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Molecular genetics of tooth development

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Organogenesis depends upon a well-ordered series of inductive events involving coordination of molecular pathways that regulate the generation and patterning of specific cell types. Key questions in organogenesis involve the identification of the molecular mechanisms by which proteins interact to organize distinct pattern formation and cell fate determination. Tooth development is an excellent context for investigating this complex problem because of the wealth of information emerging from studies of model organisms and human mutations. Since there are no obvious sources of stem cells in adult human teeth, any attempt to create teeth *de novo* will probably require the reprogramming of other cell types. Thus, the fundamental understanding of the control mechanisms responsible for normal tooth patterning in the embryo will help us understand cell fate specificity and may provide valuable information towards tooth organ regeneration.

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Introduction

Teeth, like all epithelial appendages, form via a sequential and reciprocal series of inductive signals transmitted between the epithelium and neural crest derived mesenchyme. Each tissue layer instructs the other to differentiate in a precisely determined manner leading to the formation of highly specialized structures, such as incisors, canines, premolars and molars. Each of these groups of teeth derives from different parts of the oral epithelium and, depending on the species, teeth can be formed from both endoderm and ectoderm or from ectoderm only [1,2••].

Morphologically, tooth development commences with a thickening of the dental epithelium to form a structure known as the dental lamina (Figure 1). Within this band of thickened epithelium the cells start to proliferate and to invaginate in certain positions to form the placodes. After this fundamental step in development, further epithelial invagination and convolution form the bud, cap and bell stages of tooth morphogenesis (Figure 1). During these stages, the constant interplay of inductive signals between the epithelium and mesenchyme (i) gives rise to distinct anatomical and functional parts of the tooth and (ii) mediates the differentiation of the epithelium into enamel-secreting ameloblasts and that of the mesenchyme into dentine-secreting odontoblasts (Figure 1).

Animal and human studies that employ the tools of contemporary molecular genetics have identified a number of genes that act at specific stages of tooth development and regulate its patterning and differentiation process (Figure 1; Tables 1 and 2; http://bite-it.helsinki.fi). The purpose of this review is to discuss in general terms some recent findings regarding genes and pathways that control tooth development and to provide new perspectives on the potential molecular mechanisms that coordinate the process of odontogenesis and tooth regeneration.

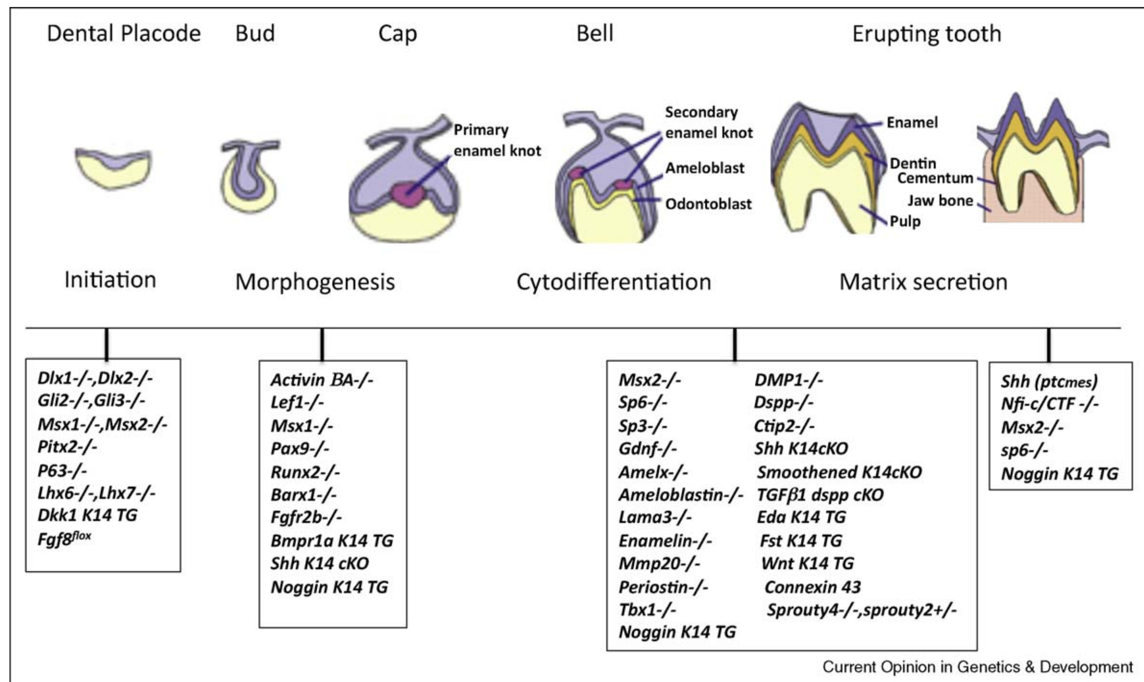
Genes and pathways involved in regulation of tooth development

Four major signaling pathways and their inhibitors control tooth formation: a fine balance that determines number and patterning

The conserved signaling pathways of BMP, FGF, SHH and WNT ligands and their receptors constitute the key pathways that are used reiteratively during tooth development and mediate the epithelial–mesenchymal interactions [3,4]. Over the past 15 years, studies using transgenic animals provided functional data showing that, in most cases, disruption of genes that are part of these signaling pathways results in severe aberrations of tooth development, such as complete tooth agenesis or arrest of tooth development at early stages of development (the lamina or bud stage of development), leading to anodontia (lack of teeth) [reviewed in [5,6•,7,8•]; Tables 1 and 2 and Figure 1]. For example, conditional inactivation of FGF8 in the dental epithelium results in arrest of tooth development at the lamina stage. Overexpression of BMPR1a in transgenic mice, or functional inactivation of FGFR2b or SHH results in arrest of tooth development at the bud stage [reviewed in [4,5]].

Recently, however, it was realized that the inhibitors of these signaling pathways also contribute to control tooth development. In most cases, when the inhibitors

Figure 1



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or mediators of these signaling pathways are perturbed, more teeth are formed with abnormal shape, ameloblast or odontoblast differentation defects and reduced matrix deposition [9–12,13•,14••]; reviewed in [8•], Tables 1 and 2].

For example, loss of *Ectodin* leads to supernumerary teeth through inhibition of BMP signaling [10]. Ectodysplasin (Eda), a WNT signaling mediator, when overexpressed can lead to supernumerary teeth [15,16]. *Apc* (*Adenomatous polyposis coli*), another WNT modifier that organizes the complex that degrades β-catenin, results in multiple tooth buds when conditionally knocked out in the oral epithelium,. Consistently, when *β-catenin* is overexpressed results in supernumerary teeth. These results suggest that overexpression of the canonical WNT signaling, either through loss of function of its inhibitors or by overexpression of its effectors leads to supernumerary teeth [11,17,18]. The importance of the tooth-inductive potential of WNT signaling manipulation is further demonstrated by the recent discovery that WNT pathway activation, even postnatally, lead to formation of extra teeth [14••]. Moreover, a member of the low-density receptor-related protein family, Lrp4, that modulates and integrates both the BMP and the canonical WNT signalling by binding the secreted BMP antagonist protein Ectodin, when mutated in mice results in supernumerary incisors and molars as well as fused molars, a phenotype identical to that of *Ectodin* mouse mutant [19].

As in the case of BMP and WNT pathways, the mediators and/or inhibitors of the SHH and FGF signaling lead to supernumerary teeth, when mutated. Primary cilia mediate SHH signaling, since mutations in their protein components affect SHH activity. Mice mutant for a cilia intraflagellar transport (IFT) protein, IFT88/polaris, result form ectopic teeth, through increase of Shh activity in the toothless region of the embryonic jaw primordia, the diastema region [20,21,22••]. Consistently, upregulation of Shh activity in mice mutant for *Gas1*, a Shh protein antagonist, results in ectopic diastema teeth [22••]. Finally, inactivation of either *Sprouty2* (*Spry2*) and/or *Sprouty 4* (*Spry4*), the inhibitors of FGF signaling, leads to supernumerary teeth in the diastema [12,13•].

These studies demonstrate that tooth formation is the result of a tight control between networks of activators and inhibitors, and that any modification of these networks leads to abnormalities in either number or patterning. Interestingly, the transcription factors that mediate such signaling networks are indispensable for early and late tooth development as well. Genetic experiments where the function of transcription factors such as *Msx1*, *2*, *Dlx1*, *2*, 5, *Runx2*, *Pax9*, *Pitx2*, *Lef1*, *Gli1*, *2*, *3*, *Lhx6*, *7*, *8*, *Prx1*, *2* and others (http://bite-it.helsinki.fi) is eliminated in mice or humans results in most cases in an arrest of tooth development at the bud stage or before during the lamina stage, leading to anodontia [Tables 1 and 2; [5,6•,8•]; Figure 1]. Exception to the rule is the case

Table 1

Abnormalities caused by mutations in transgenic mice affecting tooth formation.

Gene	Mutation	Tooth phenotype	Reference
Msxl, *Msx2*	Double mutant	Initiation stage arrest	Bei and Maas (1998)
Dlx1, *Dlx2*	Double mutant	Initiation stage arrest	Thomas *et al*. (1997)
Fgf8	*Fgf8^{flox}*	Initiation stage arrest	Trumpp *et al*. (1999)
Lhx6/Lhx7	Double mutant	initiation stage arrest	Grigoriou *et al*. (1998)
Pitx2	Null	Initiation stage arrest	Liu *et al*. (2003)
Gli2, *Gli3*	Double mutant	Initiation stage arrest	Hardcastle *et al*. (1998)
P63	Null	Initiation stage arrest	Yang *et al*. (1999)
Dkkl	K14 transgenic	Initiation stage arrest	Andl *et al*. (2002)
Pax9	Null	Bud stage arrest	Peters *et al*. (1998)
Lef1	Null	Bud stage arrest	Van genderen *et al*. (1994)
Msx1	Null	Bud stage arrest	Satokata and Maas (1994)
Runx2	Null	Bud stage arrest	Aberg *et al*. (2004)
Barx1	Null	Bud stage arrest	Tucker *et al*. (1998)
Bmpr1a	K14 transgenic	Bud stage arrest	Andl *et al*. (2004)
Fgfr2b	Null	Bud stage arrest	De Moerlooze *et al*. (2000)
Shh	K14 conditional KO	Bud stage arrest	Dassule *et al*. (2000)
Noggin	K14 TG	Bud stage arrest	Plikus *et al*. (2005)
Activin βA	Null	Bud stage arrest, lack incisors and mandibular molars	Matzuk *et al*. (1995)
Ctip2	Null	Late bell stage defect	Golonzhka *et al*. (2009)
Gli2	Null	Abnormal maxillary incisor	Hardcastle *et al*. (1998)
Gli3	Heterozygous	Maxillary incisor development arrested as a rudimentery epithelium thickening	Hardcastle *et al*. (1998), Mo *et al*. (1997)
Eda	Tabby encode eda	Small enamel knot	Tucker *et al*. (2000)
Edar	Downless	Absent enamel knot, disorgonized enamel rope	Headon and Overbeek (1999)
Fgf10	Null	Smaller tooth germ, cervical loops of the incisors are hypoplastic	Harada *et al*. [34]
Wnt/β catenin	*K14 conditional KO*	Misshappen tooth bud, ectopic teeth	Liu *et al*. [18]
Ectodin/Sostdc1/wise	Null	Supernumerary teeth, enlarge enamel knot, abnormal cusp	Kassai *et al*. [10]
Apc	K-14Cre; Apccko/cko	Supernumerary teeth	Kuraguchi *et al*. [11]
Sp6	Null	Supernumerary teeth	Nakamura *et al*. [23]
Lrp4	Null	Supernumerary teeth	Johnson *et al*. (2005)
IFT88/polaris	Null	Supernumerary teeth	Liu *et al*. (2005)
Gas1	Null	Supernumerary teeth	Ohazama *et al*. [22••]
Osr2	Null	Supernumerary teeth	Zhang *et al*. [24••]
Sprouty2, *4*	Null	Supernumerary teeth	Klein *et al*. [12]

of Sp6, a zinc finger transcription factor known as *Epfn*. *Sp6* null mice develop numerous teeth, up to 50 incisors and 8 molars, and that would be a surprise, if we did not know that Sp6 functions through upregulation of *Lef1*, a target, again of WNT signaling, whose activation leads to extra teeth, as mentioned above [23].

Another transcription factor that leads to supernumerary teeth upon mutation is odd-skipped related-2 (Osr2) [24••]. The study by Zhang *et al.* regarding the role of Osr2 transcription factor in tooth development stands alone, since most of the mouse mutants that develop extra buds/teeth do so in the toothless diastema region along the already formed single row of teeth. By contrast, *Osr2* deletion in mice leads to supernumerary teeth lingual to their molars, thus forming a second row of molars, through upregulation and expansion of the odontogenic field that is driven by the BMP4-Msx1-BMP4 pathway in the mesenchyme [25]. Thus, normally, Osr2 suppresses this pathway along the buccolingual axis to restrict molar development to one tooth row in mice [24••].

Complex networks of signaling pathways and the control of tooth diversity in evolution

The studies mentioned above indicate the importance of keeping a fine balance between signaling ligands, their receptors, inhibitors and transcription factors in regulating all aspects of tooth development, including the patterning, the size, the number and the shape. Since perturbations of these pathways lead to such fundamental changes in patterning and number, one could hypothesize that evolution favoured certain pathways versus others in promoting certain changes in the dentition of vertebrate species.

Table 2

Abnormalities caused by mutation in transgenic mice affecting tooth matrix deposition and root formation.

Gene	Mutation	Tooth phenotype	Reference
Enamel defect			
Msx2	Null	Enamel hypoplasia	Satokata *et al*. (2000)
Lama3	Null	Enamel hypoplasia	Ryan *et al*. (1999)
Sp3	Null	Enamel hypoplasia	Bowman *et al*. (2000)
Sp6	Null	Enamel hypoplasia	Nakamura *et al*. [23]
Smoothened	K14 conditional KO	Enamel hypoplasia	Gritli-Linde *et al*. (2002)
Gdnf	Null	No enamel	deVicente *et al*. (2002)
Periostin	Null	Incisor enamel defect	Rios *et al*. (2005)
TGFB1	Dspp conditional KO	Enamel hypoplasia	Haruyama *et al*. (2006)
Eda	K14 transgenic	No enamel	Mustonen *et al*. (2004)
Follistatin	K14 transgenic	No enamel	Wang *et al*. [9]
Follistatin	Null	Ectopic enamel	Wang *et al*. [9]
Wnt3	K14 transgenic	No enamel	Millar *et al*. (2003)
Amelx	Null	Enamel hypoplasia	Gibson *et al*. (2001)
Ameloblastin	Null	No enamel	Fukumoto *et al*. (2005)
Tbx1	Null	Enamel free teeth	Caton *et al*. (2009)
Enamelin	Null	Enamel hypoplasia/aplasia	Hu *et al*. (2008)
Mmp20	Null	Enamel hypoplasia	Caterina *et al*. (2002)
Connexin 43	Dominant negative	Enamel hypoplasia	Dobrowolski *et al*. (2008)
Sprouty2, *4*	Spry2+/−, Spry4−/−	Ectopic enamel	Klein *et al*. [13•]
Periostin	Null	Thinner enamel layer,	Rios *et al*. (2005)
Noggin	K14 transgenic	Abnormal ameloblast	Plikus *et al*. (2005)
Dentine defect			
Dspp	Null	Dentinogenesis imperfecta	Thyagarajan *et al*. (2001)
DMP1	Null	Abnormal dentine tubule system	Lu *et al*. (2007)
Msx2	Null	Dentinogenesis imperfecta	Aioub *et al*. (2007)
Sp6	Null	Abnormal dentine stucture	Nakamura *et al*. [23]
Sp3	Null	Dentine defect	Bowman *et al*. (2000)
Noggin	K14 transgenic	Abnormal dentinoblast	Plikus *et al*. (2005)
Root defect			
Msx2	Null	Root malformation	Satokata *et al*. 2000
Shh	Ptc^{mes}	Shorter root	Nakatomi *et al*. (2006)
Nfi-c/CTF	Null	Lacking root	Steele-perkins *et al*. (2003)
Sp6	Null	Defect in root formation	Nakamura *et al*. [23]
Noggin	K14 transgenic	Failed to form multiple root	Plikus *et al*. (2005)

For example, many non-mammalian vertebrates, such as fish or reptiles, replace their teeth throughout their life, have multi-rowed dentition and their teeth are all of simple shape, while mammalian vertebrates develop teeth in a single row, replace their teeth once or not at all, and the teeth acquire different shapes and forms such as incisors, canines, premolars and molars [1,26,27]. Mice are examples of mammalian vertebrates that possess molars and incisors only, they are monophyodonts (one set of teeth) and they develop their teeth in a single raw. The ancestors of mice, however, the Glires, a clade including rodents and lagomorphs, possessed premolars and canines similar to the dentition observed in non-rodent species [28]. Interestingly, some rodents, such as squirrels and guinea pigs, still have premolar teeth, suggesting that the genetic information specifying premolar and canine tooth shapes or tooth replacement processes is still in place.

The phenotypes of numerous knock out and transgenic mice that form either additional teeth in the diastema region, a region where normally premolars and canines would have exist, or multiple *de novo* teeth, or multi-rowed dentition just by perturbing a signaling pathway, support such a hypothesis (Tables 1 and 2).

Stem cells in teeth and their potential to be regenerated

Despite the progress made from genetic studies described above, the processes involved in the formation of extra teeth and tooth replacement are still not known. Some studies suggest that *Pitx2* and *Bmp4* are key molecules associated with continuous tooth replacement in fish [26]. Mammals, such as mice, that do not replace their teeth, form *de novo* teeth when the WNT signaling is overexpressed [11,14••,17,18]. Could any of these pathways be the key to regenerate teeth in humans? Can we use the pathway network knowledge to drive stem cell differentiation processes towards *de novo* tooth formation?

Currently, efforts towards that goal are concentrated in an attempt to discover adult stem cells in human or mouse

teeth. Mesenchymal stem cells have been identified in adult human teeth in the dental pulp (DPSCs) and in the dental follicle (DFSCs) [29,30]. These cells have stem cell properties, can be cultured as stem cells *in vitro*, can form colonies and differentiate *in vivo* into odontoblasts, cementoblasts and periodontal ligament cells [31]. Dental epithelial cells, from the quiescent Epithelial Cell Rests of Malassez (ERM)-the only dental epithelium remaining after root formation, located within the periodontal ligament (PDL)-were isolated from pigs and differentiated into ameloblast-like cells producing enamel *in vivo,* when co-seeded with dental pulp cells [32]. Using the continuously growing incisor of the mouse as a model for the study of adult epithelial stem cells, researchers have shown that label-retaining cells were localized in the epithelially derived cells of the cervical loop, and this population of cells has been proposed to constitute the mouse incisor stem cell niche [33]. Further studies indicated that members of the FGF family of ligands, namely FGF3 and FGF10, derived from mesenchyme, promote the proliferation and survival of the incisor epithelial stem cell niche [33,34,35••]. Consistently, FGF10 is down-regulated in teeth that do not grow continuously, such as the mouse molar, while the addition of FGF10 to cultured mouse molars promotes the maintenance of their cervical loops [36].

Although these studies have provided some insight on potential sources of tooth stem cells in pigs and mice, the fact remains that adult human teeth do not grow continuously and no human stem cell niches have yet been identified. The epithelial cell rests of Malassez (the only remaining epithelial cells after the tooth completes its development), the dental pulp and dental follicle stem cells that have been isolated from humans are promising, but their stemness is not yet well established and, more importantly, there is no evidence yet that they are capable to direct tooth morphogenesis.

Future directions: reprogramming of adult differentiated cells and the search for the unique molecular identity of teeth

Recent advances in adult cell reprogramming through the creation of induced pluripotent stem (iPS) cell lines from adult differentiated cells offer the possibility to produce pluripotent stem cells from patient's own tissue [37•,38]. iPS cells are created by forced expression of defined transcription factors as Oct4, Sox2, cmyc and Klf4, which have been shown to induce pluripotency in somatic fibroblast cells [39••]. In another study, iPS cells were generated from human fibroblasts using Oct4, Sox2, Nanog and Lin28 [40]. Although the four reprogramming factors were different in these two studies, all of them were transferred into the cells by means of retrovirus gene transfer, which holds some risk to cause insertion mutations. In addition, since some of the reprogramming factors are oncogenes as well, the risk of tumour induction is another potential limitation to be considered. A recent breakthrough study by the group of Doug Melton succeeded to directly reprogram pancreatic exocrine cells to insulin producing beta cells [41••]. This method is more advanced to the reprogramming of any adult differentiated cells to iPS cells, because the pancreatic exocrine cells share a common genomic and epigenomic identity with those of insulin producing beta cells. The latter approaches could be promising and suggest that tooth-specific cell types, such as ameloblasts or dental follicle cells, with matrix producing capability could be produced through reprogramming of adult cell types. Whether reprogramming of adult cells to iPS and then programming of iPS can be used to generate, instead of specific cell types, a rather complex three-dimensional organ like the tooth, is not yet known.

The tooth is a complex organ and its development a process controlled by a sequence of cellular and molecular networks that act at particular places and times to guide pluripotent cells to restricted dental cell fates. The mechanisms that control and determine the history of the cells so that the differentiation program is properly executed during embryonic development are not known. Despite the fact that genetics and epistasis analysis have led to the discovery of numerous genes and pathways involved during different stages of tooth development, the same genes are required for the development of other tissues, especially that of other ectodermal organs such as hairs, nails and glands [Figure 1 and Tables 1 and 2]. In addition, perturbations of these genes affect not only tooth development but, in most cases, the development of other ectodermal organs, thus leaving open the obvious question of cell fate specificity [8•,42].

What it is known, however, is that transcription factors control cell fate through a selective regulation of target genes, and that the target gene specificity is achieved through context-dependent selective protein interactions. Recent studies suggest that in early tooth morphogenesis, a network of transcription factors operate in the dental mesenchyme to regulate a specific transcriptional output and that their combinatorial action, along with their target specificity is further modified by epigenetic mechanisms, such as sumoylation (M Bei, unpublished). The need of such a comprehensive analysis at the molecular level, along with extensive whole-genome data sets (including ChIP-chip and ChIP-Seq of multiple transcription factors), maps of epigenetic states, expression profiling of genetically manipulated cells, would help determine the molecular identity of the tooth and permit controlling of the iPS programming towards the generation of *de novo* teeth.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Smith MM: **Vertebrate dentitions at the origin of jaws: when and how pattern evolved**. *Evol Dev* 2003, **5**:394-413.

2. •• Soukup V, Epperlein H, Horacek I, Cerny R: **Dual epithelial origin of vertebrate oral teeth**. *Nature* 2008, **455**:795-796.
Using transgenic axolotls with a combination of fate-mapping approaches, the authors provided evidence for oral teeth derived from both the ectoderm and the endoderm and, moreover, they demonstrated teeth with a mixed ecto/endodermal origin.

3. Mikkola ML: **Genetic basis of skin appendage development**. *Semin Cell Dev Biol* 2007, **18**:225-236.

4. Thesleff I: **Epithelial-mesenchymal signalling regulating tooth morphogenesis**. *J Cell Sci* 2003, **116**:1647-1648.

5. Fleischmannova J, Matalova E, Tucker AS, Sharpe PT: **Mouse models of tooth abnormalities**. *Eur J Oral Sci* 2008, **116**:1-10.

6. • Bei M: **Molecular genetics of ameloblast cell lineage**. *J Exp Zool (Mol Dev Evol)* 2009, **312B(5)**:437-444.
In this review the author discusses the role of genes that play definitive role on the determination of ameloblast cell fate and life cycle based on studies in transgenic animals. Mutations in both mouse and humans reveal the involvement of several classes of genes in ameloblast life cycle and its function, a process that emerges as a model for elucidating the molecular regulatory cascades that operate in other developmental systems as well.

7. Caton J, Tucker AS: **Current knowledge of tooth development: patterning and mineralization of the murine dentition**. *J Anat* 2009, **219**:502-515.

8. • Tummers M, Thesleff I: **The importance of signal pathway modulation in all aspects of tooth development**. *J Exp Zool (Mol Dev Evol)* 2009, **312B(5)**:309-319.
In this review the authors discuss the complexity of the regulatory network involved in all facets of tooth development. This complexity leads to the availability of numerous developmental solutions to the same problem, all of which are accomplished by regulatory tinkering. We conclude that the complexity and nature of the signalling network probably facilitated the change of tooth shapes and patterns during evolution.

9. Wang XP, Suomalainen M, Jorgez CJ, Matzuk MM, Werner S, Thesleff I: **Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation**. *Dev Cell* 2004, **7**:719-730.

10. Kassai Y, Munne P, Hotta Y, Penttila E, Kavanagh K, Ohbayashi N, Takada S, Thesleff I, Jernvall J, Itoh N *et al.*: **Regulation of mammalian tooth cusp patterning by ectodin**. *Science* 2005, **309**:2067-2070.

11. Kuraguchi M, Wang XP, Bronson RT, Rothenberg R, Ohene-Baah NY, Lund JJ, Kucherlapati M, Maas RL, Kucherlapati R: **Adenomatous polyposis coli (APC) is required for normal development of skin and thymus**. *PLoS Genet* 2006, **2**:146.

12. Klein OD, Minowada G, Peterkova R, Kangas A, Yu BD, Lesot H, Peterka M, Jernvall J, Martin GR: **Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial–mesenchymal FGF signaling**. *Dev Cell* 2006, **11**:181-190.

13. • Klein OD, Lyons DB, Balooch G, Marshall GW, Basson MA, Peterka M, Boran T, Peterkova R, Martin GR *et al.*: **An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors**. *Development* 2008, **135**:377-385.
The authors show that sprouty genes, which encode antagonists of receptor tyrosine kinase signaling, function to ensure that enamel-producing ameloblasts are generated on only one side of the tooth by inhibiting the formation of ectopic ameloblasts from self-renewing stem cells, and that they do so by preventing the establishment of an epithelial–mesenchymal FGF signaling loop. These data reveal that the generation of differentiated progeny from a particular stem cell population can be differently regulated in the embryo and adult.

14. •• Wang XP, O'Conell DJ, Lund J, Saadi I, Kuraguchi M, Turbe-Doan A, Cavallesco R, Kim H, Park PJ, Harada H *et al.*: **Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood**. *Development* 2009, **136**:1939-1949.
In this study the authors demonstrate that adult dental tissues can form new teeth in response to either epithelial Apc loss-of-function or beta-catenin activation, and that the effect of Apc deficiency is mediated by beta-catenin. The formation of supernumerary teeth via Apc loss-of-function is non-cell-autonomous.

15. Mustonen T, Pispa J, Mikkola ML, Pummila M, Kangas AT, Pakkasjarvi L, Jaatinen R, Thesleff I: **Stimulation of ectodermal organ development by Ectodysplasin-A1**. *Dev Biol* 2003, **259**:123-136.

16. Pispa J, Mustonen T, Mikkola ML, Kangas AT, Koppinen P, Lukinmaa PL, Jernvall J, Thesleff I: **Tooth patterning and enamel formation can be manipulated by misexpression of TNF receptor Edar**. *Dev Dyn* 2004, **231**:432-440.

17. Jarvinen E, Salazar-Ciudad I, Birchmeier W, Taketo MM, Jernvall J, Thesleff I: **Continuous tooth generation in mouse is induced by activated epithelial Wnt**. *Proc Natl Acad Sci U S A* 2006, **103**:18627-18632.

18. Liu F, Chu EY, Watt B, Zhang Y, Gallant NM, Andl T, Yang SH, Lu MM, Piccolo S, Schmidt-Ullrich R *et al.*: **Wnt/beta-catenin signaling directs multiple stages of tooth morphogenesis**. *Dev Biol* 2008, **313**:210-224.

19. Ohazama A, Johnson EB, Ota MS, Choi HJ, Porntaveetus T, Oommen S, Itoh N, Eto K, Gritli-Linde A, Herz J *et al.*: **Lrp4 modulates extracellular integration of cell signaling pathways in development**. *PLos One* 2008, **3**:4092.

20. Liu A, Wang B, Niswander LA: **Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors**. *Development* 2005, **132**:3103-3111.

21. Zhang Q, Murcia NS, Chittenden LR, Richards WG, Michaud EJ, Woychik RP, Yoder BK: **Loss of the Tg737 protein results in skeletal patterning defects**. *Dev Dyn* 2003, **227**:78-90.

22. •• Ohazama A, Haycraft CJ, Seppala M, BlackburnJ, Ghafoor S, Cobourne M, Martinelli MC, Fan CM, Peterkova R, Lesot H *et al.*: **Primary cilia regulate Shh activity in the control of molar tooth number**. *Development* 2009, **136**:897-903.
In this study the authors show that in mice mutant for a cilia intraflagellar transport (IFT) protein, IFT88/polaris, Shh activity is increased in the toothless diastema mesenchyme of the embryonic jaw primordia. This results in the formation of ectopic teeth in the diastema, mesial to the first molars. Interestingly, the ectopic teeth adopt a size and shape characteristic of premolars, a tooth type that was lost in mice around 50–100 million years ago.

23. Nakamura T, deVega S, Fukumoto S, Jimenez L, Unda F, Yamada Y: **Transcription factor epiprofin is essential for tooth morphogenesis by regulating epithelial cell fate and tooth number**. *J Biol Chem* 2008, **283**:4825-4833.

24. •• Zhang Z, Lan Y, Chai Y, Jiang R: **Antagonistic actions of Msx1 and Osr2 pattern mammalian teeth into a single row**. *Science* 2009, **323**:1232-1234.
In this study the authors show that mice lacking the transcription factor odd-skipped related-2 (Osr2) develop supernumerary teeth lingual to their molars because of expansion of the odontogenic field. Osr2 was expressed in a lingual-to-buccal gradient and restricted expression of bone morphogenetic protein 4 (Bmp4), an essential odontogenic signal in the developing tooth mesenchyme. Expansion of the odontogenic field in Osr2-deficient mice required Msx1, a feedback activator of Bmp4 expression. These findings suggest that the Bmp4-Msx1 pathway propagates mesenchymal activation for sequential tooth induction and that spatial modulation of this pathway provides a mechanism for patterning vertebrate dentition.

25. Bei M, Kratochwil K, Maas RL: **BMP4 rescues a non-cell autonomous function of Msx1 in tooth development**. *Development* 2000, **127**:4711-4718.

26. Fraser GJ, Berkovitz BK, Graham A, Smith MM: **Gene deployment for tooth replacement in the rainbow trout (*Oncorhynchus mykiss*): a developmental model for evolution of the osteichthyan dentition**. *Evol Dev* 2006, **8**:446-457.

27. Fraser GJ, Bloomquist RF, Streelman JT: **A periodic pattern generator for dental diversity**. *BMC Biol* 2008, **6**:32.

28. Yamanaka A, Yasui K, Sonomura T, Uemura M: **Development of heterodont dentition in house shrew (*Suncus murinus*)**. *Eur J Oral Sci* 2007, **115**:4334-4340.

29. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S: **Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo***. *Proc Natl Acad Sci U S A* 2000, **97**:13625-13630.

30. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi ST: **Investigation of multipotent postnatal stem cells from human periodontal ligament**. *Lancet* 2004, **364**:149-155.

31. Yao S, Pan F, Prpic V, Wise G: **Differentiation of stem cells in the dental follicle**. *J Dent Res* 2008, **87**:767-771.

32. Shinmura Y, Tsuchiya S, Hata K, Honda MJ: **Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells**. *J Cell Physiol* 2008, **217**:728-738.

33. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I: **Localization of putative stem cells in dental epithelium and their association with notch and FGF signaling**. *J Cell Biol* 1999, **147**:105-120.

34. Harada H, Toyono T, Toyoshima K, Yamasak M, Itoh N, Kato S, Sekine K, Ohuchi H: **FGF10 maintains stem cell compartment in developing mouse incisors**. *Development* 2002, **129**:1533-1541.

35. •• Wang X, Suomalainen M, Felszeghy S, Zelarayan LC, Alonso MT, Plikus MV, Maas RL, Chuong C, Schimmang T, Thesleff I: **An integrated gene regulatory network controls stem cell proliferation in teeth**. *Plos Biol* 2007, **5**:1324-1333.

In this study the authors show that spatially restricted and balanced effects of BMP, FGF and Activin signaling networks can regulate stem cell proliferation in the niche and account for asymmetric organogenesis. They also demonstrate that subtle variations in this or related regulatory networks may explain the different regenerative capacities of various organs and animal species.

36. Yokohama-Tamaki T, Ohshima H, Fujiwara N, Takada Y, Ichimori Y, Wakisaka S, Ohuchi H, Harada H: **Cessation of Fgf10 signaling, resulting in a defective dental epithelial stem cell compartment, leads to the transition from crown to root formation**. *Development* 2006, **133**:1359-1366.

37. • Nishikawa S, Goldstein RA, Nierras CR: **The promise of human induced pluripotent stem cells for research and therapy**. *Nat Rev Mol Cell Biol* 2008, **9**:725-729.

In this review the authors discuss the possibility to generate induced pluripotent stem (iPS) cells from human somatic cells that have been reprogrammed to a pluripotent state. They also discuss how iPS can serve as a research tool, how iPS cell technology provides opportunities to study normal development and to understand reprogramming and how iPS cells can have an immediate impact as models for human diseases, including cancer.

38. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilic J, Pekarik V, Tiscornia G *et al.*: **Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes**. *Nat Biotechnol* 2008, **26**:1276-1284.

39. •• Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors**. *Cell* 2006, **126**:663-676.

40. Yu J, Vodyanik M, Smuga-Otto K, Antosiewicz-Bourget J, Frane J, Tian S, Nie J, Jonsdottir G, Ruotti V, Stewart R *et al.*: **Induced pluripotent stem cell lines derived from human somatic cells**. *Science* 2007, **318**:1917-1920.

41. •• Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA: ***In vivo* reprogramming of adult pancreatic exocrine cells to beta cells**. *Nature* 2008, **455**:627-632.

Using a strategy of re-expressing key developmental regulators *in vivo*, the authors identified a combination of the three transcription factors Ngn3 (also known as Neurog3, Pdx1 and Mafa that reprogrammes differentiated pancreatic exocrine cells from adult mice into cells that closely resemble beta cells. The induced beta cells are indistinguishable from endogenous islet beta cells in size, shape and ultrastructure. They express genes essential for beta cell function and can ameliorate hyperglycaemia by remodelling local vasculature and secreting insulin. This study provided an example of cellular reprogramming using defined factors in an adult organ and suggests a general paradigm for directing cell reprogramming without reversion to a pluripotent stem cell state.

42. Duverger O, Morasso MI: **Role of homeobox genes in the patterning, specification, and differentiation of ectodermal appendages in mammals**. *J Cell Physiol* 2008, **216**:337-346.