.

A 'dental gene network' and odontode deep homology

Recent data (reviewed above) demonstrate that teeth form from epithelium of ectoderm, endoderm and even a combination of the two, when properly combined with neural crest-derived ectomesenchyme. These observations suggest that the cellular derivation of dental epithelium is not an informative means to sort between types of teeth. Using similar logic, Fraser and colleagues [47] asked if the oral (presumed ectodermal) and pharyngeal (presumed endodermal) teeth of cichlid fishes developed under the control of similar or different gene co-expression groups (CEGs). Despite important differences between oral and pharyngeal teeth (*i.e.* pharyngeal teeth express Hox genes), the main observation was that, regardless of location in the oro-pharyngeal cavity, teeth develop using a common set of genes. Integrating across other studies of vertebrate dentitions (both oral and pharyngeal), the authors delineated a 'core' dental gene set used to make all teeth. This dental CEG includes the well-known molecules β-*catenin*, *bmp2*, *bmp4*, *dlx2*, *eda*, *edar*, *fgf3*, *fgf10*, *notch2*, *pitx2*, *runx2* and *shh* (Table 1).

Considering these recent reports in combination, a slightly different picture of tooth origins emerges. Developmental data from the axolotl [42] and molecular data from fishes [33, 35, 36, 78–82] strongly suggest that the germ-layer origin of dental epithelium does not matter and that teeth derived from ectoderm, endoderm and a mixed origin, exhibit similar morphogenesis. Moreover, the expression of genes does not differ significantly between oral (supposedly ectoderm) and pharyngeal (supposedly endoderm) dentitions in osteichthyans [33, 35, 36, 47, 78–82]. These data imply that we should not view teeth as originating from any single cell type in any single place. Rather, teeth should be seen as derivatives of epithelial-mesenchyme interplay driven by reciprocal interactions among signalling networks (Fig. 2). Teeth develop wherever such networks are expressed, regardless of germ layer distribution. Such a viewpoint holds that there is strong

Table 1. Conserved and coordinated gene expression during the initiation stage of divergent epithelial-contributed structures

Gene	Taste bud	cLL placodes	Tooth	Scale	Gill rakers
β-cat	☑	☑ ☑	☑ ☑	☑ ☑	☑ ☑
bmp2	☑	☑ ☑	☑ ☑	☑ ☑	☑ ☑
bmp4	☒	☑ ☑	☑ ☑	☑ ☑	☑ ☑
dlx2	☒	☒	☑ ☑	☒	☒
eda	☑	☑ ☑	☑ (☑)	☑	☑ ☑
edar	☒	☑ ☑	☑	☑	☑
fgf10	☑	☑ ☑	☑	☑	☑ ☑
fgf3	☑	☑ ☑	☑ (☑)	☑	☑ ☑
notch2	☑	☑ ☑	☑ ☑	☑	☑ ☑
pax9	☑	?	☑	☒	☒
pitx2	☑	☑	☑	☒	☒
runx2	☒	?	☑	☑	☑
sema3F	☑ ☑	☑	☑ ☑	☒	☒
shh	☑	☒	☑	☑	☑
sox2	☑	☑ ☑	☒	☒	☒
stra13	☑	☑ ☑	☑	☒	☒
tbx1	☑	?	☑	☒	?
wnt10a	☑	?	☑ ☑	☑	?
wnt7b	?	☑	☑	?	?

Taste bud [101–103], tooth [34, 47], gill raker [47], scale [NB, within this discussion we address the early evolution of denticles, for which expression data are as yet unavailable; this screen was performed on the developing scales of Malawi cichlids, scales present on teleosts are not the same in structure or development as placoid scales (skin denticles) of chondrichthyans], and the cephalic lateral line (cLL). Bracketed genes refer to differential gene expression among vertebrate groups. Expression data from the teleost (Cichlidae) cLL placode (Fraser, Milholland and Streelman) and scale (Fraser and Streelman) are currently unpublished.
☑ - Epithelial gene expression; ☑ - Mesenchymal gene expression; ☒ - No expression recorded; ? - Unknown expression.

molecular homology among all teeth (and perhaps all odontodes) and that one of the goals of future research is to solve the structure of the networks involved. Note that gene expression data for odontodes are derived almost exclusively from dentitions; it therefore remains to be determined whether skin denticles exhibit a similar degree of shared gene expression (Table 1).

An 'inside and out' model of odontode evolutionary development

The classic hypotheses described above ('outside-in', 'inside-out' and the 'modified outside-in' hypotheses) rely heavily on phylogenetic reconstruction of ancient events, fossil material and a false dichotomy between endoderm and ectoderm dental epithelium. Here, we attempt to push beyond these classic arguments by proposing a new perspective informed by new data. We suggest that odontodes evolved when and wherever epithelial and neural crest derived ectomesenchymal gene networks came into association during development (Fig. 2). We call this the 'inside and out' model to (i) focus attention away from an argument about primacy of location and/or cell type and (ii) clarify that an appreciation of deep homology among odontodes may broaden our evolutionary understanding of these structures, as with animal appendages [83–85].

This alternative perspective of odontode development and evolution has the advantage that some of its tenets should be experimentally testable in extant organisms (see Table 1, Fig. 3 and below). Our perspective incorporates important aspects of the classic hypotheses. We infer homology among all odontodes as does the 'outside-in' theory, although we favour deep molecular homology. Similarly, we agree with the idea of co-option of patterning information, as stated by the 'inside-out' theory. Our proposal is focused on: (1) the presumptive odontode gene regulatory network (oGRN) as the homologous unit rather than specific cell populations; (2) the inference that tissue mixing and incorporation between endoderm and ectoderm to 'place' the cellular competence for odontode development may have occurred, but is unnecessary; (3) the essential components for odontode formation are neural crest-derived ectomesenchyme and any epithelium and (4) the suggestion that an epithelial placode co-expression group (epCEG) linking all odontodes may have had an ancestral sensory function (Fig. 3). Thus, we speculate that the sequence of events was as follows: an epCEG together with the neural crest-derived mesenchymal CEG (mesCEG) came to form the oGRN, common to all odontodes regardless of location; this common oGRN

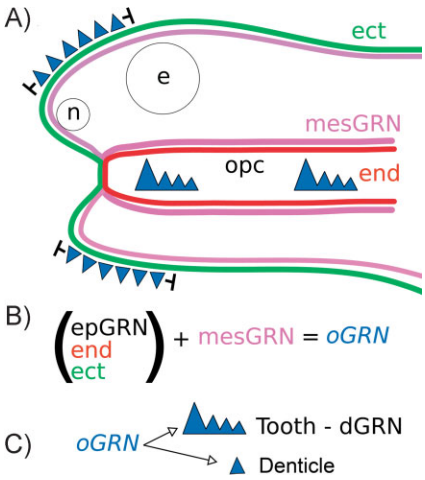

Figure 2. The inside and out gene regulatory hypothesis for odontode evolution. **A**: Schematic diagram represents a generalised early vertebrate/fish in lateral/sagittal view: we propose that regardless of tissue origin (endoderm or ectoderm), the ingredients for odontode evolution, instigated by the appearance of the putative odontode gene regulatory network (oGRN), involved the collaboration of two pre-existing gene co-expression groups: (i) the neural crest-derived ectomesenchymal co-expression group (mesCEG) and (ii) the epithelial co-expression group (epCEG), which operates within both the endoderm and ectoderm (**B**). **C**: The evolution of both skin denticles and teeth were separate operations of the combination of epCEG and mesCEG in alternative locations, the epidermis and the oro-pharyngeal cavity (opc). Within the opc, co-option of the oGRN potential was transferred to the oral jaws during the transition from jawless (agnathans) to jawed vertebrates (gnathostomes). Each CEG (mesCEG and epCEG) must have acted as part of larger yet currently unknown GRN (mesGRN and epGRN).

within the oro-pharyngeal region formed the dental GRN [47] (Figs. 2 and 3).

It is plausible to think that a CEG was already acting within a thickened epCEG for a sensory papillae-like developmental fate (similar to taste bud, Table 1 and Fig. 3). Thus, we speculate that these sensory papillae-like structures evolved ahead of both taste buds and odontodes. With the inclusion of signals from differentiating neural crest (mesCEG), the fate possibilities for these precursor structures were greatly enhanced. Presently, we know little of the functional interrelationships of these CEGs leading to possible precursor gene networks. *De novo* 'instigator networks' are unlikely. Rather, the advent of odontodes was probably the result of 'innovative consolidation' (where two or more pre-existing signalling networks joined to form a new collaborative network) of two or more separate regulatory networks triggered by the wandering neural crest. We envision that an oGRN evolved as the association of: (1) an 'epithelial thickening/placode' CEG (epCEG); in place due to sensory structure development on both the epidermis, *e.g.* related to electro-receptive organs or lateral line extensions and within the oro-pharynx, such as during the development of taste bud-like structures and (2) elements of a neural crest-derived ectomesenchyme CEG (mesCEG) [68, 69, 71]. This may have galvanised the evolution of odontodes for both oro-pharyngeal feeding (teeth) and epidermal protection/armour (denticles) (Fig. 3).

Agenda for future discovery

During the course of evolution, single-surface odontodes formed in the epidermis, retained their function and were modified into odontocomplexes or scales for hydrodynamic enhancements and armour, and those forming in the oro-pharyngeal cavity became utilised for food-processing activities and predatory advantage. The common question 'How did skin denticles become teeth or *vice versa*?' is difficult to answer given the current constraints of the data. We hope to shift thinking by suggesting that gene networks active across multi-cell lineages (ectoderm, endoderm and neural crest) instigated the evolution of odontodes within previously unrelated tissues, in numerous locations.

Notably, our perspective should allow inference of the necessary, sufficient and distinct components of the epCEG, the mesCEG and thus the oGRN (Table 1, Fig. 3). Specifically, research might be directed toward a deeper understanding of the molecular homology among all odontodes, whether the skin denticles of sharks, the scales of fishes or the molar teeth of mammals. For instance, we observe that many of the genes expressed as part of the 'core' dental network are active in the initiation of teleost scales, and sensory structures like the cephalic lateral line placodes and taste buds (Table 1, Fig. 3; and G.J.F./J.T.S., unpublished).

We are intrigued by the idea that the epCEG for odontodes had as its precursor an ancient sensory structure similar to a taste bud. Teeth and oro-pharyngeal denticles are co-localised with taste buds (and related papillae) throughout the oro-pharyngeal cavity of chondrichthyan (personal observation, and ref. 15) and osteichthyan fish [33, 36, 86, 87] and it is becoming clear that both of these now-distinct structures share a similar developmental ground plan (Table 1, Fig. 3). When co-localised, teeth and taste buds both develop from a thickened epithelial placode, from a common epithelium, with teeth forming ahead of taste buds in time [33]. Co-localisation is decoupled in some vertebrates, like mammals. Decoupled co-localisation is also observed in select teleost fish, *e.g.* zebrafish, which have lost oral teeth but have retained the capacity to develop oral taste buds and gained further extra-oral sites [88], although teeth and taste buds are co-localised in the zebrafish posterior pharynx. Taste buds develop from (ectodermal and endodermal) epithelia and it is somewhat unclear what signals are necessary from the underlying mesenchyme for their development [57, 89]. Taste buds might not recruit neural crest-derived mesenchyme for their patterning [89], although mesenchyme might be involved in directing epithelial evaginations and attracting innervation [90]. Innervation is not required for the initiation of teeth [91] or taste buds [92, 93] – in fact, first-generation teeth lack innervation in non-mammalian vertebrates [94] – but it is necessary for subsequent morphogenesis and continued replacement [95].

Importantly, a guidance mechanism exists during development that could account for (i) the link between taste bud-like sensory structures and odontodes and (ii) the transition to tooth/odontode from a sensory precursor. Neuropilin and semaphorin molecules are known to repel/attract both axons and neural crest cells in vertebrate embryos [96–98]. We speculate that guidance mechanisms of this type, already in

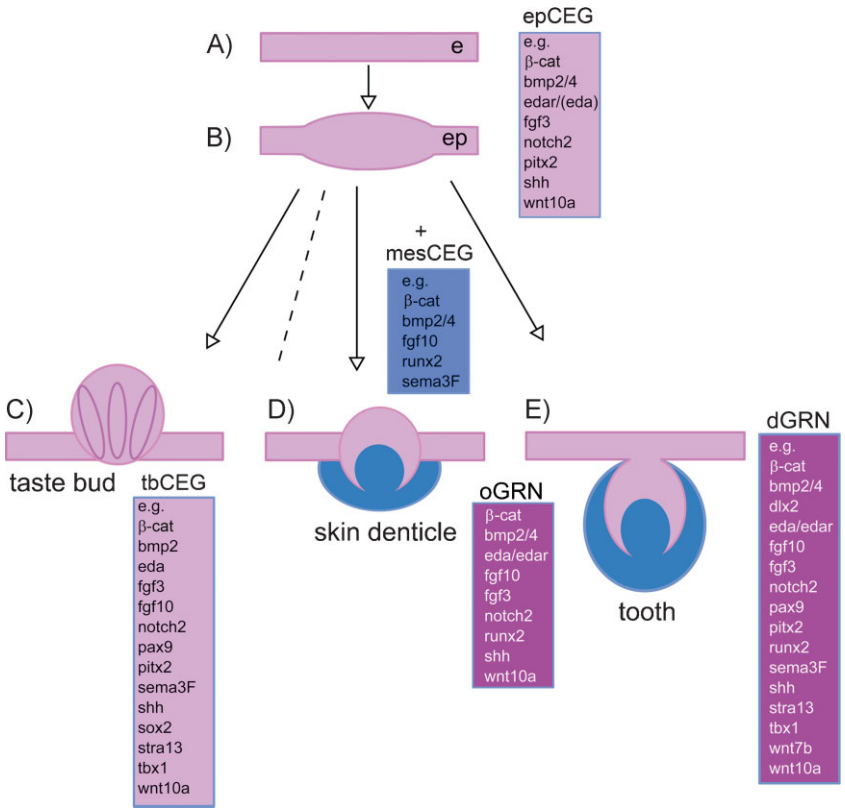

Figure 3. Epithelial transitions and innovative network consolidation. **A**: A generalised epithelium from which a thickened epithelial placode initiates. **B**: The genes expressed within this thickening can be described as the epithelial co-expression group (epCEG). From this thickened epithelial placode the epithelium can transition into a number of structures: (**C**) a taste bud, a similar sensory unit, is a superficial epithelial element posing a unique epithelial gene expression signature (tbCEG); (**D**) a denticle and (**E**) a tooth recruit the underlying mesenchyme that contains the neural crest-derived cell population and the set of genes associated with the neural crest-derived ectomesenchyme (mesCEG). Note that this collection of genes is related to and influenced by the ncGRN. For a more complete list of genes that interact in the ncGRN, embryo-wide, see Refs. [68, 69]. We propose the mesCEG collaborated with the epCEG to provide the ingredients for the oGRN (see also Fig. 2). Skin denticles and teeth are born from the odontode GRN as they are both odontodes by definition. The tooth itself houses a unique subset of genes (collectively the dental GRN). This coordinated gene network contains genes that are not shared with scales and thus we assume dermal denticles, highlighting their evolutionary and developmental separation. The divergence between members of the oGRN and dGRN reflects those genes only expressed in the dentition *versus* those expressed across odontodes determined from expression during teleost scale development; it remains to be tested whether these expression trends hold for denticles of extant sharks and rays.

place for axonal navigation, were co-opted by neural crest cells during the elaboration of vertebrate odontodes. This may in fact be the means with which pioneer neural crest cells 'found' the ancestral epithelial placodes. It must be clarified that we are not suggesting teeth evolved from taste buds, but rather that both odontodes and taste buds may share a common primitive sensory unit that relied on the development of an innervated epithelial placode (Fig. 3B).

Another vital question for future research is the degree to which the ncGRN and the reactive mesenchyme (ectomesenchyme – mesCEG) provide a necessary trigger/cue to push the fate of a taste bud-like sensory structure toward an odontode. A dissection of the molecular controls that distinguish a tooth from a taste bud after the formation of the common epithelial thickening is necessary for these future investigations. Such questions can be addressed *via* manipulation studies. For instance, can we shift the fate of taste bud primordia during early development toward dental/odontode fate, *via* collective overexpression techniques, artificial (*in vitro*) cell combination or tissue recombination assays? Interestingly, loss of follistatin [90] (a mesenchymal, secreted polypeptide that regulates the size, patterning and innervation of taste papillae) in mice can lead to an invaginated lingual epithelium (dysplastic epithelium) into the underlying mesenchyme, reminiscent of the invaginated epithelial thickening during tooth initiation (taste epithelia evaginate to produce the characteristic superficial papillae). Follistatin is expressed during tooth development and contributes to dental epithelial morphogenesis [99] and enamel formation, naturally inhibiting ameloblast differentiation [100]. It will be important to understand how follistatin functions in dentitions co-localised with taste buds, such as those in most non-mammalian vertebrates.

In addition, can we force the neural crest signalling cascades (once identified) to induce odontode fate from endodermal and ectodermal epithelia in isolation? Furthermore, can we defer the promotion of an odontode epithelial thickening for the development of a basic, taste bud-like sensory structure? We note a particularly promising candidate from the data presented in Table 1 [101–103]; *sox2* is expressed solely in taste buds and the cephalic lateral line and appears to label the sensory nature (neuromast cells) of these epithelial placodes. Advancement will also involve dissection of the replacement capacities of each of these structures, teeth, denticles and taste buds. Can the molecular biology of renewal and regeneration be modified to promote alternative fates? Can a mammalian taste bud be re-programmed during replacement to form an odontode or a keratinised tooth-like structure similar to the filiform papillae found in association with taste buds on mammalian tongues [90]?

To sum up, we argue that odontodes originated when two evolving CEGs (a neural crest-derived mesCEG and an ancestral epCEG; Fig. 3) came together in embryological time and space. This union galvanised an 'odontode explosion' during early vertebrate evolution, filling the oro-pharyngeal cavity and covering the epidermis of jawless vertebrates. We hope this perspective will point the focus of future research on those signalling interactions that specify, maintain and delineate odontodes, their possible precursors and their subsequent descendants.

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References

1. **Donoghue PJC.** 2002. Evolution of development of the vertebrate dermal and oral skeletons: unraveling concepts, regulatory theories, and homologies. *Paleobiology* **28**: 474–507.
2. **Ørvig T.** 1967. Phylogeny of tooth tissues: evolution of some calcified tissues in early vertebrates. In Miles AEW. ed; *Structural and Chemical Organisation of Teeth*. New York/London: Academic Press. p 45–110.
3. **Ørvig T.** 1977. A survey of odontodes ('dermal teeth') from developmental, structural, functional and phyletic points of view. In Mahala Andrews S, Miles RS, Walker AD. ed; *Problems in Vertebrate Evolution*. New York: Academic Press. p 53–75.
4. **Reif W-E.** 1982. Evolution of dermal skeleton and dentition in vertebrates: the odontode-regulation theory. *Evol Biol* **15**: 287–368.
5. **Donoghue PC**, **Sansom IJ.** 2002. Origin and early evolution of vertebrate skeletonization. *Microsc Res Technol* **59**: 352–72.
6. **Donoghue PC**, **Forey PL**, **Aldridge RJ.** 2000. Conodont affinity and chordate phylogeny. *Biol Rev Camb Philos Soc* **75**: 191–251.
7. **Donoghue PC**, **Sansom IJ**, **Downs JP.** 2006. Early evolution of vertebrate skeletal tissues and cellular interactions, and the canalization of skeletal development. *J Exp Zool B Mol Dev Evol* **306**: 278–94.
8. **Purnell MA.** 1995. Microwear in conodont elements and macrophagy in the first vertebrates. *Nature* **374**: 798–800.
9. **Smith MM**, **Coates MI.** 1998. Evolutionary origins of the vertebrate dentition: phylogenetic patterns and developmental evolution. *Eur J Oral Sci* **106**: 482–500.
10. **Sire J-Y**, **Donoghue PCJ**, **Vickaryous MK.** 2009. Origin and evolution of the integumentary skeleton in non-tetrapod vertebrates. *J Anat* **214**: 409–40.
11. **Smith MM**, **Johanson Z.** 2003. Separate evolutionary origins of teeth from evidence in fossil jawed vertebrates. *Science* **299**: 1235–6.
12. **Johanson Z**, **Smith MM.** 2003. Placoderm fishes, pharyngeal denticles, and the vertebrate dentition. *J Morphol* **257**: 289–307.
13. **Johanson Z**, **Smith MM.** 2005. Origin and evolution of gnathostome dentitions: a question of teeth and pharyngeal denticles in placoderms. *Biol Rev* **80**: 303–45.
14. **Miyake T**, **Vaglia JL**, **Taylor LH**, *et al.* 1999. Development of dermal denticles in skates (Chondrichthyes, Batoidea): patterning and cellular differentiation. *J Morphol* **241**: 61–81.
15. **Nelson GJ.** 1970. Pharyngeal denticles (placoid scales) of sharks, with notes on the dermal skeleton of vertebrates. *American Museum Novitates* **2415**: 1–26.
16. **Davidson EH**, **McClay DR**, **Hood L.** 2003. Regulatory gene networks and the properties of the developmental process. *Proc Natl Acad Sci USA* **100**: 1475–80.
17. **Davidson EH**, **Rast JP**, **Oliveri P**, *et al.* 2002. A genomic regulatory network for development. *Science* **295**: 1669–78.
18. **Niehrs C**, **Pollet N.** 1999. Synexpression groups in eukaryotes. *Nature* **402**: 483–7.
19. **de Beer GR.** 1971. *Homology: An Unsolved Problem*. London: Oxford University Press.
20. **Wagner GP.** 1989. The biological homology concept. *Annu Rev Ecol Syst* **20**: 51–69.
21. **Reif WE.** 1980. Development of dentition and dermal skeleton in embryonic *Scyliorhinus canicula*. *J Morphol* **166**: 275–88.
22. **Sire JY**, **Huysseune A.** 2003. Formation of dermal skeletal and dental tissues in fish: a comparative and evolutionary approach. *Biol Rev Camb Philos Soc* **78**: 219–49.
23. **Smith MM**, **Coates MI.** 2001. The evolution of vertebrate dentitions: phylogenetic pattern and developmental models (palaeontology, phylogeny, genetics and development). In Ahlberg PE. ed; *Major Events in Early Vertebrate Evolution*. London and New York: Taylor and Francis. p 223–40.
24. **Smith MM**, **Hall BK.** 1990. Developmental and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. *Biol Rev Camb Philos Soc* **65**: 277–374.
25. **Jolie M.** 1968. Some implications of the acceptance of a delamination principle. In Orvig T. ed; *Current Problems of Lower Vertebrate Phylogeny*. Stockholm: Almqvist and Wiskell. p 89–108.
26. **Schaeffer B.** 1977. The dermal skeleton in fishes. In Andrews SM, Miles RS, Walker AD. ed; *Problems in Vertebrate Evolution*. London: Academic Press. p 25–52.
27. **Romer AS.** 1936. *Vertebrate Paleontology*. Illinois, Chicago: The University of Chicago Press. p 492.
28. **Smith MM.** 2003. Vertebrate dentitions at the origin of jaws: when and how pattern evolved. *Evol Dev* **5**: 394–413.
29. **Smith MM**, **Coates MI.** 2000. Evolutionary origins of teeth and jaws:developmental models and phylogenetic patterns. In Teaford MF, Smith MM, Ferguson MWJ. ed; *Development, Function and Evolution of Teeth*. Cambridge: Cambridge University Press. p 133–51.
30. **Sire JY.** 2001. Teeth outside the mouth in teleost fishes: how to benefit from a developmental accident. *Evol Dev* **3**: 104–8.
31. **Sire JY**, **Akimenko MA.** 2004. Scale development in fish: a review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). *Int J Dev Biol* **48**: 233–47.
32. **Sire JY**, **Allizard F**, **Babiar O**, *et al.* 1997. Scale development in zebrafish (*Danio rerio*). *J Anat* **190**: 1545–61.
33. **Fraser GJ**, **Berkovitz BK**, **Graham A**, *et al.* 2006. Gene deployment for tooth replacement in the rainbow trout (*Oncorhynchus mykiss*): a developmental model for evolution of the osteichthyan dentition. *Evol Dev* **8**: 446–57.
34. **Fraser GJ**, **Bloomquist RF**, **Streelman JT.** 2008. A periodic pattern generator for dental diversity. *BMC Biol* **6**: 32.
35. **Fraser GJ**, **Graham A**, **Smith MM.** 2004. Conserved deployment of genes during odontogenesis across osteichthyans. *Proc R Soc Lond B Biol Sci* **271**: 2311–27.
36. **Fraser GJ**, **Graham A**, **Smith MM.** 2006. Developmental and evolutionary origins of the vertebrate dentition: molecular controls for spatiotemporal organisation of tooth sites in Osteichthyans. *J Exp Zool B Mol Dev Evol* **306**: 183–203.
37. **der Brugghen WV**, **Janvier P.** 1993. Denticles in thelodonts. *Nature* **364**: 107–7.
38. **Huysseune A**, **Sire JY**, **Witten PE.** 2009. Evolutionary and developmental origins of the vertebrate dentition. *J Anat* **214**: 465–76.
39. **Graveson AC**, **Smith MM**, **Hall BK.** 1997. Neural crest potential for tooth development in a urodele amphibian: developmental and evolutionary significance. *Dev Biol* **188**: 34–42.
40. **Sellman S.** 1946. Some experiments on the determination of the larval teeth in *Ambystoma mexicanum*. *Odont Tidskr* **54**: 1–54.
41. **Wilde CE.** 1955. The urodele neuroepithelium. I. The differentiation *in vitro* of the cranial neural crest. *J Exp Zool* **130**: 573–91.
42. **Soukup V**, **Epperlein HH**, **Horacek I**, *et al.* 2008. Dual epithelial origin of vertebrate oral teeth. *Nature* **455**: 795–8.
43. **Edwards LF.** 1929. The origin of the pharyngeal teeth of the carp (*Cyprinus carpio* Linnaeus). *Ohio J Sci* **29**: 93–130.
44. **Kardong KV.** 1995. *Vertebrates. Comparative Anatomy, Function, Evolution*. New York, NY: McGraw-Hill.
45. **Romer AS.** 1962. *The Vertebrate Body*. 3rd Edition. Philadelphia: W.B. Saunders.
46. **Armstrong HA**, **Smith CJ.** 2001. Growth patterns in euconodont crown enamel: implications for life history and mode-of-life reconstruction in the earliest vertebrates. *Proc R Soc Lond B* **268**: 815–20.

47. **Fraser GJ**, **Hulsey CD**, **Bloomquist RF**, *et al.* 2009. An ancient gene network is co-opted for teeth on old and new jaws. *PLoS Biol* **7**: e31.
48. **Purnell MA.** 2002. Feeding in extinct jawless heterostracan fishes and testing scenarios of early vertebrate evolution. *Proc R Soc Lond B Biol Sci* **269**: 83–8.
49. **Graham A.** 2001. The development and evolution of the pharyngeal arches. *J Anat* **199**: 1133–41.
50. **Graham A**, **Smith A.** 2001. Patterning the pharyngeal arches. *BioEssays* **23**: 54–61.
51. **Balinsky BI.** 1975. *An Introduction to Embryology*. Philadelphia: W.B. Saunders.
52. **Dickinson AJ**, **Sive H.** 2006. Development of the primary mouth in *Xenopus laevis*. *Dev Biol* **295**: 700–13.
53. **Waterman RE.** 1985. Formation and perforation of closing plates in the chick embryo. *Anat Rec* **211**: 450–7.
54. **Waterman RE**, **Schoenwolf GE.** 1980. The ultrastructure of oral (buccopharyngeal) membrane formation and rupture in the chick embryo. *Anat Rec* **197**: 1441–70.
55. **Graham A.** 2003. Development of the pharyngeal arches. *Am J Med Genet A* **119**: 251–6.
56. **Graham A.** 2008. Deconstructing the pharyngeal metamere. *J Exp Zool B Mol Dev Evol* **310**: 336–44.
57. **Barlow LA**, **Northcutt RG.** 1995. Embryonic origin of amphibian taste buds. *Dev Biol* **169**: 273–85.
58. **Pispa J**, **Thesleff I.** 2003. Mechanisms of ectodermal organogenesis. *Dev Biol* **262**: 195–205.
59. **Tucker A**, **Sharpe P.** 2004. The cutting-edge of mammalian development; how the embryo makes teeth. *Nat Rev Genet* **5**: 499–508.
60. **Imai H**, **Osumi N**, **Eto K.** 1998. Contribution of foregut endoderm to tooth initiation of mandibular incisor in rat embryos. *Eur J Oral Sci* **106**: 19–23.
61. **Stock DW.** 2001. The genetic basis of modularity in the development and evolution of the vertebrate dentition. *Philos Trans R Soc Lond B Biol Sci* **356**: 1633–53.
62. **Huysseune A**, **Van der Heyden C**, **Verreijdt L**, *et al.* 2002. Fish dentitions as paradigms for odontogenic questions. *Connect Tissue Res* **43**: 98–102.
63. **De Beer GR.** 1947. The differentiation of neural crest cells into visceral cartilages and odontoblasts in Amblystoma, and a re-examination of the germ-layer theory. *Proc R Soc Lond B* **134**: 377–98.
64. **Donoghue PC**, **Graham A**, **Kelsh RN.** 2008. The origin and evolution of the neural crest. *BioEssays* **30**: 530–41.
65. **Graham A.** 2004. Evolution and development: rise of the little squirts. *Curr Biol* **14**: R956–8.
66. **Jeffery WR**, **Strickler AG**, **Yamamoto Y.** 2004. Migratory neural crest-like cells form body pigmentation in a urochordate embryo. *Nature* **431**: 696–9.
67. **Baker CV.** 2008. The evolution and elaboration of vertebrate neural crest cells. *Curr Opin Genet Dev* **18**: 536–43.
68. **Sauka-Spengler T**, **Meulemans D**, **Jones M**, *et al.* 2007. Ancient evolutionary origin of the neural crest gene regulatory network. *Dev Cell* **13**: 405–20.
69. **Sauka-Spengler T**, **Bronner-Fraser M.** 2006. Development and evolution of the migratory neural crest: a gene regulatory perspective. *Curr Opin Genet Dev* **16**: 360–6.
70. **Sauka-Spengler T**, **Bronner-Fraser M.** 2008. Evolution of the neural crest viewed from a gene regulatory perspective. *Genesis* **46**: 673–82.
71. **Sauka-Spengler T**, **Bronner-Fraser M.** 2008. A gene regulatory network orchestrates neural crest formation. *Nat Rev Mol Cell Biol* **9**: 557–68.
72. **Baker CV**, **Bronner-Fraser M.** 1997. The origins of the neural crest. Part II: an evolutionary perspective. *Mech Dev* **69**: 13–29.
73. **Blentic A**, **Tandon P**, **Payton S**, *et al.* 2008. The emergence of ectomesenchyme. *Dev Dyn* **237**: 592–601.
74. **Chai Y**, **Jiang X**, **Ito Y**, *et al.* 2000. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* **127**: 1671–9.
75. **Imai H**, **Osumi-Yamashita N**, **Ninomiya Y**, *et al.* 1996. Contribution of early-emigrating midbrain crest cells to the dental mesenchyme of mandibular molar teeth in rat embryos. *Dev Biol* **176**: 151–65.
76. **Sire J-Y**, **Marin S**, **Allizard F.** 1998. Comparison of teeth and dermal denticles (odontodes) in the teleost *Denticeps clupeoides* (clupeomorpha). *J Morphol* **237**: 237–55.
77. **Smith M**, **Hickman A**, **Amanze D**, *et al.* 1994. Trunk neural crest origin of caudal fin mesenchyme in the zebrafish *Brachydanio rerio*. *Proc R Soc Lond B* **256**: 137–45.
78. **Debiais-Thibaud M.** 2007. Development of oral and pharyngeal teeth in the medaka (*Oryzias latipes*): comparison of morphology and expression of eve1 gene. *J Exp Zool B Mol Dev Evol* **308**: 693–708.
79. **Jackman WR**, **Draper BW**, **Stock DW.** 2004. Fgf signaling is required for zebrafish tooth development. *Dev Biol* **274**: 139–57.
80. **Laurenti P**, **Thaeron C**, **Allizard F**, *et al.* 2004. Cellular expression of eve1 suggests its requirement for the differentiation of the ameloblasts and for the initiation and morphogenesis of the first tooth in the zebrafish (*Danio rerio*). *Dev Dyn* **230**: 727–33.
81. **Stock DW**, **Jackman WR**, **Trapani J.** 2006. Developmental genetic mechanisms of evolutionary tooth loss in cypriniform fishes. *Development* **133**: 3127–37.
82. **Wise SB**, **Stock DW.** 2006. Conservation and divergence of Bmp2a, Bmp2b, and Bmp4 expression patterns within and between dentitions of teleost fishes. *Evol Dev* **8**: 511–23.
83. **Shubin N**, **Tabin C**, **Carroll S.** 1997. Fossils, genes and the evolution of animal limbs. *Nature* **388**: 639–48.
84. **Shubin N**, **Tabin C**, **Carroll S.** 2009. Deep homology and the origins of evolutionary novelty. *Nature* **457**: 818–23.
85. **Wagner GP.** 2007. The developmental genetics of homology. *Nat Rev Genet* **8**: 473–9.
86. **Linser PJ**, **Carr WE**, **Cate HS**, *et al.* 1998. Functional significance of the co-localization of taste buds and teeth in the pharyngeal jaws of the largemouth bass, *Micropterus salmoides*. *Biol Bull* **195**: 1273–81.
87. **Kumari U**, **Yashpal M**, **Mittal S**, *et al.* 2005. Morphology of the pharyngeal cavity, especially the surface ultrastructure of gill arches and gill rakers in relation to the feeding ecology of the catfish *Rita rita* (Siluriformes, Bagridae). *J Morphol* **265**: 197–208.
88. **Hansen A**, **Reutter K**, **Zeiske E.** 2002. Taste bud development in the zebrafish *Danio rerio*. *Dev Dyn* **223**: 483–96.
89. **Barlow LA**, **Northcutt RG.** 1997. Taste buds develop autonomously from endoderm without induction by cephalic neural crest or paraxial mesoderm. *Development* **124**: 949–57.
90. **Beites CL**, **Hollenbeck PL**, **Kim J**, *et al.* 2009. Follistatin modulates a BMP autoregulatory loop to control the size and patterning of sensory domains in the developing tongue. *Development* **136**: 2187–97.
91. **Lumsden AGS**, **Buchanan JAG.** 1986. An experimental study of timing and topography of early tooth development in the monse embryo with an analysis of the role of innervation. *Arch Oral Biol* **31**: 301–11.
92. **Barlow LA**, **Chien CB**, **Northcutt RG.** 1996. Embryonic taste buds develop in the absence of innervation. *Development* **122**: 1103–11.
93. **Ito A**, **Nosrat IV**, **Nosrat CA.** 2010. Taste cell formation does not require gustatory and somatosensory innervation. *Neurosci Lett* **471**: 189–94.
94. **Sire JY**, **Davit-Beal T**, **Delgado S**, *et al.* 2002. First-generation teeth in nonmammalian lineages: evidence for a conserved ancestral character? *Microsc Res Technol* **59**: 408–34.
95. **Kollar EJ**, **Lumsden AG.** 1979. Tooth morphogenesis: the role of the innervation during induction and pattern formation. *J Biol Buccale* **7**: 49–60.
96. **Gammill LS**, **Gonzalez C**, **Gu C**, *et al.* 2006. Guidance of trunk neural crest migration requires neuropilin 2/semaphorin 3F signaling. *Development* **133**: 99–106.
97. **Rohm B**, **Ottemeyer A**, **Lohrum M**, *et al.* 2000. Plexin/neuropilin complexes mediate repulsion by the axonal guidance signal semaphorin 3A. *Mech Dev* **93**: 95–104.
98. **Yu HH**, **Moens CB.** 2005. Semaphorin signaling guides cranial neural crest cell migration in zebrafish. *Dev Biol* **280**: 373–85.
99. **Wang XP**, **Suomalainen M**, **Jorgez CJ**, *et al.* 2004. Modulation of activin/bone morphogenetic protein signaling by follistatin is required for the morphogenesis of mouse molar teeth. *Dev Dyn* **231**: 98–108.
100. **Wang XP**, **Suomalainen M**, **Jorgez CJ**, *et al.* 2004. Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation. *Dev Cell* **7**: 719–30.
101. **Iwatsuki K**, **Liu HX**, **Gronder A**, *et al.* 2007. Wnt signaling interacts with Shh to regulate taste papilla development. *Proc Natl Acad Sci USA* **104**: 2253–8.
102. **Liu F**, **Thirumangalathu S**, **Gallant NM**, *et al.* 2007. Wnt-beta-catenin signaling initiates taste papilla development. *Nat Genet* **39**: 106–12.
103. **Okubo T**, **Pevny LH**, **Hogan BL.** 2006. Sox2 is required for development of taste bud sensory cells. *Genes Dev* **20**: 2654–9.