



# From molecules to mastication: the development and evolution of teeth

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Teeth are unique to vertebrates and have played a central role in their evolution. The molecular pathways and morphogenetic processes involved in tooth development have been the focus of intense investigation over the past few decades, and the tooth is an important model system for many areas of research. Developmental biologists have exploited the clear distinction between the epithelium and the underlying mesenchyme during tooth development to elucidate reciprocal epithelial/mesenchymal interactions during organogenesis. The preservation of teeth in the fossil record makes these organs invaluable for the work of paleontologists, anthropologists, and evolutionary biologists. In addition, with the recent identification and characterization of dental stem cells, teeth have become of interest to the field of regenerative medicine. Here, we review the major research areas and studies in the development and evolution of teeth, including morphogenesis, genetics and signaling, evolution of tooth development, and dental stem cells. © 2012 Wiley Periodicals, Inc.

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## MORPHOGENESIS AND DEVELOPMENT

The formation of a head with complex jaws and networked sensory organs was a central innovation in the evolution of vertebrates, allowing the shift to an active predatory lifestyle.<sup>1</sup> The earliest vertebrates were jawless fish (agnathans); the jaw-bearing gnathostomes arose later and have been more successful evolutionarily. An important event in head evolution was the emergence of dentition. To function properly in grasping and crushing food, teeth must be of adequate hardness, proper shape, and anchored to underlying bone (Figure 1). Tooth number, shape, and size vary significantly among species, because

of natural selection in response to the environmental pressures provided by various types of food (Figure 2).

Teeth, or tooth-like structures called odontodes or denticles, are present in all vertebrate groups, although they have been lost in some lineages. Most fish and reptiles, and many amphibians, possess dentitions that contain a large number of teeth (polyodont) of similar shape (homodont) that undergo continuous replacement (polyphyodont<sup>2</sup>). These teeth are comprised of dentin and enamel or an enamel-like structure, are rootless, and are attached directly to bone by ankylosis or fibrous tissue. In contrast, most mammalian teeth are rooted and are connected to the jaws through interactions between the periodontal ligaments and alveolar sockets (Figure 1).

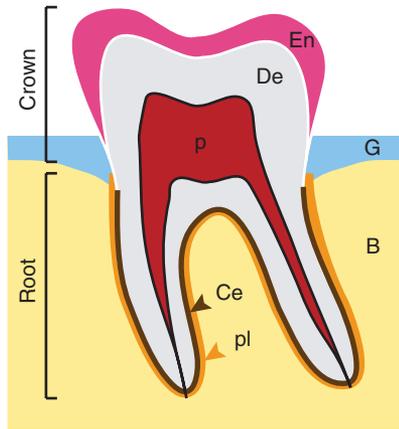
Egg-laying monotremes, the most basal living mammals, possess a rudimentary unpaired egg tooth, similar to reptiles and birds, for use during hatching.<sup>3</sup> Adult monotremes have horny plates as opposed to teeth. Therian mammals, which include all living mammals except monotremes, typically are heterodonts, meaning that the teeth have different shapes. Four types of teeth are present in mammals:

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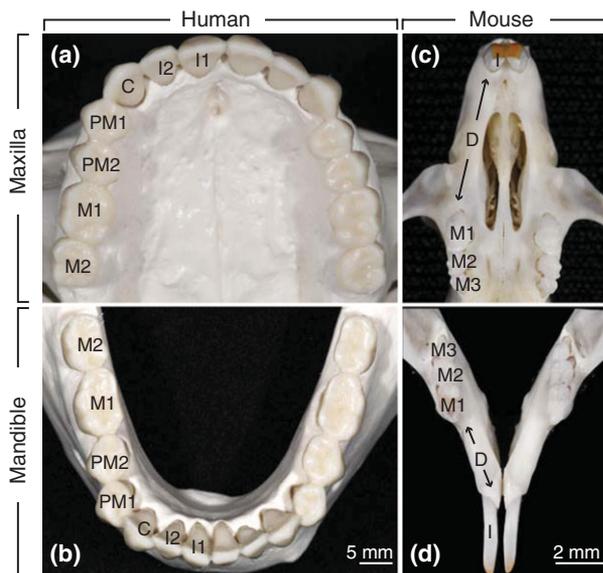
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**FIGURE 1** | Cartoon depiction of a first lower molar from a human adult. The crown (part of the tooth covered by enamel) and the root are shown. The tooth and its supporting structure, the periodontium, contain all four mineralized tissues in the body, bone (B), cementum (Ce), dentin (De), and enamel (En). The tooth is attached to the underlying bone via periodontal ligaments (pl). The pulp chamber (p) houses the blood vessels and nerves (not shown) as well as the putative odontoblast stem cells. The gingiva (G) is the oral mucosa that overlies alveolar bone (B).



**FIGURE 2** | Human and mouse dentitions. The maxillary (a, c) and mandibular (b, d) dental arches show the reduced dentitions in adult human (a, b) and mouse (c, d). Both species are derived from a common mammalian ancestor that is thought to have had six incisors, two canines, eight premolars, and six molars in each dental arch. The third molar or wisdom tooth (M3) is absent in the human specimen. I, incisor; I1, central incisor; I2, lateral incisor; C, canine; PM1, first premolar; PM2, second premolar; M1, first molar; M2, second molar; M3, third molar; D, diastema. Images are courtesy of Dr. Kyle Burke Jones (UCSF).

incisors, canines, premolars, and molars. In contrast to nonmammalian vertebrates, only two generations of teeth (diphyodonty) are present in most mammals.

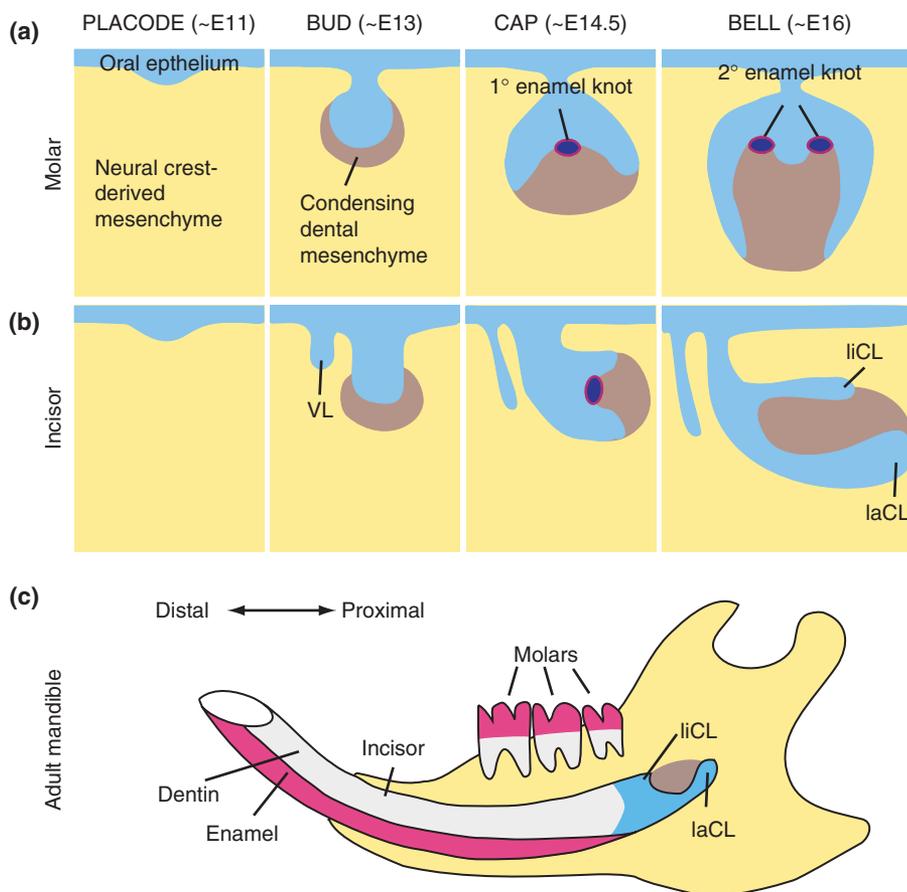
The ancestral dental pattern for eutherian placental mammals in each quadrant is three incisors, one canine, four premolars, and three molars, and the premolars and molars are typically multicuspid.<sup>4</sup> In most extant mammals, tooth number is reduced relative to this ancestral pattern (e.g. Figure 2).

The dentition is highly specialized in mice, which are the most commonly used model to study tooth development. In each quadrant, a single incisor is separated from three molars by a toothless region called the diastema (Figure 2(c) and (d)). Rodent incisors are unusual because they grow continuously throughout the life of the animal, a property attributed to the presence of populations of adult stem cells. Such stem cell-fueled continuous growth of rodent teeth is discussed in more detail below. Also, rodents possess no replacement teeth, unlike humans, who have two sets of teeth.

### Stages of Tooth Development

The tissues required for tooth development originate from two principal sources. The epithelium is derived from oral ectoderm and potentially pharyngeal endoderm,<sup>5,6</sup> whereas the mesenchyme is derived from cranial neural crest cells. Neural crest cells arise from the margins of the neuro-epithelium and migrate laterally and ventrally to fill the facial prominences with mesenchyme.<sup>7</sup> The neural crest-derived mesenchyme (hereafter referred to as mesenchyme) eventually forms the facial and jaw skeletons, as well as most of the soft and hard tissues in teeth, including dentin, dental pulp, alveolar bone, and periodontal ligament;<sup>8</sup> these tooth-specific tissues are discussed in greater detail below.

In mice, initiation of tooth development occurs between embryonic day (E) 8.5–10, when the sites of tooth formation are first apparent based on the expression of several genes.<sup>a</sup> The first morphological sign of odontogenesis is a thickening of the oral epithelium at E11 in the mouse (week 7 of gestation in humans) (Figure 3). During the subsequent bud stage at E12.5–E13.5, cells of the thickened oral epithelium proliferate and form a dental lamina that invaginates into the mesenchyme. Mesenchymal cells condense underneath the forming epithelial bud to generate the dental papilla. During the cap stage at E14, the epithelial bud extends further and begins to surround the dental papilla. Cells of the dental mesenchyme located adjacent to the dental papilla or outside the epithelial organ form the dental follicle or sac. Also, the primary enamel knot, a transient signaling center that regulates tooth shape, is present in the dental epithelium. During the bell stage beginning at E16, the tooth germ increases further in size, and the final shape



**FIGURE 3** | Tooth development. The various stages of mouse molar (a) and incisor (b) development, and the adult mouse mandible (c) are depicted in sagittal views. The oral epithelium thickens at the placode stage and invaginates into the neural crest-derived mesenchyme. Mesenchymal condensation occurs at the bud stage and the enamel knot, a central signaling area, first appears at the cap stage. The extracellular matrices of dentin and enamel are secreted with the differentiation of ameloblasts and odontoblasts during the bell stage. The matrix will eventually mineralize forming the tooth crown and is followed by tooth eruption. Similar developmental events occur in incisors and molars with notable differences being the presence of a vestibular lamina (VL), as well as the labial and lingual cervical loops (laCL and liCL, respectively), during incisor development, and the presence of secondary enamel knots, the future site of cusps, during molar development.

of the tooth crown becomes increasingly apparent. In the forming molars, secondary enamel knots, which are the signaling centers in areas of epithelial folding whose initiation is controlled by the primary enamel knot, determine the sites of tooth cusp formation.<sup>9</sup> Finally, tooth-specific cell types, such as ameloblasts and odontoblasts, begin to differentiate.

The enamel-producing ameloblasts are generated from epithelial cells adjacent to the dental papilla called the inner enamel epithelium (IEE), and these cells secrete enamel matrix that eventually mineralizes. Dentin-producing odontoblasts differentiate from the outermost layer of the dental papilla and gradually migrate to the center of the dental papilla as they secrete dentin matrix. Root formation coincides with tooth eruption after formation of the crown, which is the part of the tooth covered by enamel.<sup>10</sup> Cementum, periodontal ligament, and alveolar bone are all

derived from the dental follicle, which has a mesenchymal origin, and are involved in anchoring the teeth to the jaws.<sup>11,12</sup> Rodent incisors do not have a typical crown or root but rather possess a crown-like labial (near the lip) surface covered by enamel and a root-like lingual (near the tongue) surface where enamel is absent.<sup>13</sup> The first teeth to erupt in mice, the mandibular incisors, become visible at around postnatal day (P) 9, followed shortly by the maxillary incisors. Eruption of molar teeth begins at P15 with the first mandibular molars.

### Epithelial–Mesenchymal Interactions: The Tooth as a Model for Developmental Biologists

Reciprocal interactions between the epithelium and the underlying mesenchyme regulate tooth

morphogenesis, and studies of these interactions have made the tooth an important model for developmental biologists. The ability to form teeth, also called the odontogenic potential, was shown by classical tissue recombination experiments to reside in the epithelium at the placode stage. In these studies, oral epithelium of murine embryos between E9 and E11.5 induced tooth formation in nondental mesenchyme.<sup>14,15</sup> After E11.5, the dental mesenchyme was able to induce nondental epithelia to participate in odontogenesis,<sup>16</sup> whereas the ability by the epithelium to induce tooth formation appeared to be lost at this stage. Thus, these early experiments suggested that, during early tooth development, the odontogenic potential shifts from the epithelium to the mesenchyme. During the cap stage and beyond, the dental mesenchyme regulates tooth shape formation and can induce formation of ameloblasts and enamel matrix secretion in nondental epithelium.<sup>16,17</sup>

### Adult Teeth and Tooth-Specific Mineralized Tissues

In terms of hard tissues, the tooth is comprised of enamel, dentin and cementum, and it is anchored to the alveolar bone by periodontal ligaments; thus, the tooth and supporting structure, called the periodontium, contain all of the four types of mineralized tissues found in the vertebrate body (Figure 1). The two main parts of an adult tooth are the crown and the root. The crown is covered by enamel, which is a highly mineralized, acellular substance secreted by cells derived from dental epithelium. Enamel is the hardest structure found in the body, and it consists primarily of hydroxyapatite, a crystalline calcium phosphate, which is also the major component of dentin, cementum, and bone. The organization of mineral in enamel is unique, as this material is formed of rods of hydroxyapatite crystals running from the dentin–enamel junction to the surface of the tooth. Underlying dentin supports both the enamel layer of the tooth crown and the cementum layer of the tooth root. Dentin is less mineralized and less brittle than enamel and is necessary for the support of enamel in the crown and cementum in the roots. The individual collagen fibrils of the periodontal ligaments originate from the cementum and cementum–dentin junction and attach directly to the alveolar bone of the jaws<sup>18</sup> (Figure 1). The dental pulp is a mass of vascularized connective tissue enclosed by dentin in the central part of the tooth. The apical foramen, an opening in the area of the root apex, allows the supply of the dental pulp with blood vessels and nerves.

## GENETICS AND SIGNALING

A number of signaling pathways work in concert to orchestrate tooth development, and this section summarizes some of the major pathways. Signaling cascades involved in development, response to intercellular signals and environment, cell cycle control, and pathogenesis require transcription factors that interact with DNA to regulate gene expression; some of the transcription factors involved in tooth development are summarized. Newly discovered functions for microRNAs are also briefly discussed. Expression data of the molecular factors discussed here at various stages of tooth development can be found at [www.bite-it.helsinki.fi](http://www.bite-it.helsinki.fi).

### FGFs

*Fgf8* and *Fgf9* are among the earliest genes to be expressed in the oral epithelium. The conditional inactivation of *Fgf8* in ectoderm caused defects in structures derived from the first pharyngeal arch including teeth, jaws, lateral skull wall, and middle ear, as well as part of the tongue and other soft tissues.<sup>19</sup> Although molars and the proximal mandible were absent, the distal-most structures such as lower incisors were present. These results suggested that a large proximal derivative of the first pharyngeal arch primordium is specified by FGF8, but a small distal region depends on other signaling molecules for its outgrowth and patterning.<sup>19</sup> In mice over-expressing a dominant negative form of *Fgfr2b*, tooth development did not progress beyond the bud stage.<sup>20</sup> *Fgf4* and *Fgf9*, which are expressed in the enamel knot, are thought to stimulate proliferation in adjacent epithelial and mesenchymal tissues.<sup>9,21</sup> Deletion of *Fgf3* and *Fgf10* in mice resulted in smaller teeth with aberrant cusp morphology,<sup>22</sup> but *Fgf3* and *Fgf10* did not appear to be required individually for ameloblast differentiation.<sup>22,23</sup> The inactivation of sprouty genes, which are inhibitors of FGF signaling, resulted in the formation of supernumerary teeth<sup>24</sup> and the generation of ectopic enamel on the lingual surface of the incisor.<sup>25</sup> FGF signaling is also important in zebrafish tooth morphogenesis, and decreases in FGF signaling have been proposed to lead to the loss of oral teeth.<sup>26,27</sup>

### BMPs

BMPs function at multiple stages during odontogenesis. BMP4, in particular, is an important mediator of signaling between epithelial and mesenchymal tissues.<sup>28</sup> During initiation of tooth formation, BMP signaling in the oral epithelium antagonizes FGF

signaling, which is thought to determine the sites of tooth formation.<sup>29–31</sup> Mesenchymal BMP4 regulates *Shh* expression<sup>30</sup> and is critical for the transition from tooth bud to cap stage and for induction of the enamel knot in the epithelium.<sup>32,33</sup> The inactivation of activin or *Bmpr1a* in either epithelium or mesenchyme resulted in the arrest of tooth development after the bud stage.<sup>34–36</sup> During the cap stage, BMP4 induced the cyclin dependent kinase inhibitor, *p21*, and the expression of *Bmp4* and *p21* was associated with differentiation as well as apoptosis of the primary enamel knot.<sup>33</sup> Therefore, BMP signaling regulates patterning of the cusps and ultimately, the shape of the tooth crown. *Fst1*, which acts as an antagonist of BMP and INHBA (formerly known as activin) signaling, and *Sostdc1*, an inhibitor of both BMP and WNT signaling, are important regulators of enamel knot formation.<sup>37,38</sup> BMP signaling is also known to function during root formation<sup>39</sup> and during differentiation of odontoblasts and ameloblasts.<sup>38,40,41</sup>

## SHH

During initiation of tooth formation, *Shh* is expressed in specific regions of the epithelium of the molar and incisal placodes.<sup>42</sup> At this early stage, the mitogenic activity of SHH is thought to stimulate proliferation in the placode epithelium, which enables invagination into the underlying mesenchyme and formation of the epithelial bud.<sup>43,44</sup> Analysis of expression of the receptor *Ptch1* and the transcription factor *Gli1*, which are both downstream targets of SHH signaling, showed that SHH signals to the mesenchyme as well as the epithelium.<sup>44,45</sup> Expression of *Shh* is retained at the tip of the epithelial bud, becomes down-regulated toward the end of the bud stage, is re-induced in the enamel knot, and remains expressed in the epithelium throughout ameloblast differentiation. The regulation of these interesting expression patterns is not well understood and remains the subject of much interest.

SHH from the enamel knot regulates crown formation by stimulating proliferation in epithelial and mesenchymal tissues adjacent to the signaling center. Conditional inactivation of *Shh* or the signal transducer smoothed from the epithelium, or inhibition of signaling using an antibody against SHH, demonstrated that SHH signaling regulates tooth separation, size and morphology as well as cytological organization of matrix secreting cells.<sup>46–48</sup> Primary cilia exert a negative regulatory effect on SHH activity and function to repress tooth formation.<sup>49</sup> In zebrafish, SHH signaling is required continuously throughout tooth development from initiation to morphogenesis.<sup>50</sup> Despite gene duplication and differences in the location of where teeth form between mice and zebrafish,

the role of SHH signaling in tooth development appears to be conserved between these two species.<sup>50</sup>

## WNTs

The modulation of WNT signaling leads to variations in tooth number. Supernumerary teeth arise with the up-regulation of WNT signaling. Multiple ectopic teeth in the molar region were observed after constitutive activation of the transcriptional effector CTNNB1 (beta-catenin),<sup>51,52</sup> and mis-expression in epithelial tissues of *Lef1*, the binding partner of CTNNB1, resulted in multiple tooth-like structures in the oral epithelium.<sup>53</sup> Overexpression of the zinc finger protein-encoding gene, *Sp6*, led to an increase in WNT signaling and mice with up to 50 teeth.<sup>54</sup> Inactivation of WNT antagonists such as *Apc*<sup>55,56</sup> and *Sostdc1*<sup>37,57,58</sup> also led to increases in the number of teeth. Several of these studies demonstrated that the dental epithelium undergoes multiple invaginations leading to the formation of extra enamel knots and ultimately, supernumerary teeth.<sup>51,54,56,59</sup> Conversely, there is evidence in humans that decreases in WNT signaling lead to tooth loss.<sup>60,61</sup>

The mechanism by which WNT signaling regulates tooth number is still unclear. Surprisingly, *Msx1*, which is required for normal tooth development, was dispensable for WNT-mediated supernumerary tooth formation, whereas *Fgf8* was identified as a direct target of WNT signaling.<sup>56</sup> WNT signaling regulates *Shh*<sup>48,62</sup> and *Bmp4* expression,<sup>63</sup> and it affects multiple stages of tooth development such as bud to cap transition, formation of the enamel knot, molar tooth size, and dentinogenesis.<sup>41,52,54,62,64</sup> Conditional inactivation in the dental mesenchyme of *Smad4*, which encodes a BMP/TGF $\beta$  signal transducer, led to an up-regulation of *Ctnnb1* and down-regulation of WNT antagonist genes such as *Dkk1* and *Sfrp1*.<sup>41</sup> In these mice, enamel appeared normal whereas dentin formation was compromised, an observation that challenges the traditional notion that ameloblast differentiation is dependent upon odontoblast differentiation.<sup>65</sup>

## Notch

Components of the Notch signaling pathway, which include four transmembrane Notch receptors (*Notch1–4*) and 5 transmembrane ligands (*Jag1*, *Jag2*, *Dll1*, *Dll3*, and *Dll4*), are expressed during tooth development and affect several aspects of tooth formation. Notch signaling was demonstrated to regulate tooth morphogenesis and ameloblast differentiation.<sup>66</sup> Specifically, inactivation of the Notch-interacting domain of JAG2 in mice caused abnormal molar shapes, additional cusps, and inhibition of ameloblast differentiation and enamel matrix deposition.<sup>66</sup>

The stratum intermedium (SI), a layer of cells that is subjacent to the ameloblast layer during enamel formation and whose function is still unclear, expresses *Notch1* and its downstream target, *Hes1*, whereas the IEE and ameloblasts express *Jag1*.<sup>67</sup> In HAT-7 cells, a dental epithelial cell line, treatment with exogenous JAG1 led to the differentiation of SI cells, and this effect was neutralized with an anti-JAG1 antibody, pointing to the importance of Notch signaling in the SI.<sup>67</sup>

## EDA

Ectodysplasin-A (*Eda*) is a member of the tumor necrosis factor (TNF) superfamily of signaling molecules. The EDA-A1 isoform, its receptor EDAR, and the adapter protein EDARADD act in a linear fashion and activate canonical NF $\kappa$ B signaling as well as other pathways.<sup>68</sup> The EDA-A2 isoform signals through another receptor, EDA2R (formerly known as XEDAR), rather than EDAR, and activates similar pathways but appears to play a less important role in development.<sup>69</sup> Mice with mutations in *Eda*, *Edar* or *Edaradd* (initially found as the spontaneous mutants *tabby*, *downless*, and *crinkled*, respectively) all have a decrease in the number of teeth with abnormal cusp morphology.<sup>68</sup>

During early odontogenesis, EDA signaling is crucial for determining the size of the tooth field and the number of teeth generated. Specifically, mutations in *Eda* or *Edar* resulted in formation of smaller teeth and frequently the absence of third molars.<sup>70</sup> In contrast, increased levels of *Eda* expression, or expression of a constitutively active form of *Edar*, led to the formation of a supernumerary tooth in the diastema region.<sup>71,72</sup> In zebrafish, mutations in *eda* and *edar* led to defects in ectodermal structures such as scales and glands and partial or complete loss of pharyngeal teeth.<sup>73</sup>

EDA signaling also affects tooth shape. Mutations in any of the three pathway components in mice result in molars with reduced cusp number and rounded cusps. *Eda* is expressed in oral and dental epithelium throughout tooth formation, whereas *Edar* and *Edaradd* are expressed in the enamel knot. The enamel knots in *tabby* or *crinkled* mutants were smaller,<sup>74,75</sup> whereas loss of *Edar* in *downless* mice led to the formation of an elongated-rope like enamel knot.<sup>76</sup> Interestingly, overexpression of *Edar* but not of *Eda* resulted in formation of extra cusps.<sup>71,72</sup> It is clear that the loss of function of *Eda* versus *Edar* has distinct effects on tooth size and morphology. This may be due to activation of the EDA2R pathway, which is influenced by *Eda* but not *Edar* expression,

to interaction between EDAR and a yet unidentified protein, and/or to a ligand-independent activity for EDAR.<sup>77</sup>

## Transcription Factors

The initial patterning as well as the coordinated interplay of signals at each step of tooth development is greatly dependent on the actions of transcription factors. Here, some of the general concepts and recent advances in our understanding of the roles of transcription factors in tooth development are discussed.

At E8.5, prior to any morphological signs of tooth development, *Pitx2* is expressed in the stomatodeal epithelium, the precursor to oral and dental epithelium, and it is considered to be the earliest transcription factor expressed during tooth development.<sup>30,78</sup> *Pax9* expression has been shown to specify the mesenchymal regions at the prospective sites of all teeth at E10.<sup>29</sup> The direct regulation by PITX2 of *Dlx2*, a gene that is expressed at E9.5, is attenuated by a physical interaction between DLX2 and PITX2.<sup>79</sup> At later stages, DLX2 and FOXJ1, a transcription factor expressed in the oral epithelium that plays a fundamental role in embryonic development, activate transcription of amelogenin, a tooth-specific protein required in enamel formation and mineralization.<sup>80</sup>

During initiation of tooth development, epithelial FGF8 and BMP4 induce the expression of numerous transcription factor genes including *Barx1*, *Dlx1*, *Dlx2*, *Msx1*, *Msx2*, *Pax9*, *Pitx1*, and *Pitx2*.<sup>29–31</sup> The expression in prospective mesenchyme of many non-HOX homeobox-containing genes, such as *Barx1*, *Dlx1*, *Dlx2*, *Dlx3*, *Dlx4*, *Dlx5*, *Dlx6*, *Lhx6*, *Lhx7*, *Msx1*, and *Msx2*,<sup>81–83</sup> led to the proposal of the odontogenic homeobox code model, which postulates that expression of specific combinations of homeobox gene directs the formation of specific tooth types.<sup>84</sup>

*Msx1* and *Pax9* act in the dental mesenchyme to maintain expression of *Bmp4*, which is crucial for establishing the enamel knot.<sup>32,33</sup> Absence of either of these transcription factors led to an arrest in tooth development at the bud stage, similar to that reported in *Lef1*-null embryos.<sup>64</sup> Recently, it was shown that during early tooth formation, mesenchymal condensation (i.e., compression of mesenchyme) alone could regulate expression of *Msx1* and *Pax9*, as well as *Bmp4*.<sup>85</sup>

## MicroRNAs

There is an emerging role for microRNAs (miRNAs) in the development and evolution of teeth. Small RNAs,

and miRNAs in particular, have important effects on development and disease through modulation of specific signaling pathways.<sup>86</sup> miRNAs are endogenously expressed, short (~21 nucleotides), noncoding RNA molecules that affect protein synthesis by posttranscriptional mechanisms.<sup>87</sup> The involvement of miRNAs in various ectodermal derivatives has been demonstrated in skin,<sup>88,89</sup> hair,<sup>90</sup> and teeth.<sup>91–94</sup> *Pitx2-Cre;Dicer* deleted mice showed a multiplication of enamel-free incisors, demonstrating the importance of miRNAs in ameloblast differentiation as well as their role in the regulation of ameloblast stem cells<sup>91</sup>; *Dicer* is an RNase III enzyme required for conversion of pre-miRNAs to mature miRNAs.<sup>95,96</sup> *Krt14-Cre; Dicer* deleted mice showed milder changes in tooth shape, epithelial homeostasis, and enamel formation.<sup>93</sup> The differences in phenotype between the two mutant mice are likely due to the early expression of *Pitx2* in stomatodeal epithelium compared to *Krt14* expression. The expression of miRNAs in distinct regions of the mouse incisor and pulp was profiled using microarray experiments, laying the groundwork for future investigations.<sup>93,94</sup> These initial studies indicate that there is much work ahead in understanding the roles of miRNAs during tooth development.

## EVOLUTION OF TOOTH DEVELOPMENT

A fundamental question in evolutionary developmental biology is how genetic changes contribute to morphologic variations that are subjected to natural selection. Teeth or tooth-like structures such as odontodes and denticles are invaluable in the study of evolutionary developmental biology for several reasons. First, teeth are ancient structures that are found in multiple locations in the vertebrate body such as the posterior pharynx of extinct jawless fish and extant fish, the dermal surface of sharks and rays, the oral cavity of rodents and humans, and lining the oro-pharyngeal cavity of fish in association with gill arches.<sup>97,98</sup> Second, there is great variation in the shape, size, number, and rows of teeth, and these variations are relatively easy to characterize. Third, teeth are readily fossilized vertebrate structures with excellent preservation of morphology because of the hardness of enamel, and thus they provide a large number of specimens for comparative genomic, anatomic, and phylogenetic studies. This section provides an overview of the main ideas and current research in the evolution of tooth development and highlights some of the multidisciplinary approaches that can be utilized to answer important questions in the evolution and development of teeth.

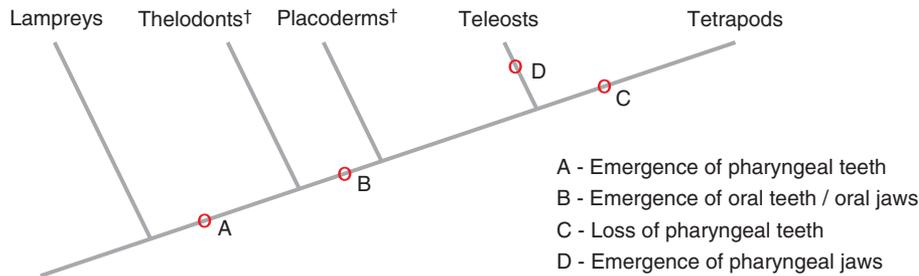
## The Origin of Teeth in Vertebrates

Teeth are an ancient and key vertebrate innovation, and their origin is a hotly debated question. The first occurrence of tooth-like structures is believed to be in the posterior pharynx of jawless fishes more than 500 million years ago.<sup>98,99</sup> With the evolution of jawed vertebrates, teeth developed on oral jaws and helped to establish the dominance of gnathostomes on land and in water.

It is still unclear whether oral teeth evolved with jaws for predation and mastication or first appeared as external dental armor as protection from predation. At least two opposing theories have been put forth regarding the evolution of oral teeth. The ‘outside-in’ theory posits that teeth evolved from ectoderm-derived, skin denticles that folded and integrated into the mouth.<sup>100</sup> The ‘inside-out’ theory suggests that teeth originated from endoderm, with the formation of pharyngeal teeth in jawless vertebrates and moved anteriorly to the oral cavity with the evolution of jaws.<sup>101</sup> However, recent studies suggest that neither theory may be entirely correct.<sup>102</sup>

Fate-mapping approaches using transgenic axolotls showed that teeth formed normally regardless of whether the oral epithelium was derived from ectoderm or endoderm.<sup>6</sup> Experiments utilizing chicken embryos, which have lost the ability to form teeth,<sup>103</sup> have demonstrated the dominant role of mesenchyme in the initiation of tooth development. Specifically, transplantation of mouse neural crest cells into developing chicken embryos showed the formation of tooth germ-like structures.<sup>104</sup>

Some extant fish, such as certain cichlids, possess both oral and pharyngeal teeth (Figure 4). Pharyngeal teeth develop on discrete pharyngeal jaws in hox-positive, endoderm-derived sites, whereas oral teeth develop in hox-negative, ectoderm-derived regions.<sup>99</sup> Pharyngeal teeth of jawless vertebrates appear to utilize an ancient gene network that predates the origin of oral jaws, oral teeth, and ectodermal appendages.<sup>99</sup> During mouse development, expression of various genes such as *claudin6*, *Foxa2*, alpha-fetoprotein, *Esrp1* (formerly known as *Rbm35a*), and *Sox2* is observed in the presumptive molar region but not in the incisor region.<sup>5</sup> In *Chuk-* (formerly known as *Ikka*) null mice, there was abnormal epithelial evagination in incisors but not in molars.<sup>105</sup> These and other studies suggest differences in the epithelium from which incisor and molar teeth develop. However, despite distinct developmental environments, which suggest different molecular mechanisms that result in heterodont dentition, both oral and pharyngeal teeth also show striking similarities in their gene regulatory networks.<sup>99</sup>



**FIGURE 4** | Simplified evolutionary progression of dentitions and jaws. Point A indicates the origin of pharyngeal teeth in extinct (†) jawless fish. Oral teeth and jaws are thought to have arisen at point B. The pharyngeal teeth were lost in common ancestors to tetrapods at point C. In some extant teleosts such as cichlids, both oral and pharyngeal teeth are present and pharyngeal jaws are thought to have arisen at point D.

Taken together, the studies using axolotl, cichlids, chicken, and mice demonstrate that teeth can form despite different epithelial origins and demonstrate the important role of mesenchyme in the initiation of tooth development, challenging the primacy of oral ectoderm in this role.<sup>14</sup> These studies also demonstrate the conservation of gene regulatory networks across lineages with origins in different germ layers and the role of deep homology<sup>106</sup> in the evolution and development of teeth. Thus, teeth appear to have evolved both ‘inside and out’, wherever and whenever the odontogenic-specific gene network of the mesenchyme was present.<sup>97</sup>

### Evolution of Tooth Shape, Size, Number, and Rows

Both humans and rodents evolved from a common mammalian ancestor that is thought to have had a full complement of teeth comprising three incisors, one canine, four premolars, and three molars in each dental quadrant that replaced its teeth a single time.<sup>4</sup> During mammalian evolution, teeth were lost along the lineages that gave rise to both rodents and humans (Figure 2). Humans have all four of the major classes of teeth but have lost members of several of these classes; for example, we only have two incisors and two premolars (Figure 2(a) and (b)). Rodent ancestors underwent a further reduction in dental formula, such that mice have only one incisor and three molars per quadrant, and no replacement teeth (Figure 2(c) and (d)). Rodent teeth are considered to be deciduous teeth that do not undergo replacement,<sup>107–109</sup> but the potential for replacement teeth in mice appears to have been retained.<sup>109–111</sup>

In addition to modifications in the number of teeth, the morphology of mammalian teeth is enormously diverse. These modifications involve variations in cusp shape and crest organization, and in the case of a number of species, the evolution of stem cell-fueled continuous growth, as discussed

below. Comparative studies of tooth morphology have been greatly advanced by improvements in three-dimensional (3D) imaging techniques such as high-resolution micro-computed tomography. Some recent studies regarding the regulation of tooth shape, size, number, and rows are discussed below.

The various tooth shapes observed in heterodont animals are believed to have evolved from ancestral conical teeth, perhaps similar to canines, through the addition of cones and grooves.<sup>112</sup> Relatively little is known regarding the molecular mechanisms underlying such changes, and therefore they are the subject of much current interest. Decreasing BMP signaling in the incisor region can lead an incisor to acquire a molar-like phenotype.<sup>113</sup> However, it has recently been proposed that the molar-like phenotype was a result of the splitting of the incisor placode rather than a change in tooth identity.<sup>114</sup> *Lrp4*-null mice displayed enamel grooves on the labial surface of incisors that exhibited similar molecular characteristics as molar cusps, suggesting that WNT signaling may be involved in cusp development.<sup>112</sup>

Two recent studies have provided important information about the developmental regulation of the relative size and number of molars. By using mouse molar cultures, it was proposed that a combination of activators and inhibitors governs the relative relationship between size and number of teeth.<sup>115</sup> Detailed studies of tooth shape indicated that the complexity of the cusps directly reflects the animal’s diet across many mammalian species.<sup>115,116</sup> These studies pointed to higher order, generalizable principles that govern tooth shape and size.

Several studies have shown that alterations in signaling pathways can lead to variation in tooth number, and such studies point to mechanisms that may have determined tooth number during mammalian evolution. An example of dramatic tooth loss was highlighted in the cypriniform fish, a group including zebrafish. Zebrafish possess pharyngeal teeth, and fossil evidence suggests that zebrafish lost

their oral teeth 50 million years ago<sup>117</sup> (Figure 4). This was associated with the loss of *dlx2a* and *dlx2b* expression in the oral epithelium. Because DLX genes are required for tooth development in mice,<sup>118</sup> changes in trans-acting regulators of DLX genes that may be downstream of FGF signaling have been proposed as candidates responsible for the loss of cypriniform oral teeth.<sup>27</sup> Interestingly, a region in the upstream regulatory element of *dlx2b* was retained that drives specific expression in the oral epithelium, and the retention of this cis-regulatory element is attributed to its requirement in other tissues, as the DLX genes have pleiotropic effects.<sup>119</sup> These studies suggest that teeth lost from specific regions may be relatively easy to reacquire during evolution<sup>119</sup> and they are exciting because they challenge Dollo's Law of the Irreversibility of Evolution, which states that an organism can never exactly return to a previous evolutionary state<sup>120</sup> because a lost structure cannot reappear in evolution.

A number of mouse mutants with changes in tooth number or pattern have provided tantalizing hints about the evolution of dentition. Supernumerary teeth present in the diastema region of mice (Figure 2(c) and (d)), a species with reduced dentition, may represent the revival of teeth present in ancestral species. The following experiments in mice led to supernumerary teeth in the diastema region of the mandible: epithelial overexpression of *Eda* under the control of the *Krt14* promoter;<sup>71</sup> inactivation of the receptor tyrosine kinase antagonists, *Spry2* and *Spry4*;<sup>24</sup> production of a hypomorphic allele of the gene encoding Polaris, a protein involved in SHH signaling;<sup>121</sup> a null mutation of the SHH antagonist, *Gas1*;<sup>49</sup> and inactivation of the BMP/WNT pathway inhibitor gene, *Sostdc1*.<sup>37,57,58,122</sup> Diastema teeth in *Krt14*-*Eda* mutants and in *Spry2*-, *Spry4*-, *polaris*-, and *Gas1*-null mice have a size and shape characteristic of premolars, a tooth type that was lost in mice around 50–100 million years ago. Interestingly, diastema teeth in *Sostdc1*-null mice showed a molar-like phenotype, as well as enlarged enamel knots and altered cusp patterns.<sup>37,57,58,122</sup> In the diastema region, it was previously observed that the tooth primordium was present but failed to further develop because it does not maintain *Shh* expression.<sup>123–126</sup> These studies demonstrate how loss of teeth from specific regions may be relatively easy to reacquire during evolution.

Mice carrying mutations in *Sostdc1*,<sup>58,122</sup> *Lrp4*,<sup>127</sup> or inheriting the *Di* (duplicate incisor) trait<sup>128</sup> have supernumerary upper or lower incisors that are located lingual to the normal incisor. Decreases in sprouty gene dosages also led to

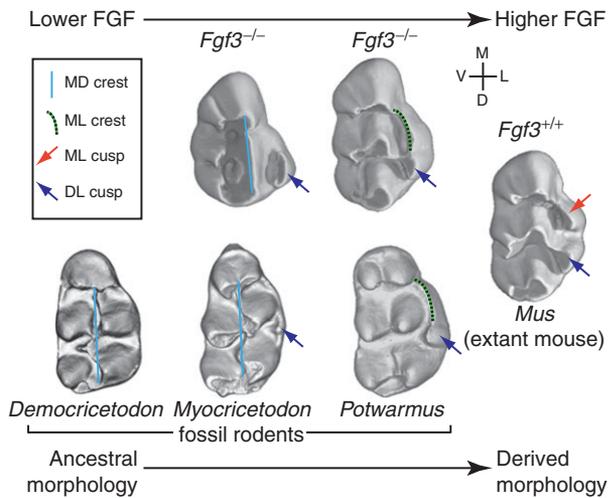
increasing numbers of incisors.<sup>129</sup> The detailed study of *Sostdc1* mutants indicated that the supernumerary incisors corresponded to replacement teeth.<sup>127</sup> Splitting of the incisor placode has been observed in *Sostdc1/Fst1* double-null mice, which have bifid incisors.<sup>114</sup> The potential mechanisms by which supernumerary incisors arise in mice include: failure of integration of the ancestral dental primordia;<sup>109</sup> development of replacement teeth;<sup>127</sup> splitting of a large placode into smaller elements;<sup>114</sup> or development of supernumerary tooth germs.<sup>130</sup> These examples of supernumerary teeth may also reflect ancestral rodent dentition, in which a larger number of incisors was found, and highlight potential mechanisms by which humans and mice have evolved their reduced dentition.

Mammals possess a single row of teeth in the upper and lower jaws, unlike the multiple rows observed in some nonmammalian species such as fish and snakes. Teeth are replaced only once in most mammals, whereas in many nonmammalian species, teeth are continuously replaced. Additionally, in rodents, there are no replacement teeth, but there is continuous, stem cell-fueled growth of incisors, as well as of molars in species such as voles.<sup>131</sup> Interestingly, supernumerary teeth developed lingual to the first molars in mice with inactivation of *Osr2* (odd-skipped related-2), a gene homologous to the *Drosophila* transcriptional repressor *odd-skipped*.<sup>110</sup> *Osr2* limits the odontogenic field by suppressing the BMP4-MSX1 signaling cascade.<sup>110</sup> The development of supernumerary teeth in *Osr2* mutants may represent a second row of teeth similar to the multiple rows observed in some fish, and it may represent a reawakening of replacement teeth in mice.

## Comparative Tooth Morphology and Mammalian Evolution

Because of the highly mineralized nature of enamel, there is excellent preservation of detailed dental features in teeth from extant and extinct species. Using this vast repository of specimens, detailed 3D images can be constructed to compare subtle differences in tooth morphology. This information can be applied in interesting ways to further our understanding on the evolution of tooth development.

Comparative morphologic studies of mutant mice and various extinct and extant species have shed light on the role of specific genes in the evolution and development of tooth morphology. One such study showed that varying dosages of the *Fgf3* gene caused morphological changes in teeth of mutant mice and in human patients<sup>132</sup> (Figure 5). Using comparisons between mice and humans carrying *Fgf3*



**FIGURE 5** | Dental morphology of *Fgf3* mutant mice and fossil rodents. As *Fgf3* dosage is decreased in mice, the mesio-lingual (ML) cusp of the upper first molar is transformed into the ML crest (*Fgf3*<sup>+/-</sup>) and is eventually lost (*Fgf3*<sup>-/-</sup>), whereas the mesio-distal (MD) crest appears (*Fgf3*<sup>-/-</sup>). Comparisons of mutant and wild-type mice with fossil rodents such as *Democricetodon*, *Myocricetodon*, and *Potwarmus* show several features: during the transition from ancestral to derived morphologies, there is a loss of the MD crest, an emergence of the DL cusp, and an emergence of the ML crest that is transformed into the ML cusp in mice. The arrow indicating the relative levels of FGF signaling applies only to the allelic series of *Fgf3* mutant mice, as the expression levels of *Fgf3* in muroid ancestors is unknown. The following abbreviations are used for orientation: M, mesial; D, distal; V, vestibular; L, lingual. (Reprinted with permission from Ref 132. Copyright 2009 PNAS)

mutations with primitive rodent and primate fossils, it was observed that decreases in *Fgf3* dosage led to tooth phenotypes that resembled the progressive reappearance of ancestral morphologies<sup>132</sup> (Figure 5). Progressive decreases in sprouty dosage caused increasing numbers of incisors, mimicking the dentition of rodent ancestors.<sup>129</sup> Multidisciplinary approaches that integrate development and evolution can thus help to correlate subtle dental modifications with genetic mutations in a variety of mammalian lineages.

A large amount of information can be extracted from the analysis of fossilized teeth. For example, a record of growth can be attained from the enamel and dentin that allows the reconstruction of the developmental history and the timing of crown and root formation. Measurements of daily enamel cross-striations can be used to infer information about the timing and rate of enamel/crown formation; accentuated neonatal lines in the enamel of deciduous and permanent molars may denote the time of birth; incremental markings in the dentin indicate the timing of root completion; and the quality of the enamel-dentin junction, where the crown meets the root, provides a

window to tooth development and the actions of the enamel knot.<sup>133</sup> Using such techniques, tooth development in Neanderthals was shown to closely resemble that of human populations, underscoring the similarities between humans and Neanderthals.<sup>133</sup>

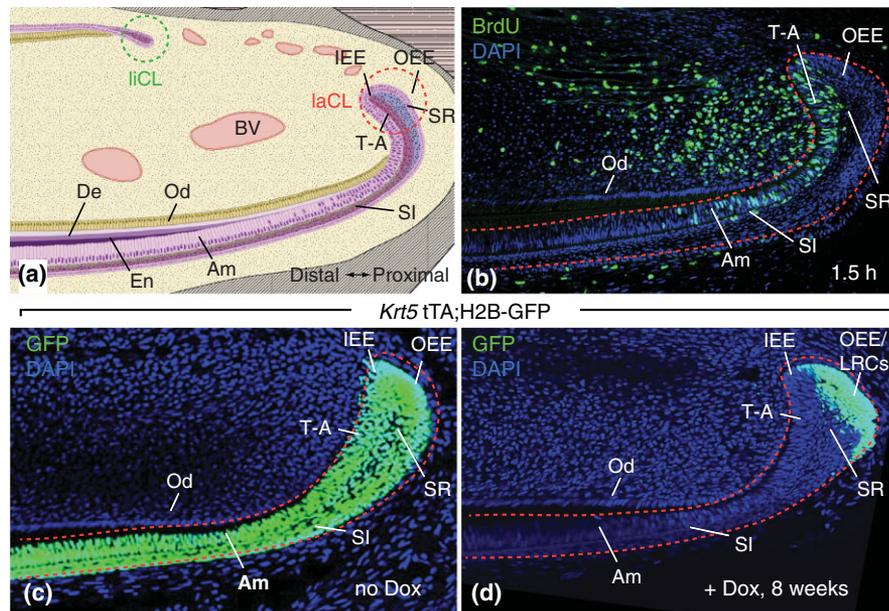
In another study utilizing fossilized teeth to understand the evolution of species, the worn cusp apices of teeth (mesowear) from North American horses for the past 55.5 million years was analyzed. Hypsodonty (high-crowned teeth) was correlated with mesowear, thereby strengthening the argument that the evolution of hypsodonty relative to brachydonty (short-crowned teeth) was adapted for abrasive diets associated with the spread of grasslands in North America.<sup>134</sup> In brachydont species such as humans, the tooth crown is entirely above the level of the jaw bone upon initial eruption, whereas in hypsodont species, some of the tooth crown is retained below the level of the jaw bone.<sup>135</sup> In rodents, hypsodonty is posited to be an intermediate stage on the evolutionary path toward hypselodonty (ever-growing teeth).<sup>135</sup>

Thus, by utilizing model and nonmodel organisms to analyze genetic and signaling pathways, along with detailed 3D reconstructions of teeth in extant and extinct species and in combination with ecological data, some of the exciting multidisciplinary studies discussed above are at the forefront of research in the evolution of tooth development.

## Dental Stem Cells

The regenerative ability of many adult tissues is dependent on tissue-specific stem cell populations that maintain stable numbers by self-renewal and that possess the capacity to differentiate into distinct cell lineages. Regeneration and renewal in adult mammals has been studied in several organs, including the blood, gut, brain, skin, and hair. Here, we describe the advent of the continuously growing mouse incisor as an adult stem cell model system. Although the study of incisor stem cells is a relatively new field, advances have recently been made in the identification of these cells, the understanding of their function, and the characterization of molecular mechanisms that regulate their behavior.

In mice, both molars and incisors go through similar developmental stages at early stages of odontogenesis, but incisors continue to grow throughout postnatal life, whereas molars cease growth in the perinatal period. The ability of the incisor to grow continuously is dependent on the presence of epithelial and mesenchymal stem cells that have the capacity to self-renew and differentiate into all of the cell types of the adult tooth, including ameloblasts, odontoblasts, and



**FIGURE 6** | The adult mouse incisor. The lower incisor is shown in sagittal view (a–d). (a) The diagram indicates the two stem cell compartments in the lingual (liCL) and labial (laCL) cervical loops. Also shown are the inner enamel epithelium (IEE), from which the transit-amplifying (T-A) cells and ameloblasts (Am) arise, the outer enamel epithelium (OEE) that houses the enamel stem cells in the laCL, stratum intermedium (SI), odontoblasts (Od), dentin (De), enamel (En), and blood vessels (BV). (b) Adult mice were injected with BrdU for 1.5 h. BrdU-positive cells indicate rapidly proliferating cells in the T-A region. (c and d) Images from incisors of *Krt5-tTA; H2B-GFP* mice. In the absence of doxycycline (no Dox; C), GFP is present in all the cells expressing *Krt5*, which includes the OEE, IEE, SR, SI, and Am. In the presence of doxycycline (+Dox; D) for 8 weeks, H2B-GFP expression was turned off, leading to the retention of GFP in the slowly proliferating label-retaining cells (LRCs) of the OEE. The LRCs are putative dental epithelial stem cells.

the SI. Importantly, in the wild-type rodent incisor, the labial CL, but not the lingual CL, contains stem cells that give rise to ameloblasts and the SI (Figure 6). Labeling experiments demonstrated that cells in the dental epithelium move in a proximal to distal direction.<sup>136</sup> In the labial CL, the stem cell progeny contribute to a population of transit amplifying (T-A) cells (Figure 6(b)). T-A cells undergo several rounds of cell division before they move distally and differentiate. Early labeling experiments examining the rate of ameloblast and odontoblast migration in mice and rats gave the first clues that turnover of these specialized cells was rapid, underlining the need for progenitor pools to resupply differentiated cell populations.<sup>136,137</sup> Mouse incisor epithelia appear to function as a ‘conveyor belt’, moving cells from a proximal, undifferentiated source to regularly repopulate the tooth with specialized cell types. Initial studies using explant cultures of the CL region from 2-day-old mice showed that new epithelial structures could be generated in culture, indicating that the labial CL housed the dental stem cells that give rise to ameloblasts and the SI.<sup>138</sup>

Identification of organ-specific adult stem cell populations can be challenging, because stem cells often reside in heterogeneous niches intermingled with support cells. A useful character of stem cells

that has aided in their identification *in vivo* is the relatively slow cell-division kinetics of many stem cells relative to surrounding tissue.<sup>139</sup> Slow-cycling cell populations have largely been identified through label retention experiments, traditionally utilizing BrdU incorporation, because cells that divide slowly do not dilute the BrdU label as quickly as their rapidly dividing neighbors. Using this technique, BrdU label-retaining cells (LRCs) were identified in the labial CL of cultured perinatal incisors and in adult incisors *in situ*.<sup>138,141</sup> Another approach to label retention is the use of transgenic mice harboring a tetracycline-sensitive, histone H2B-GFP cassette under the control of a tissue specific trans-activator<sup>140</sup> (Figure 6(c) and (d)). Expression of H2B-GFP is initially activated in all cells of the tissue of interest followed by a ‘chase’ period when the transgene is repressed by exposure of the animal to doxycycline, such that rapidly dividing cells dilute the label. This technique was used to identify LRCs in the outer enamel epithelium (OEE) of the adult labial CL.<sup>141</sup> The LRCs of the dental epithelium expressed *Gli1*, a target of SHH signaling, and lineage tracing experiments demonstrated that the *Gli1*-expressing cells were indeed stem cells.<sup>141</sup> More recently, identification of LRCs in nonmammalian vertebrates has been pursued.<sup>142,143</sup>

Understanding the regulation of adult stem cell populations is key to future utilization of such cells for clinical therapies. How stem cells are maintained at the appropriate number, what signals regulate their differentiation, and how they are established within the context of the developing organism are important questions in stem cell research. Many signaling molecules and pathways are implicated in development and homeostasis of the incisor, including WNTs, BMPs and FGFs. One theme that has emerged from several recent studies is the convergence of distinct FGF signaling pathways that maintain the size and shape of the CL through regulation of cell division and death. Expression analyses first indicated that components of the FGF pathway may play major roles in the mouse incisor.<sup>22,138</sup> Specifically, *Fgf3* and *Fgf10* are expressed in the mesenchyme immediately adjacent to and surrounding the labial CL, whereas *Fgfr1b* and *Fgfr2b* are enriched in the epithelium. Incisors of *Fgf3* null mice at P0 are indistinguishable from those in wild-type mice, yet *Fgf3*<sup>-/-</sup>;*Fgf10*<sup>+/-</sup> compound mutants reveal a severely hypoplastic labial CL, indicating that precise levels of FGF signaling are responsible for regulating the size and shape of the stem cell niche.<sup>22</sup> Consistent with this result, attenuation of signaling via FGFR2B in the embryo using a tetracycline-inducible dominant negative system gave similar results in E18.5 embryos.<sup>144</sup>

It appears that several additional signaling pathways either directly or indirectly converge on FGF signaling via regulation of *Fgf3* to control proliferation in developing incisors. Mis-expression of *Fst* throughout the dental epithelium under the control of the *Krt14* promoter resulted in complete down-regulation of mesenchymal *Fgf3* expression with reduction in epithelial proliferation and severely hypoplastic labial CLs.<sup>22</sup> Conversely, loss of *Fst* in the epithelium of incisors led to up-regulated *Fgf3* expression in mesenchyme adjacent to the lingual CL, causing increased proliferation and expansion of the lingual CL.<sup>22</sup> Deletion of *Tgfbr1* (formerly known as *Alk5*) in the mesenchyme led to down-regulation of *Fgf3*, *Fgf9*, *Fgf10*, and a reduced labial CL size, likely due to proliferation defects.<sup>145</sup> Notably, fewer LRCs survived in this mutant, and the defect could be reversed by the addition of exogenous FGF10. Thus, several lines of evidence indicate that FGF signaling is involved in the maintenance of incisor stem cell number.

The sprouty genes encode negative feedback regulators of FGF signaling that are expressed in both the lingual and labial epithelia as well as in the mesenchyme adjacent to the labial CL. Loss of sprouty function in the incisor resulted in up-regulation of FGF gene expression in the lingual epithelium and

mesenchyme and the presence of ameloblasts in the lingual epithelium.<sup>25</sup> This study highlighted the importance of balanced FGF signaling in the incisor to maintain asymmetric production of ameloblasts on the labial side, while preventing ameloblast formation and activity on the lingual side.

In addition to FGF signaling, incisor stem cells require the activity of the Notch pathway to ensure development and survival. Three Notch receptors are expressed in the CL regions in the developing incisor. *Notch1* and *Notch2* are expressed in the epithelium and mesenchyme, whereas *Notch3* is restricted to the mesenchyme.<sup>138</sup> The Notch ligand encoded by *Jag2* is strongly expressed in the epithelium, and *Jag2*-null mice show incisors with defects in cellular morphology.<sup>66</sup> Inhibition of Notch signaling with DAPT led to a reduction in the size of the labial CL in explant experiments.<sup>146</sup>

Comparative analysis of two different rodent species provides a potential mechanism for the evolution of ever-growing teeth. The sibling vole (*Microtus rossiaemeridionalis*), in contrast to mice, possesses ever-growing incisors and molars.<sup>131</sup> During development, CLs in mouse molars undergo a transition to the root fate and cease producing enamel. Vole molars do not undergo such a transition, and enamel is continuously generated for the life of the animal.<sup>131</sup> Interestingly, mouse and vole molars are practically identical in morphology and distribution of developmental markers until E17, when the molars are in the late bell stage. Given the roles of FGF and Notch signaling in maintenance of the stem cell niche in the mouse incisor, these pathways were compared at stages during which mouse molars initiate root formation.<sup>131</sup> The development of roots in the mouse coincides with loss of epithelial Notch and mesenchymal FGF signaling, whereas vole molars continue to express key signaling components and partially bypass the root fate. This idea is substantiated by evidence showing that the maintenance of Notch signaling in the cervical loops of mouse molars grown *in vitro* resulted in continuous crown development in lieu of root formation.<sup>147</sup> Although correlative, these studies suggest that vole molars have evolved mechanisms that maintain the necessary morphology for continuous growth fueled by molar cervical loops, which may be analogous to the stem cell niche of the mouse incisor.

## CONCLUDING REMARKS

Important advances have been made in our understanding of the development and evolution of teeth. The tooth provides a valuable model for the elucidation of major biological questions, and the

identification and initial characterization of dental stem cells have been exciting recent developments. Many tooth-specific defects in model and nonmodel organisms mirror conditions present in humans and provide a means to study their genetics, development, and pathology. For a comprehensive review on the diseases of the tooth, please refer to the review in this series by Sharpe et al. Much work remains to be done, and the utilization of cellular, molecular, and genetic approaches, as well as anthropological and paleontological techniques, will enable continued progress. The rapid increases in our understanding

of dental development in extant and extinct vertebrate species using techniques including 3D imaging, genetic manipulations, omics analyses, and genome-wide association studies make this an exciting time to study the development and evolution of teeth.

## NOTE

<sup>a</sup>Gene and protein names in the mouse and human genome databases are available from the NCBI website, <http://www.ensembl.org/index.html>.

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