Published in final edited form as:

Curr Top Dev Biol. 2015 ; 115: 321–333. doi:10.1016/bs.ctdb.2015.07.005.

Developmental plasticity of patterned and regenerating oral organs

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Abstract

In many aquatic vertebrates, including bony and cartilaginous fishes, teeth and taste buds co-localize on jaw elements. In these animals, taste buds are renewed continuously throughout life, whereas teeth undergo cyclic whole organ replacement by various means. Recently, studies of cichlid fishes have yielded new insights into the development and regeneration of these dental and sensory oral organs. Tooth and taste bud densities co-vary positively across species with different feeding strategies, controlled by common regions of the genome and integrated molecular signals. Developing teeth and taste buds share a bipotent epithelium during early patterning stages, from which dental and taste fields are specified. Moreover, these organs share a common epithelial ribbon that supports label-retaining cells during later stages of regeneration. During both patterning and regeneration stages, dental organs can be converted to taste bud fate by manipulation of BMP signaling. These observations highlight a surprising long-term plasticity between dental and sensory organ types. Here, we review these findings and discuss the implications of developmental plasticity that spans the continuum of craniofacial organ patterning and regeneration.

Keywords

tooth; taste bud; patterning; stem cell; development & regeneration

Introduction and background

Vertebrate dentitions differ in their location, pattern and regenerative capacity. Birds and turtles lack true teeth entirely; some bony fishes such as zebrafish possess teeth only in the pharynx, whereas others (e.g., cichlids) have numerous rows of teeth on both oral and pharyngeal jaws (Fraser *et al.* 2009). Sharks and rays exhibit teeth (often in multiple rows) or tooth plates on their cartilaginous oral jaws, and mammalian teeth are confined to a single row at the oral jaw margin. Mammalian teeth may vary in shape and function along the tooth row (e.g., incisor, molar) but non-mammalian dentitions tend to be homodont (i.e., each tooth is the same shape in a row; tooth shape may differ across rows). Non-mammalian teeth are replaced *de novo* throughout life via various mechanisms (Tucker and Fraser 2014), but

most mammals have lost this regenerative ability. For example, humans replace each tooth only once and mice never replace their teeth.

In sharks, snakes, lizards, crocodiles and mammals that undergo limited replacement, the earliest embryonic teeth are typically smaller versions of their adult counterparts. However, in ray-finned fishes, lungfishes and salamanders, first generation teeth are small and simple unicuspid (i.e., one-cusp teeth), lacking neurovascular bundles [NVBs] (Sire *et al.* 2002). In these latter dentitions, shape and increased complexity (e.g., size, curvature, multiple cusps) arise through the process of replacement. In cichlid fishes, the particular oral jaw tooth pattern -- that is, the size and spacing of teeth -- is set with the initiation of the first generation, prior to the development of complex tooth shape (Fraser *et al.* 2008). This occurs differently in the pharyngeal dentition of sticklebacks (Cleves *et al.* 2014). Cichlids exhibit one-for-one tooth replacement (like humans, unlike sharks), with each oral tooth replaced in 30–100 day cycles (depending on age and size), never in adjoining tooth positions (Tuisku and Hildebrand 1994). This establishes a continuum of lifelong dental patterning and regeneration that integrates key molecular pathways of odontogenesis and organ renewal (Fraser *et al.* 2013).

Mammalian and non-mammalian dentitions differ in at least one other important aspect. In mammals, teeth are confined to a single row on the oral jaw, and taste buds are located separately on the tongue and palate. In other vertebrate groups, including bony and cartilaginous fishes, teeth and taste buds are often co-localized on jaw elements (Figure 1).

We highlight recent advances in our understanding of the development and regeneration of these co-localized oral organs, focusing on cichlid fishes from Lake Malawi. These studies reveal a surprising and long lasting plasticity between dental and sensory organs that spans the continuum of craniofacial organ patterning and regeneration. In what follows, we review recent findings and discuss the implications of long-term developmental plasticity for the evolution and bio-inspired design of oral organs.

The continuum of oral organ patterning and regeneration

Whole organ replacement of dentitions in a one-for-one fashion, while maintaining fidelity of pattern, requires (i) signals of dental competence to specify tooth vs. non-tooth, (ii) an odontogenesis program that can be repeatedly recruited within a tooth family, (iii) signals/sources of renewal that can be cyclically and spatially restricted, and (iv) a clock mechanism to coordinate timing in (alternate) tooth positions. Cichlid teeth execute largely conserved developmental programs of competence and morphogenesis (Fraser *et al.* 2013) in an environment filled with taste buds (Figure 1).

Recently, the evolutionary developmental interplay between these placode-derived craniofacial organs was examined. There is an adaptive positive correlation between oral jaw densities of teeth and taste buds in adult Lake Malawi cichlids. Piscivorous and planktivorous species, which hunt with acute vision and swallow prey without processing it in the mouth, exhibit reduced tooth and taste bud densities. Algivores and insectivores, on the other hand, which actively search for appropriate food and acquire it with the mouth, possess many teeth and taste buds. Using an intercross design (i.e., a cross between a

planktivore and an algivore species) and genetic linkage mapping, Bloomquist and colleagues identified common regions of the genome that contribute to this phenotypic correlation (Bloomquist *et al.* 2015a).

To explore this association during development, they next used *in situ* hybridization (ISH) at 5 days post fertilization (5dpf), when the oral jaws first become apparent, to chart the spatial activity of tooth and taste bud markers. The earliest oral lamina expresses *pitx2* (a marker of dental epithelium), *shh* (dental and taste epithelium), and *sox2* (taste epithelium) in overlapping patterns (Figure 2A). A day later, the first *pitx2*⁺, *shh*⁺ dental placodes are detectable and *calb2*⁺ (a marker of mature taste buds with more distinct expression than *sox2*) taste buds lingual to first generation teeth are initiated. Subsequently, a labial band of taste buds appears flanking the first tooth row. As seen in dorsal views of whole mount ISH jaws, *pitx2*, *shh* and *sox2* continue to be expressed in these increasingly distinct fields (Figure 2B).

Small molecule manipulation of the Wnt, Hh and BMP pathways, during the critical developmental window (5-6dpf) when organ fields differentiate one from the other, provides clues to the regulatory logic of cichlid tooth and taste bud co-patterning. Wnt signaling couples tooth and taste bud density and acts upstream of Hh and BMP, which together mediate organ plasticity. LDN treatment, which inhibits BMP signaling, results in a striking phenotype wherein tooth density is reduced and taste buds infiltrate the tooth field (Figure 6A–B'). Thus, these studies and others suggest that cichlid teeth and taste buds are tightly co-patterned, under common genetic and developmental control (Bloomquist *et al.* 2015a). Developmental plasticity or bipotency may be a general feature of organ systems like teeth and taste buds that function together -- as previously noted for liver/pancreas (Xu *et al.* 2011), and heart/lung (Peng *et al.* 2013).

Although teeth and taste buds are typically studied in isolation because of their separate location in mammals, vertebrate teeth and taste buds exhibit significant gene synexpression and may share an ancestral origin (Fraser *et al.* 2010). Moreover, a common epithelial ribbon links cichlid teeth and taste buds as they regenerate (Figure 3).

As seen in sagittal section of the lower oral jaw, the successional lamina for each replacement tooth (outlined in red) is contiguous with oral epithelium that houses taste buds (outlined in orange) and their support cells. Both organs express members of FGF, Hh and Wnt pathways (see also Fraser *et al.* 2013). BMP pathway genes are biased toward expression in dental epithelium and mesenchyme, whereas BMP antagonists (e.g., *fst*, *osr2*) are active in non-dental mesenchyme, subjacent to taste buds (Bloomquist *et al.* 2015a). Notably, at these later stages, *pitx2* remains confined to teeth and *calb2* marks taste buds, but other genes thought to be organ-specific are not. *sox2* is expressed in both organs, as is *foxa2*, previously described as an endodermal taste bud marker (Beites *et al.* 2009; Luo *et al.* 2009). *foxa2* activity in these most rostral teeth and taste buds thought to be derived from ectoderm is reminiscent of previous observations that putatively endoderm-derived pharyngeal dentitions express the ectodermal factors *eda* and *edar* (Fraser *et al.* 2009).

The integration of teeth and taste buds over ontogeny prompted examination of the expression of adult stem cell markers in these adjoining structures undergoing renewal (taste buds) and replacement (teeth). Chosen markers have known roles in (i) regenerating reptile teeth, (ii) the labial stem cell niche of mouse incisors, (iii) taste bud and tongue epithelium renewal, (iv) dental mesenchymal homeostasis/repair, as well as (v) intestinal villi and (vi) hair follicle regeneration (Okubo *et al.* 2009; Handrigan *et al.* 2010; Juuri *et al.* 2013; Biehs *et al.* 2013; Tanaka *et al.* 2013; Yee *et al.* 2013; Kaukua *et al.* 2014; intestine and hair follicle data reviewed in Blanpain and Fuchs 2014 and Krieger and Simons 2015).

Visualized in sagittal section, adult stem cell markers are expressed in the successional lamina, at the base of taste buds, in the cervical loop domains and at the tip of replacement teeth, in the dental follicle, in dental mesenchyme where teeth will be penetrated by NVBs, and in the mesenchyme subjacent to taste buds (Figure 4). Stem cell marker expression mirrors the location of label-retaining cells (LRCs) after long-term BrdU pulse-chase experiments. Immunohistochemical (IHC) co-staining of LRCs with select adult stem factors (e.g., Sox2, Bmi1) further supports the location of multiple stem niches in adjacent taste bud and dental territories (Figure 5; Bloomquist *et al.* 2015b). Overall, cichlid teeth and taste buds exhibit sustained cellular and molecular connectivity -- from early patterning into regeneration stages.

The plasticity of stem-based organ regeneration

Recent work in models of stem cell driven organ renewal (e.g., tooth, intestine, hair follicle) has revealed surprising plasticity and noteworthy context dependence of cellular behavior. In mouse incisor mesenchyme for instance, pericytes can be reprogrammed to form odontoblasts upon injury (Feng *et al.* 2011), whereas neural crest derived glia (Kaukua *et al.* 2014) and Gli1-expressing periarterial cells associated with the NVB (Zhao *et al.* 2014) contribute to dental tissue homeostasis. Similarly in the intestinal crypt and the hair follicle, cells from differentiated organ zones can regain stem cell competence and ultimately repopulate the organ upon targeted ablation (Blanpain and Fuchs 2014). Notably, epithelial cells from outside the hair follicle can migrate to the follicular stem cell niche. Once in position, these cells behave like endogenous stem cells and promote regeneration (Rompolas *et al.* 2013). Taken together, these studies highlight the importance of the niche environment, which can impinge upon internal molecular programs to mediate reversible behavior of cells (Nieto 2013).

Given the developmental bipotency observed for cichlid teeth and taste buds (Bloomquist *et al.* 2015a and above), recent studies addressed whether this plasticity persists into later regeneration stages. Intriguingly, after brief LDN (BMP inhibitor) treatment of juvenile animals at organ regeneration stages, *shh* expression is abrogated from the cervical loop regions of cichlid teeth and *calb2* is activated in these domains (Bloomquist *et al.* 2015b; Figure 6A”–B”).

These data suggest that a change in the local environment is sufficient to switch the competence of progenitor cells from a dental to sensory fate. It is generally unknown whether the reverse is true; whether taste buds cells can adopt dental competence.

Interestingly, Beites *et al.* (2009) showed that tongue taste buds in Follistatin (a BMP antagonist) null mice invaginated rather than evaginated, recapitulating at least a morphogenetic hallmark of tooth development. Overall, these findings reveal a striking and potentially long lasting plasticity between dental and taste bud tissues, raising the possibility that these oral organs can be inter-converted throughout the time window from developmental patterning to adult regeneration. Such inter-organ reprogramming is a unique extension of the intra-organ conversion discussed above for intestine and hair follicle models (Blanpain and Fuchs 2014).

Cichlids combine the features of renewing and replacing oral organ systems

Teeth and taste buds of cichlid fishes are co-patterned across ontogeny, from an early bipotent epithelium to cellular connectivity during regeneration stages. These organs may share developmental as well as adult progenitors, and remarkable inter-organ plasticity is observed across the lifespan (Bloomquist *et al.* 2015a, b; Figure 6). Cichlid taste buds persist in the mouth and are continuously renewed, probably fueled by label-retaining stem cells located at their base and throughout the oral epithelium (see Figure 5), similar in large part to taste bud regeneration on the mammalian tongue (Okubo *et al.* 2009). Cichlid taste buds are innervated throughout the lifetime of the organ (Figure 10.3 in Streelman 2013; Bloomquist *et al.* 2015a). Cichlid teeth, on the other hand, exhibit one-for-one cycled replacement, roughly every 5 weeks per tooth. Label-retaining stem cells are located in the cervical loop regions, at the tooth tip and in dental mesenchyme (Bloomquist *et al.* 2015b; Figure 5, 6). Teeth and taste buds share expression of adult stem cell markers *igfbp5*, *sox2*, *lgr4*, *lgr6*, *bmi1* and *hopx*, also observed in other regenerating systems like intestinal villi and hair follicles.

Cichlid teeth exhibit intramedullary replacement -- within a bony crypt of the jaw (Figure 7). Whole-tooth replacement thus entails significant jaw remodeling. Given that some individuals possess hundreds of oral (and pharyngeal) teeth (Fraser *et al.* 2008; 2009), all undergoing patterned replacement, cichlid jaws face a constant biomechanical demand as a result of this turnover. Whole-tooth replacement also requires NVB pathfinding and re-innervation, as functional teeth are shed and replacement organs take their place (Figure 7).

The trigger of cycled tooth replacement and re-innervation has yet to be identified. The recent finding that hair follicle 'plucking' initiates a sequential immune cascade, which mobilizes stem-based regional regeneration (Chen *et al.* 2015), may provide a model. Whole-tooth replacement would then be viewed as a natural case of experimental wounding or ablation (also Rompolas *et al.* 2013).

Generally, the proximity of cichlid teeth and taste buds puts two distinct types of regenerating organ systems together. Taste buds undergo continuous renewal but not organ replacement; teeth are cyclically replaced *in toto*. This effectively locates a system of homeostatic renewal and continuous turnover just superficial to one characterized by cycled whole-organ replacement (Figure 7). This is analogous to placing many of the features and molecular markers of intestinal villi next to those of a hair follicle. It has been speculated

that the difference between organisms with lifelong regeneration of complex dentitions (e.g., cichlids, reptiles, sharks) and those without (mammals) lies in the continued maintenance and ultimate recruitment of stem cells superficial to the successional lamina (Handrigan *et al.* 2010; Fraser *et al.* 2013). Cichlids may solve this problem by integrating the development and regeneration of co-patterned sensory and dental organs.

Acknowledgements

The US National Institute of Dental and Craniofacial Research supports our work on tooth and taste bud evolution, development and regeneration (2R01DE019637-06 to JTS, F30DE023013 to RFB).

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Figure 1.

Co-mingling of teeth and taste buds on the oral jaws of Lake Malawi cichlid fishes. ISH of *calb2* in the cichlid lower jaw, at 4 months of age. Yellow asterisk marks rows of expression in taste buds, while rows of teeth are marked by red numerals. Note that tooth row three has not yet erupted. Dorsal view, labial to bottom of page, scale=100 μ m.

Figure 2.

Teeth and taste buds share a bipotent oral lamina. ISH of dental marker *pitx2*, taste/sensory marker *sox2*, and an early marker of both, *shh*, at two representative stages of development. Top panel: Sagittal section (18µm thickness, 40X magnification) at initial stages of jaw formation, 5dpf. Bottom panel: By 12dpf, distinct fields of teeth (marked by *pitx2*), and taste buds (*sox2*) have emerged, while *shh* is expressed in both domains. Whole mount dorsal views, labial to bottom of page.

Figure 3.

Teeth and taste buds share an epithelial ribbon and molecular marker expression during regeneration stages (~20dpf). Orange dashes outline taste buds, green outlines the functional tooth, and red outlines replacement teeth. Note expression of *fst* and *bmp4* outside of and juxtaposed to the taste bud unit. These are sagittal sections, 40X magnification; labial is to the bottom of the page, rostral to the left, scale=10μm.

Figure 4.

Expression of adult stem cell markers in cichlid tooth and taste bud renewal. In A-G, orange dashes outline taste buds, green outlines the functional tooth, and red outlines replacement teeth. These are sagittal sections, 40X; labial is to the bottom of the page, rostral to the left, scale in B=20μm. Sox10 IHC (H) in green with replacement tooth epithelium outlined in white. LRC after 100 days chase (I) marked with IHC against BrdU, where LRC clusters are seen at cervical loops, tip of tooth, and in taste bud and surrounding epithelium; replacement tooth outlined in white, 20x objective.

Figure 5. Identification of LRCs/stem cells in cichlid tooth and taste bud regeneration. Double label for LRC after 90 days chase (red) and IHC of adult stem marker Sox2 (blue). This is a sagittal section; 20x objective; labial is to the bottom of the page, rostral to the left.

Figure 6.

Inter-organ plasticity between cichlid teeth and taste buds at patterning and regeneration stages. *calb2* ISH was used to mark taste buds. A-B' show infiltration of taste buds into the tooth field and reduced tooth density in early (5-6dpf) LDN-treated animals. In the middle panel, midline of cleared and stained fishes is marked by a white line, teeth in each half of the dentary are marked by white numerals. A-B', these are dorsal views of dentaries, labial to bottom of page. At later regeneration stages (20dpf), LDN treatment results in *calb2* expression in the cervical loops of replacement tooth germs (B''), which is never present in controls (A''). A''-B'' are sagittal sections, 40x objective. For experiments at patterning stages, animals were treated for 24 hours at 5-6dpf, allowed to recover for a week, then sacrificed and assayed. For experiments at regeneration stages, animals were treated for 48 hours at 20dpf and then immediately sacrificed and assayed.

Figure 7.

Cichlid teeth and taste buds combine the features of regenerating and replacing organ systems. Here, layers of epithelium are indicated in blue and green. Light pink at the base of the replacement tooth shows dental papilla. Areas of bone are depicted in white, with broken white lines representing remodeling of the bony crypt. Dark pink NVBs are shown penetrating the functional tooth and taste bud, with broken lines in areas of re-innervation. Putative stem cell niches are indicated by crosshatching (mesenchyme) and parallel lines (epithelium). Oral to left of page; labial to bottom.