Continuous tooth replacement: the possible involvement of epithelial stem cells

Ann Huysseune[1]* and Irma Thesleff[2]

Summary
Epithelial stem cells have been identified in integumental structures such as hairs and continuously growing teeth of various rodents, and in the gut. Here we propose the involvement of epithelial stem cells in the continuous tooth replacement that characterizes non-mammalian vertebrates, as exemplified by the zebrafish. Arguments are based on morphological observations of tooth renewal in the zebrafish and on the similarities between molecular control of hair and tooth formation. Dissection of the molecular cascades underlying the regulation of the epithelial stem cell niche might open perspectives for new regenerative treatment strategies in clinical dentistry. *BioEssays* 26:665–671, 2004.

Introduction

The dentition of vertebrates consists of repetitive units, which are usually replaced during the lifetime of the animal. This replacement occurs throughout life in most non-mammalian vertebrates, but only once in most mammals, including part of the human dentition,[1] and, in some mammals, e.g. the mouse, teeth are never replaced.

Teeth develop as a result of epithelial–mesenchymal interactions[2–4] and start their development in all vertebrates in a similar manner, by the formation of an epithelial thickening (called placode), which next invaginates into the underlying mesenchyme to form a bud. The formation of this bud is followed by several steps that vary to some extent in the different groups.[1]

Although previous work has revealed the existence of epithelial stem cells in continuously growing teeth,[5–7] their involvement in repeated tooth renewal has not been proposed. Here we consider the concept of epithelial stem cells and argue that such cells might be involved in the tooth replacement that characterizes most non-mammalian tooth-bearing vertebrates.

[1]Biology Department, Ghent University, Belgium.
[2]Developmental Biology Programme, Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Finland.
Funding agency: A.H. acknowledges a grant of the Bijzonder Onderzoeksfonds, Ghent University; Grant number: 011V1203.
*Correspondence to: Dr. Ann Huysseune, Biology Department, Ghent University, Ledeganckstraat 35, B-9000 Gent, Belgium.
E-mail: Ann.Huysseune@UGent.be
DOI 10.1002/bies.20039
Published online in Wiley InterScience (www.interscience.wiley.com).

Stem cells and tissue renewal

In many differentiated tissues of the vertebrate body, cells that die (naturally, or by accident) are replenished. Such tissues are maintained by adult stem cells, which have a somewhat more restricted differentiation repertoire compared to the virtually totipotent embryonic stem cells (but see Ref. 8). There are many definitions of stem cells but central to these is always the capacity of self-renewal (i.e. to divide, creating additional stem cells), a slow cell cycle, and resistance towards differentiation (e.g.[9,10]). In Fuchs and Raghavan's[11] recent definition, a stem cell is a cell "that can divide to produce both daughter stem cells and cells that go on to differentiate".

Several tissues harbouring stem cells are of epithelial nature, and the presence of stem cells for their regeneration and repair has been demonstrated for several decades. Stem cells have been reported in various epithelial tissues (e.g. corneal epithelium, crypts of the intestinal mucosa) or in organs with an epithelial component, such as liver, pancreas, lungs, mammary glands and hairs (see Refs. 8,12–18 for recent reviews).

Recently, the concept of stem cells has been revisited in the light of findings indicating that they are far more differentiated than was formerly assumed, and that they function in an environment that has a "crucial, but not necessarily irreversible impact" on the ability to differentiate in a particular direction.[8] If we cannot recognize a stem cell by its relatively undifferentiated state, then its identification and the elucidation of its function certainly become more problematical.

Although some adult stem cells are not located in a specific compartment (e.g. mammalian satellite myoblasts[19]), most adult stem cells are confined within a compartment, called the niche. A niche is defined as the micro-environment that allows stem cells to divide and to give rise to cells perpetuating the stem cell, and cells that enter a differentiation pathway.[10,20] In the cornea, for example, stem cells reside in the basal cell layer of the limbal epithelium.[10] In the small intestinal epithelium, stem cells are located near or at the base of crypts of Lieberkühn.[21] In hairs, the stem cell niche is located in the so-called bulge, a portion of the (epithelial) hair follicle located under the sebaceous gland.[22]

Molecular cascades underlying epithelial stem cell regulation

Skin and hairs are among the most-studied model systems to elucidate molecular cascades underlying epithelial stem cell regulation. The Wnt signaling pathway is considered to be functionally important for hair follicle morphogenesis (see Ref. 23 for a suite of arguments, and Ref. 24). Merrill et al.[25] showed that the status of the Tcf3/Lef1 (T-cell factor 3/ lymphoid enhancer binding factor 1) complexes plays a key role in controlling the genes that determine the differentiation status of skin stem cells. Hair follicle morphogenesis requires β-catenin/Lef1-mediated gene transactivation, whereas interference with this pathway and/or the relief of Tcf-mediated repression is possibly required for basal epidermal cell specification.[11] Levels of β-catenin seem to play a crucial role in the decision whether a stem cell or its progeny will contribute to structures below the bulge (hair cell types) or above the bulge (sebaceous gland, upper outer root sheath and epidermis).[23] β-catenin is a protein that plays a role both in intercellular junction formation (as part of adherens junctions), and in transcriptional regulation. In fact, there is now increasing evidence that adherens proteins, apart from their structural and mechanical role in cell adhesion (and so their indirect contribution to intercellular cell signaling) also play a more direct (inductive) role in cell commitment and differentiation through their participation in signal transduction.[20,26]

In line with the mechanical and signal transduction functions of adherens-related proteins, Jamora et al.[27] have recently discovered a link between Wnt signaling and reduced cell adhesion in the formation of the epithelial bud out of the bulge. Briefly, in response to Wnt, β-catenin is stabilized, and can bind to members of the Lef1/Tcf family of transcription factors. To activate Lef1, BMP signaling has to be inhibited by noggin. Activation of Lef1 leads to downregulation of E-cadherin and as a result to reduced cell adhesion, which is required for the reorganisation of the spatial interrelationships of the epithelial cells to produce a bud.

The only epithelial stem cell compartment identified so far in teeth is postulated to be under control of the Notch and FGF signaling pathways.[5–7] Notch-like transmembrane receptors and cell-bound ligands of the Delta, Jagged or Serrate type mediate a number of developmental processes most-thoroughly investigated in *Drosophila*, including lateral inhibition signaling, lineage decisions and boundary formation. When activated, Notch first undergoes a ligand-dependent proteolysis. An intracellular fragment of the receptor itself then translocates to the nucleus to bind to and activate transcription factors of the Hes family.[28] Experimental evidence has linked Notch activation and Delta expression in the skin with stem cell differentiation (reviewed in Ref. 29). Notch possibly plays an inhibitory role in hair formation, leading Fuchs and Raghavan[11] to suggest that the choice between hair or epidermis might be the result of a "tug of war" between Notch and Wnt pathways.

Stem cells and continuous tooth replacement

Most tooth-possessing non-mammalian vertebrates replace their teeth throughout life (see Ref. 1 for an overview and exceptions). During the functional life-stage of a fish tooth (i.e. when it is attached to the tooth-supporting bone and when its tip pierces the epithelium), an epithelial thickening develops to form the tooth bud that gives rise to the successor of the functional tooth. At least in teleost fish (such as cichlids, or the zebrafish), this thickening develops from the epithelial fold that surrounds the exposed tip of the functional tooth. The bud subsequently grows downward into the mesenchyme, and continues to develop either at the surface of the bone (extramedullary or extraosseous replacement, Fig. 1A), or penetrates into the medullary cavity of the tooth-bearing bone to continue its development in this sheltered space (intramedullary or intraosseous replacement, Fig. 1B).[30] The same

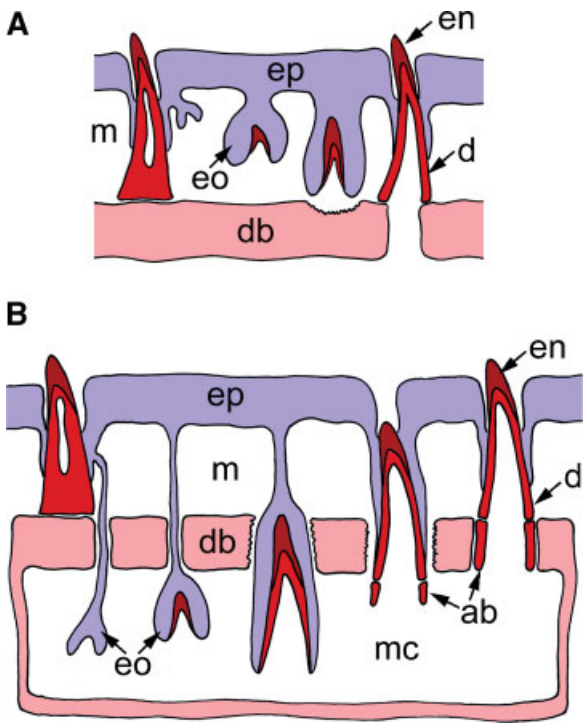

Figure 1. Schematic representation of replacement tooth formation in **A:** extramedullary or **B:** intramedullary situation, as observed in teleost fishes. In both schemes, successive stages of development of the tooth germ are shown from left to right. The predecessor (functional tooth) is only represented once (left of each figure), to show its relationship to the successor (replacement tooth). The zebrafish conforms to the upper scheme; other teleosts, e.g. cichlid fish, to the lower. Bone, pink; epithelium, purple; tooth matrix, red. Abbreviations: ab, attachment bone; d, dentine; db, dentigerous bone; ep, buccal or pharyngeal epithelium; en, enameloid; eo, enamel organ; m, mesenchyme; mc, medullary cavity.

scenario is seen whatever the position of the teeth, oral or pharyngeal.

We propose that the epithelium from which the new tooth germ buds off contains epithelial stem cells that are responsible for the continuous tooth renewal. This hypothesis is based on a number of arguments, taken largely from observations on zebrafish tooth replacement.

First, in the zebrafish (where teeth are restricted to the pharyngeal region), replacement teeth develop at the bases of the epithelial crypts that surround the erupted, functional tooth[31] (Fig. 2A). There is a considerable morphological resemblance to the epithelial crypts of the (mammalian) intestinal mucosa, although proliferation and distal cell movement typical of intestinal crypts have not yet been demonstrated.

Second, the initiation of replacement teeth in zebrafish seems to be under a different genetic control compared to the initiation of first-generation teeth. The transcription factor *eve1* has been shown to be involved in the initiation of all three first-generation teeth that develop before 6 days post-fertilization (dPF).[63] The germs of these first-generation teeth bud off directly from the pharyngeal epithelium. In contrast, the gene is not expressed during initiation of their replacement teeth, all three of which equally develop before 6 dPF, and which bud off from the crypt epithelium surrounding the exposed part of the now erupted first-generation tooth. It is possible that the epithelial stem cell niche is only established at the moment that the first replacement teeth form. The absence of *eve1* expression during initiation of replacement teeth, and the development of replacement tooth buds from crypts, both starting from the second generation onwards (i.e., the first replacement tooth generation), could be a coincidence, but could also point to a developmental link between the formation of these crypts and the establishment of stem cells. Although a domain-restricted expression pattern, such as that seen for the transcription factor *Barx-1* in mice,[32] teaches us that regional differences in expression need not be related to the presence or absence of stem cells, the spatiotemporal expression pattern of *eve1* strongly suggests such an association. Functional studies are underway to reveal the exact role of *eve1* in tooth initiation.

Third, earlier studies on cichlid replacement teeth (which develop intramedullary, i.e. at the end of an epithelial strand branching off the crypt epithelium, Fig. 2B) have revealed the presence of cells incorporating [^{3}H]thymidine in the initially club-shaped epithelial strand. Later cell divisions are restricted to the bud itself, whereas the epithelial strand connecting the bud to the crypt epithelium no longer shows any incorporation.[33] These observations fit with the hypothesis that a stem cell niche provides cues for the stem cells to generate a transiently amplifying population of cells, which subsequently withdraw from the cell cycle to give rise to terminally differentiated cells. The situation is different in zebrafish teeth, which do not form intramedullary, and therefore lack the (long)

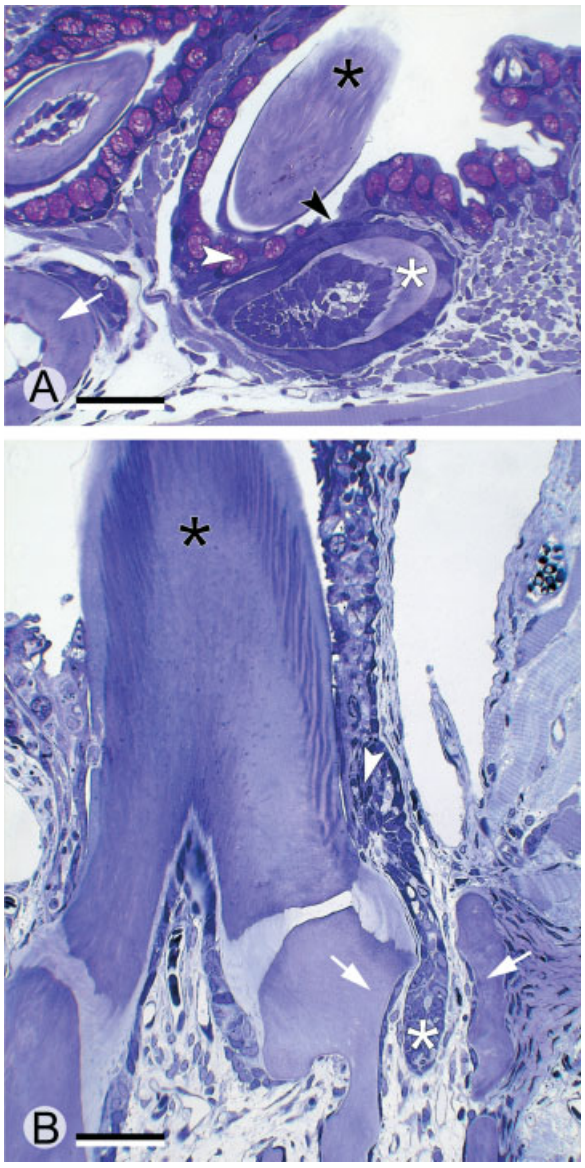

Figure 2. Micrographs of putative stem cell niche involved in **A:** extramedullary replacement tooth formation (zebrafish) and **B:** intramedullary replacement tooth formation (the cichlid fish *Hemichromis bimaculatus*). A pharyngeal tooth is shown in **B**, to match the only tooth type present in zebrafish (**A**). Functional teeth are labeled by a black asterisk; a white asterisk indicates the replacement tooth germ in **A**, and the epithelial strand giving rise to an intramedullary tooth germ in **B**. The dentigerous bone is indicated by a white arrow in **A**; two white arrows in **B** indicate the attachment bone through which a channel allows passage of the epithelial strand. The putative stem cell niche is indicated by a black arrowhead in **A** and a white arrowhead in **B**. A white arrowhead in **A** points to a mucous cell, abundantly present in the crypt epithelium. Scale bar in **A** and **B**: 20 μm.

epithelial strand. There are no studies available yet on cell proliferation in the enamel organ of the zebrafish.

Finally, there is a striking resemblance when comparing replacement tooth development, intestinal crypt renewal and hair follicle morphogenesis (Fig. 3A–C). In hair follicles and in intestinal crypts, cells are generated that can move upward (distally) or downward (proximally), that can enter multiple differentiation pathways, and that rely on mesenchymal signals for their maintenance and the differentiation of their progeny. In the small intestinal epithelium, stem cells give rise to rapidly but transiently proliferating cells that move distally and next differentiate into either enterocytes, mucous-producing goblet cells or enteroendocrine cells. Basally, they can differentiate into Paneth cells.[8] In the hair follicle, bulge cells give rise to sebaceous gland, upper outer root sheath and epidermis above the bulge, and to hair cell types below the bulge.[8] A similar process may occur in zebrafish. In the zebrafish pharyngeal epithelium, we may speculate that cells in the putative stem cell compartment give rise distally to a range of cells of the pharyngeal (crypt) epithelial lining, many of which are mucous-producing cells of various types,[34] whereas proximally, they give rise to the enamel organ of a new tooth germ. When the hair follicle regresses, the pocket of dermal papilla cells is dragged up to the bulge. One or more stem cells in the bulge respond to a stimulus from the dermal papilla to start to regenerate the follicle.[8] A process that brings epithelial stem cells and competent mesenchymal cells together to induce the formation of a new tooth germ can also be envisaged during tooth replacement in the zebrafish. However, rather than mesenchymal cells moving upward, it is more likely that epithelial cells are moved downward. Indeed, during eruption, the epithelium around the tip of a tooth that is about to erupt retracts[31] and, in this way, may displace the putative epithelial stem cell niche (Fig. 4).

Taken together, the morphological features of replacement tooth formation in zebrafish and other teleosts, and its strong resemblance to hair and intestinal crypt formation, strengthen the hypothesis of the involvement of stem cells in the process of continuous tooth renewal.

Support for this hypothesis also comes from experimental (in vitro) data. In zebrafish, first-generation teeth are initiated

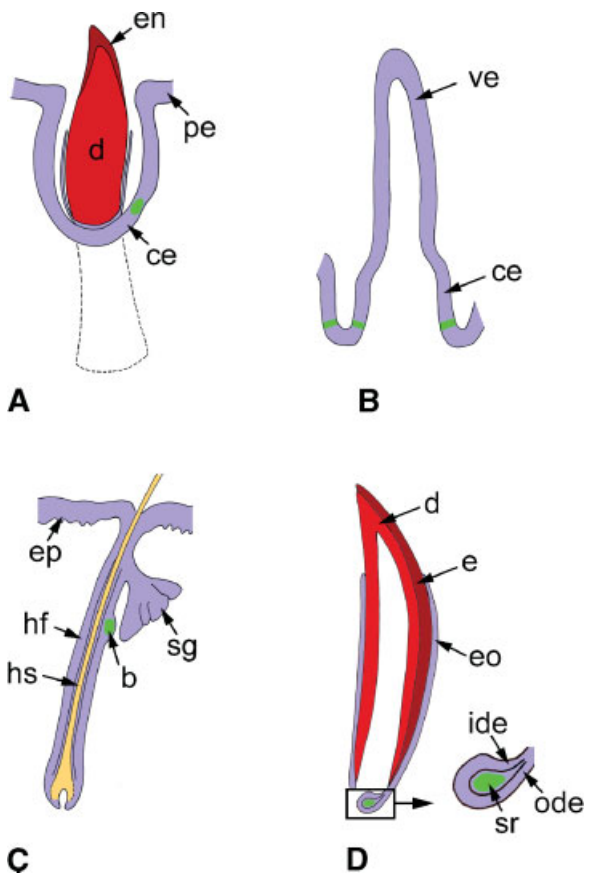

Figure 3. Schematic representation of **A:** location of putative stem cell niche (green area) involved in tooth replacement in the zebrafish, and **B–D:** comparison to stem cell niches in mammalian intestinal crypt epithelium (**B**), hair follicle (**C**), and enamel epithelium of a continuously growing rodent tooth (**D**). In the intestinal epithelium, the stem cell niche is located near the base of the crypts of Lieberkühn (ce); in the hair follicle stem cells are located in the bulge region (b); in the continuously growing rodent tooth, the stem cell niche is located in the stellate reticulum (sr) of the cervical loop at the labial side. Epithelium, purple; hair shaft, yellow; tooth matrix, red. Abbreviations: ce, crypt epithelium; d, dentine; e, enamel; en, enameloid; eo, enamel organ; ep, epidermis; hf, hair follicle; hs, hair shaft; ide, inner dental epithelium; ode, outer dental epithelium; pe, pharyngeal epithelium; sg, sebaceous gland; ve, villus epithelium.

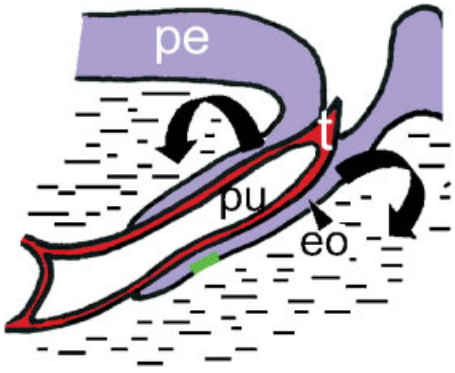

Figure 4. Schematic representation of movement (arrows) of the epithelial cell layers (pharyngeal epithelium, pe, and remains of the enamel organ, eo) during tooth eruption in the zebrafish. Epithelium, purple; tooth matrix, red. Abbreviations: pu, tooth pulp; t, tooth matrix. The small area marked in green is the putative stem cell niche.

in vitro, whereas their replacement teeth are not (Van der heyden and A.H., unpublished data). Also, the first-generation teeth fail to erupt. Eruption in vivo seems to be accomplished by reduced cell adhesion in the overlying epithelium.[31] Interestingly, it has been recently shown that the formation of a hair bud is accomplished by reduced cell adhesion.[27,35] The formation of replacement teeth possibly also depends on reduced cell adhesion, and the formation of hairs and teeth may depend on similar cascades. The cell adhesion molecule E-cadherin might well be involved in zebrafish replacement tooth formation, as it is in mammalian hair follicle formation. Zebrafish E-cadherin (*cdh1*) has been recently cloned, and its expression reported in various tissues.[36] E-cadherin is expressed in zebrafish at 72 hours in the pharyngeal epithelium and enamel organs of first-generation teeth (A.H. and Liu, unpublished data). The first replacement tooth starts to form, however, at around 80 hours.[37] Detailed observations on serially sectioned embryos are needed to examine whether *cdh1* is downregulated in that part of the crypt epithelium that gives rise to a replacement tooth bud, as would be expected if a signaling pathway similar to the one leading to hair follicle development is involved.

Support for the supposition that the development of hair and teeth is possibly regulated by the same molecular cascades comes from studies on human malformation syndromes, collectively known as hypohidrotic (or anhidrotic) ectodermal dysplasia (HED), and their mouse mutant models, *Tabby, downless, Sleek* and *crinkled*. HED as well as the mouse mutants are characterized by missing or poorly developed hair, teeth and sweat glands, as well as defects in other exocrine glands and in nails (reviewed in Refs. 38, 39). Mouse *Tabby* mutants develop only one of the four types of hairs normally present and show tooth defects. The gene responsible for this mutation encodes ectodysplasin (Eda), a signaling molecule belonging to the tumor necrosis factor (TNF) superfamily.[40,41] Mutations in the Eda receptor (Edar), and in the intracellular adapter protein (Edaradd) provoke similar phenotypes as Eda mutations in humans and mice.[42,43] In fact, growing evidence indicates that many ectodermal organs are dependent on the Eda–Edar pathway for their initiation, morphogenesis and/or differentiation. Interestingly, several lines of evidence indicate that *Eda* is regulated by Wnt signaling[39] which, as we saw above, is also involved in stem cell specification. In addition, overexpression of Eda in the ectoderm of transgenic mice affects hair cycling,[44] and *Eda* is expressed in the visceral and definitive endoderm,[41] an important finding in view of the supposed endodermal, not ectodermal, origin of zebrafish teeth.[1,45]

If, as the above studies seem to indicate, similar molecular cascades underlie hair and tooth cycling, they might provide additional support for a role of stem cells in the formation of tooth buds during the process of continuous tooth renewal.

Stem cells and continuously growing teeth

Although the possibility that epithelial stem cells might be involved in repeated tooth renewal has not been raised previously, putative stem cells have been identified in non-replacing teeth in rodents, in particular mouse and rat incisors and rabbit and vole molars.[5–7,46] All these teeth share one important characteristic: they grow continuously, meaning that tooth matrix is produced apically, whilst the tooth permanently wears off distally. Recruitment of cells in the apical part of the tooth is sustained by the presence of a niche of stem cells located in the stellate reticulum of the cervical loop (the most apical part of the enamel organ), where the outer dental epithelium merges into the inner dental epithelium (Fig. 3D). There is circumstantial evidence indicating that cells of the stellate reticulum translocate into the dental epithelium, transiently amplify and undergo their terminal differentiation. The maintenance of these stem cells is under the control of mesenchymal signals, one of which is FGF10.[5,6] Functional evidence for an epithelial stem cell compartment in the cervical loop of continuously growing teeth comes from *Fgf10*-deficient mice.[6] In these newborns, the cervical loop is only a rudiment and no putative stem cells are present. It is interesting to note that, in addition to stimulating cell division, FGF10 may regulate fate determination of epithelial stem cells, by affecting the Notch signaling pathway via stimulation of *lunatic fringe* expression in the cells of the cervical loop.[5] Notch receptors and their ligands have been attributed a role in selecting among the multiple differentiation pathways followed by stem cell descendants in the rat incisor (inner enamel epithelium (ameloblasts), stratum intermedium, stellate reticulum and outer enamel epithelium).[47]

We should point out that the structure of the enamel organ in zebrafish (and in most bony fish) is different from that of mammals in that there is no stellate reticulum or a stratum intermedium, and that inner and outer dental epithelium are directly apposed to each other.[1]

Conclusions and perspectives

Summarizing, we have presented evidence, albeit circumstantial, for the hypothesis that epithelial stem cells underlie the process of continuous tooth renewal in non-mammalian vertebrates, and that the cycling of teeth and hairs is possibly regulated by similar, if not the same, molecular cascades. That similar molecular control mechanisms may underlie non-homologous features, like hairs and teeth, is not unusual, and can be considered an example of so-called generative homoplasy.[48]

The identification of putative epithelial stem cells in tooth replacement in zebrafish or other non-mammalian vertebrates will not be an easy task. As with many stem cells, their study is hampered by the expected rarity of these cells (even when adult animals are considered), and by the assumed lack of positive markers (cf. Ref. 49). Stem cells might also be

interspersed between differentiated cells, as in the intestinal crypts.[50] We now also know that stem cells are not the typical 'undifferentiated' cells that they were classically regarded as, and that, on the contrary, they display quite a repertoire of molecules often held to characterize differentiated stem cells (as it is the case for cytokeratin (K14) in hair follicle stem cells,[51] or other keratins in intestinal stem cells[52]). Certainly, the study of stem cells will have to rely upon appropriate techniques for their isolation and analysis.

Support for the involvement of the Wnt signaling pathway as a putative regulator of stem cell function can be collected by examining tooth replacement in transgenic animals in which reporter constructs are incorporated capable of showing activation of the Wnt signaling pathway. In addition, the introduction of specific hormone-inducible chimeric constructs, of the type described by Vleminckx et al.,[53] allows the endogenous Wnt signaling pathway to be inhibited or ectopically activated in a time- and spatial-specific manner. Such studies are underway and will be highly informative on the possible function of Wnt signaling in tooth renewal.

If no support can be gathered for the hypothesis of stem cell involvement in continuous tooth replacement, other, non-stem cell-based processes will have to be considered. These might include a budding/branching process, similar to what is observed in lung or gland morphogenesis, involving antagonistic actions of paracrine factors, and implying extracellular matrix molecules.[54]

This paper does not address the possible existence of a mesenchymal stem cell compartment. Given the presumed neural crest origin of all vertebrate odontoblasts (reviewed in Ref.1), it seems likely that a pool of mesenchymal stem cells must reside in the proximity of dentigerous bones to secure a lifelong supply of pre-odontoblasts. However, given that at least intramedullary tooth formation in teleosts starts with an extremely long epithelial downgrowth through the bone, the epithelial primacy in tooth formation is likely. If a stem cell compartment is involved in the process of repeated tooth initiation, it is therefore considered most likely to reside in the epithelium.

Although further research will be primarily focused on elucidating the role of putative epithelial stem cells in continuous tooth replacement characteristic of many non-mammalian vertebrates, in particular the zebrafish, it is clear that unraveling of the molecular cascades that control the set-up and maintenance of such stem cells has far broader implications. In particular, it may help us to understand why mammals have lost the capacity of replacing their teeth throughout life, and instead can replace their teeth only once, at the most. Similarly, it might shed light on human syndromes characterized by abnormal tooth numbers, such as hypodontia (missing teeth), or hyperdontia (supernumerary teeth). Significantly, in cases of human hypodontia, it is the permanent teeth that are almost always affected, whereas the first-generation teeth (i.e., the deciduous dentition) are rarely missing.[55] Interestingly, severe hypodontia of permanent teeth was recently shown to be caused by mutations in the Wnt signal inhibitor Axin2.[56] The findings suggest that inhibition of Wnt signaling is required for tooth replacement in humans. Since Wnt signaling regulates the differentiation of stem cells during hair cycling, it is tempting to speculate that similar molecular mechanisms are also involved in tooth replacement. In cleidocranial dysplasia (CCD), a disorder caused by the lack of expression of one allele of the transcription factor *Cbfa1* (now *Runx2*), the dentition is affected with multiple supernumerary teeth. Evidence from longitudinal observations of tooth development in children affected with CCD have indicated that the supernumerary teeth form from the secondary teeth and, in some cases, they form an almost complete third dentition.[57] The supernumerary teeth nevertheless fail to erupt because of a lack of space and the formation of abnormally dense alveolar bone. The *Runx2/Cbfa1* gene apparently acts as a negative regulator of dental lamina outgrowth.[58] Recent evidence indicates that Runx2 functions in mediation of FGF signaling[59] and further analysis of its function can be expected to shed light on the mechanism of tooth replacement.

An answer to the question of what factors drive stem cells to produce a bud and direct its cells into a particular differentiation pathway can have extremely important long-term medical implications. Therapeutic applications involving dental stem cells have so far been suggested for pulp (mesenchymal) stem cells, and, although promising, are limited in that they can generate dentine and pulp tissues only (e.g. Ref. 60; see Refs. 61,62 for recent reviews). At the risk of being preposterous, we can imagine how epithelial stem cell research in the context of repeated tooth renewal may open a window on the possible application of this knowledge to the regeneration of a tooth organ in situ.

References

1. Huysseune A, Sire JY. 1998. Evolution of patterns and processes in teeth and tooth-related tissues in non-mammalian vertebrates. Eur J oral Sci 106:437–481.
2. Peters H, Balling R, 1999. Teeth. Where and how to make them. TIG 15:59–65.
3. Jernvall J, Thesleff I. 2000. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 92:19–29.
4. Pispa J, Thesleff I. 2003. Mechanisms of ectodermal organogenesis. Dev Biol 262:195–205.
5. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. 1999. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 147:105–120.
6. Harada H, Toyono T, Toyoshima K, Yamasaki M, Itoh N, et al. 2002. FGF10 maintains stem cell compartment in developing mouse incisors. Development 129:1533–1541.
7. Tummers M, Thesleff I. 2003. Root or crown: a developmental choice orchestrated by the differential regulation of the epithelial stem cell niche in the tooth of two rodent species. Development 130:1049–1057.
8. Fuchs E, Segre JA. 2000. Stem cells: A new lease on life. Cell 100:143–155.
9. Lajtha LG. 1979. Stem cell concepts. Differentiation 14:23–34.

10. Zieske JD. 1994. Perpetuation of stem cells in the eye. Eye 8:163–169.
11. Fuchs E, Raghavan S. 2002. Getting under the skin of epidermal morphogenesis. Nat Reviews 3:199–209.
12. Daniels JT, Dart JKG, Tuft SJ, Khaw PT. 2001. Corneal stem cells in review. Wound Rep Regen 9:483–494.
13. Potten CS, Booth C, Tudor GL, Booth D, Brady G, et al. 2003. Identification of a putative intestinal stem cell and early lineage marker; Musashi-1. Differentiation 71:28–41.
14. He ZP, Tang YF, Liu YB, Feng MF. 2003. Advances in studies on hepatic stem cells. Prog Nat Sci 13:166–172.
15. Zhang Y, Bai XF, Huang CX. 2003. Hepatic stem cells: existence and origin. World J Gastroenterol 9:201–204.
16. Bonner-Weir S, Sharma A. 2002. Pancreatic stem cells. J Pathol 197: 519–526.
17. Otto WR. 2002. Lung epithelial stem cells. J Pathol 197:527–535.
18. Smith GH. 2002. Mammary cancer and epithelial stem cells: a problem or a solution? Breast Canc Res 4:47–50.
19. Miller JB, Schaefer L, Dominov JA. 1999. Seeking muscle stem cells. Curr Top Dev Biol 43:191–219.
20. Perez-Moreno M, Jamora C, Fuchs E. 2003. Sticky business: Orchestrating cellular signals at adherens junctions. Cell 112:535–548.
21. Loeffler M, Birke A, Winton D, Potten C. 1993. Somatic mutation, monoclonality and stochastic models of stem cell organization in the intestinal crypt. J Theor Biol 160:471–491.
22. Rochat A, Kobayashi K, Barrandon Y. 1994. Location of stem cells of human hair follicles by clonal analysis. Cell 76:1063–1073.
23. DasGupta R, Rhee H, Fuchs E. 2002. A developmental conundrum: a stabilized form of β-catenin lacking the transcriptional activation domain triggers features of hair cell fate in epidermal cells and epidermal cell fate in hair follicle cells. J Cell Biol 158:331–344.
24. Li B, Mackay DR, Dai Q, Li TWH, Nair M, et al. 2002. The LEF1/β-catenin complex activates *movo*1, a mouse homolog of *Drosophila ovo* required for epidermal appendage differentiation. Proc Natl Acad Sci USA 99: 6064–6069.
25. Merrill BJ, Gat U, DasGupta R, Fuchs E. 2001. Tcf3 and Lef1 regulate lineage differentiation of multipotent stem cells in skin. Genes Dev 15:1688–1705.
26. Jamora C, Fuchs E. 2002. Intercellular adhesion, signalling and the cytoskeleton. Nat Cell Biol 4:101–108.
27. Jamora C, DasGupta R, Kocieniewski P, Fuchs E. 2003. Links between signal transduction, transcription and adhesion in epithelial bud development. Nature 422:317–322.
28. Baron M, Aslam H, Flasza M, Fostier M, Higgs JE, et al. 2002. Multiple levels of Notch signal regulation. Mol Membr Biol 19:27–38.
29. Savill NJ, Sherratt JA. 2003. Control of epidermal stem cell clusters by Notch-mediated lateral induction. Dev Biol 258:141–153.
30. Trapani J. 2001. Position of developing replacement teeth in teleosts. Copeia 2001:35–51.
31. Huysseune A, Sire JY. 2004. The role of epithelial remodelling in tooth eruption in larval zebrafish. Cell Tiss Res 315:85–95.
32. Tucker AS, Matthews KL, Sharpe PT. 1998. Transformation of tooth type induced by inhibition of BMP signaling. Science 282:1136–1138.
33. Huysseune A, Aerts P, Verraes W. 1989. Pharyngeal tooth replacement in *Astatotilapia elegans*. Progress in Zoology 35:480–481.
34. Whitear M. 1986. Epidermis. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, editors. Biology of the integument. 2. Vertebrates. Berlin, Heidelberg, New York, Tokyo: Springer Verlag. p 8–38.
35. Barrandon Y. 2003. Developmental biology—A hairy situation. Nature 422:272–273.
36. Babb SG, Barnett J, Doedens AL, Cobb N, Liu Q, et al. 2001. Zebrafish E-cadherin: Expression during early embryogenesis and regulation during brain development. Dev Dynam 221:231–237.
37. Van der heyden C, Huysseune A. 2000. Dynamics of tooth formation and replacement in the zebrafish (*Danio rerio*) (Teleostei, Cyprinidae). Dev Dynam 219:486–496.
38. Thesleff I, Mikkola MJ. 2002. Death receptor signaling giving life to ectodermal organs. Science's STKE (www.stke.org/cgi/content/full/OC_sigtrans;2002/131/pe22), 4pp.
39. Mikkola MJ, Thesleff I. 2003. Ectodysplasin signaling in development. Cytokine & Growth Factor Reviews 14:211–224.
40. Srivastava AK, Pispa J, Hartung AJ, Du Y, Ezer S, et al. 1997. The Tabby phenotype is caused by mutation in a mouse homologue of the EDA gene that reveals novel mouse and human exons and encodes a protein (ectodysplasin-A) with collagenous domains. Proc Natl Acad Sci USA 94:13069–13074.
41. Mikkola ML, Pispa J, Pekkanen M, Paulin L, Nieminen P, et al. 1999. Ectodysplasin, a protein required for epithelial morphogenesis, is a novel TNF homologue and promotes cell-matrix adhesion. Mech Dev 88: 133–146.
42. Headon DJ, Overbeek PA. 1999. Involvement of a novel TNF receptor homologue in hair follicle induction. Nat Genet 22:370–374.
43. Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, et al. 2001. Gene defect in ectodermal dysplasia implicates a death domain adapter in development. Nature 414:913–916.
44. Mustonen T, Pummila M, Kangas A, Mikkola M, Pakkasjärvi L, et al. 2003. Ectodysplasin-A1 stimulates the development of ectodermal organs. Dev Biol 253:123–136.
45. Huysseune A, Van der heyden C, Verreijdt L, Wautier K, Van Damme N. 2002. Fish dentitions as paradigms for odontogenic questions. Conn Tiss Res 43:98–102.
46. Starkey WE. 1963. The migration and renewal of tritium labelled cells in the developing enamel organ of rabbits. Br Dent J 115:143–153.
47. Kawano S, Morotomi T, Toyono T, Nakamura N, Uchida T, et al. 2002. Establishment of dental epithelial cell line (HAT-7) and the cell differentiation dependent on Notch signaling pathway. Conn Tiss Res 43:409–412.
48. Wake DB. 2003. Homology and Homoplasy. In: Hall BK, Olsen WM, editors. Keywords & Concepts in Evolutionary Developmental Biology. Cambridge: Harvard University Press. p 191–201.
49. Gonzales-Reyes A. 2003. Stem cells, niches and cadherins: a view from *Drosophila*. J Cell Sci 116:949–954.
50. Stappenbeck TS, Mills JC, Gordon JI. 2003. Molecular features of adult mouse small intestinal epithelial progenitors. Proc Natl Acad Sci USA 100:1004–1009.
51. Coulombe PA, Kopan R, Fuchs E. 1989. Expression of keratin K14 in the epidermis and hair follicle: insights into complex programs of differentiation. J Cell Biol 109:2295–2312.
52. Potten CS, Booth C, Pritchard DM. 1997. The intestinal epithelial stem cell: the mucosal governor. Int J Exp Pathol 78:219–243.
53. Vleminckx K, Deroo T, Denayer T, Van Roy F. 2001. A transgenic approach for studying Wnt signaling in *Xenopus* development. Mol Biol Cell 12 Suppl S:1315.
54. Hogan BLM. 1999. Morphogenesis. Cell 96:225–233.
55. McCollum M, Sharpe PT. 2001. Evolution and development of teeth. J Anat 199:153–159.
56. Lammi L, Arte S, Somer M, Järvinen H, Lahermo P, et al. 2004. Mutations in *AXIN2* cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet 74 (in press).
57. Jensen BL, Kreiborg S. 1990. Development of the dentition in cleidocranial dysplasia. J Oral Pathol Med 19:89–93.
58. D'Souza RN, Åberg T, Gaikwad J, Cavender A, Owen M, et al. 1999. *Cbfa1* is required for epithelial-mesenchymal interactions regulating tooth development in mice. Development 126:2911–2920.
59. Åberg T, Wang X, Kim JH, Yamashiro T, Bei M, et al. Runx2 mediates FGF signalling from epithelium to mesenchyme during tooth morphogenesis. Dev Biol (in press).
60. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, et al. 2002. Stem cell properties of human dental pulp stem cells. J Dent Res 81: 531–535.
61. Chai Y, Slavkin HC. 2003. Prospects for tooth regeneration in the 21st century: A perspective. Microsc Res Techniq 60:469–479.
62. Thesleff I, Tummers M. 2003. Possibilities to improve implants and regenerate dento-alveolar tissues by tissue engineering using stem cells and growth factors. In: Ellingsen JE, Lyngstadaas SP, editors. Bio-Implant Interface. Improving biomaterials and tissue reactions. Boca Raton: CRC Press. p 205–217.
63. Laurenti P, Thaeron-Antono C, Allizard F, Huysseune A, Sire JY. 2004. The cellular expression of *eve1* suggests its requirement for the differentiation of the ameloblasts, and for the initiation and morphogenesis of the first tooth in the zebrafish (*Danio rerio*). Dev Dyn (in press).