

Formation of a successional dental lamina in the zebrafish (*Danio rerio*): support for a local control of replacement tooth initiation

ANN HUYSEUNE*

Biology Department, Ghent University, Belgium

ABSTRACT In order to test whether the formation of a replacement tooth bud in a continuously replacing dentition is linked to the functional state of the tooth predecessor, I examined the timing of development of replacement teeth with respect to their functional predecessors in the pharyngeal dentition of the zebrafish. Observations based on serial semithin sections of ten specimens, ranging in age from four week old juveniles to adults, indicate that (i) a replacement tooth germ develops at the distal end of an epithelial structure, called the successional dental lamina, budding off from the crypt epithelium surrounding the erupted part of a functional tooth; (ii) there appears to be a developmental link between the eruption of a tooth and the formation of a successional dental lamina and (iii) there can be a time difference between successional lamina formation and initiation of the new tooth germ, i.e., the successional dental lamina can remain quiescent for some time. The data suggest that the formation of a successional lamina and the differentiation of a replacement tooth germ from this lamina, are two distinct phases of a process and possibly under a different control. The strong spatio-temporal coincidence of eruption of a tooth and development of a successional dental lamina is seen as evidence for a local control over tooth replacement.

KEY WORDS: *tooth replacement, epithelial stem cells, zebrafish, Danio rerio*

Introduction

Most tooth-bearing vertebrates (the notable exception being mammals) replace their teeth throughout life. In teleost fish, patterns of adjacent or alternate tooth initiation, set up by the first-generation teeth, can easily turn into regular replacement patterns where new teeth are initiated simultaneously every second, or even every third position. We have suggested that, once established, the replacement pattern appears to be maintained, as a kind of "default" state (Huyseune and Witten, 2006). Variations and modifications in this pattern are nevertheless common and suggest that tooth replacement is under local control. A regular, albeit variable pattern of tooth replacement is also present in the zebrafish (Van der heyden *et al.*, 2001; Huyseune and Witten, 2006), a model which is increasingly used for studies on tooth patterning and replacement. In zebrafish, the teeth are nevertheless restricted to the pharyngeal region (cf. Huyseune *et al.*, 1998), which represents a derived state compared to basal osteichthyans. The plesiomorphic condition for osteichthyans is one where teeth are distributed throughout the

oral and pharyngeal cavity; more derived taxa have their dentition restricted to marginal, palatal and pharyngeal teeth. The cypriniforms, to which the zebrafish belongs, represent an even further derived state, where oral teeth have been lost as well, leaving only pharyngeal teeth.

Earlier, we have suggested that the formation of a replacement tooth in zebrafish could be triggered when a fully differentiated tooth erupts, thereby activating putative stem cells (Huyseune and Thesleff, 2004). If this link between eruption and production of a new tooth bud in the zebrafish is valid, then all erupted teeth should be associated with a tooth germ that is at least in an initiation phase. Along the same line of thought, one can speculate that there is a correlation between the state of differentiation of the replacement tooth germ and the state of functionality (i.e., an estimation of the time interval the tooth has been functional) of the erupted predecessor.

An earlier study on tooth replacement in juvenile and adult

Abbreviations used in this paper: ab, attachment bone; dl, dental lamina; ft, functional tooth; rt, replacement tooth; tb, tooth base.

*Address correspondence to: Prof. Dr. Ann Huyseune. Biology Department, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium.
Fax +32-9-264-5344. e-mail: Ann.Huyseune@UGent.be

zebrafish (Van der heyden *et al.*, 2001) revealed that not all functional teeth were associated with a replacement tooth. However, these observations were made on cleared and stained material, which reveals tooth germs only from a moment at which mineralisation has started, i.e. in an advanced state of differentiation (in the above study, also unstained enameloid caps were considered). Germs in a stage of initiation, morphogenesis, or early cytodifferentiation can only be traced on histological sections.

In the present study, I used serial semithin sections through the dentition of zebrafish ranging in age from four week old juveniles to adults (9 to 32 mm SL) to examine (1) whether every erupted tooth is associated with a replacement tooth germ and (2) whether there is a correlation between the state of functionality of a tooth and of its successor.

Results

Histological characteristics of the different categories of functional and replacement teeth

Erupted teeth could be assigned to one of the following distinctive categories: attaching, young, mature, old, or in resorption (Fig. 1). Teeth in the process of attachment have a very cellular pulp cavity, tall, columnar and polarized odontoblasts in the tip of the pulp cavity, somewhat plumper, but active odontoblasts along the walls of the pulp cavity and polarized and active osteoblasts at the level of the attachment bone, which is still thin (Fig. 2A). Despite the little amount of attachment bone matrix, such teeth are erupted, i.e., have their tip exposed in the pharyngeal cavity. In young functional teeth, tall, polarized odontoblasts are still present in the tip of the pulp cavity. The latter is still quite cellular but less than in teeth in the process of attachment. Mature functional teeth have a much more empty-looking pulp cavity, filled with a loose network of stellate cells. At the tip of the pulp cavity, polarized cells nevertheless subsist (Fig. 2B). In old functional teeth, the pulp cavity is lined by flattened cells, if any. The pulp cavity gives a very loose and quite empty appearance. Often, the dentine

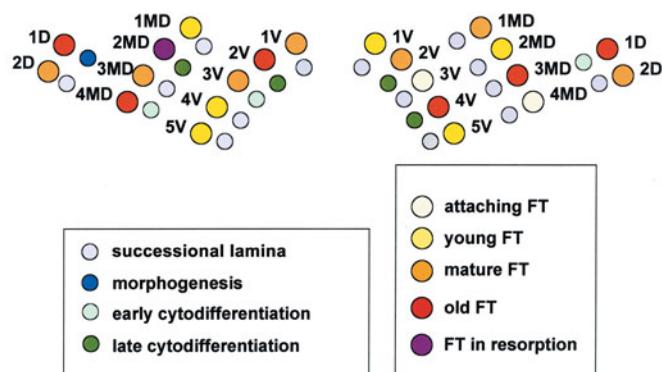


Fig. 1. Schematic representation of the pharyngeal dentition of a 11 mm SL zebrafish. The dentition was scored for the state of functionality of the erupted teeth (FT) (shades of yellow to red) and state of differentiation of the replacement teeth (grey, blue and shades of green). 1V-5V: 5 loci of the ventral (V) tooth row; 1MD-4MD: 4 loci of the mediadorsal (MD) tooth row; 1D-2D: 2 loci of the dorsal (D) tooth row.

TABLE 1

RELATIONSHIP BETWEEN THE STATE OF FUNCTIONALITY OF THE ERUPTED TEETH (FT) AND STATE OF DIFFERENTIATION OF THE REPLACEMENT TEETH (RT)

Functionality of erupted tooth	Stage of associated RT	V	MD	D	Total	Percentage
attaching FT	SL	8	7	2	17	100.0
attaching FT	M	0	0	0	0	0.0
attaching FT	ED	0	0	0	0	0.0
attaching FT	LD	0	0	0	0	0.0
young FT	SL	17	11	2	30	66.7
young FT	M	6	1	0	7	15.6
young FT	ED	4	0	1	5	11.1
young FT	LD	2	0	0	2	4.4
mature FT	SL	5	9	3	17	28.3
mature FT	M	2	1	3	6	10.0
mature FT	ED	10	2	2	14	23.3
mature FT	LD	15	5	3	23	38.3
old FT	SL	0	11	2	13	28.9
old FT	M	2	0	3	5	11.1
old FT	ED	1	4	6	11	24.4
old FT	LD	7	5	4	16	35.6
FT in resorption	SL	0	0	0	0	0.0
FT in resorption	M	0	1	0	1	7.7
FT in resorption	ED	0	0	0	0	0.0
FT in resorption	LD	4	5	3	12	92.3
FT absent	SL	0	0	0	0	0.0
FT absent	M	0	0	0	0	0.0
FT absent	ED	1	1	0	2	7.7
FT absent	LD	8	12	4	24	92.3
Total		92	75	38	205	

Abbreviations: D, dorsal tooth row; ED, early cytodifferentiation; FT, functional tooth; LD, late cytodifferentiation; M, morphogenesis; MD, mediadorsal tooth row; RT, replacement tooth; SL, successional lamina; V, ventral row tooth.

shows a metachromatic line, indicative of a secondary deposition (cf. Huysseune *et al.*, 1998). Finally, in the most advanced category, obvious signs of resorption are visible: an irregular surface of the matrix, with resorption lacunae and the distinct presence of osteoclasts (Fig. 2C) (cf. Witten *et al.*, 2001).

Replacement teeth were assigned to one of the four following stages: successional lamina stage (Fig. 2 D,E), morphogenesis (Fig. 2F), early cytodifferentiation, late cytodifferentiation. Detailed observations revealed that tooth germs develop from an epithelial structure in the shape of an invagination stretching over approximately 60 μm in antero-posterior direction and penetrating, in average, around 45 μm into the underlying mesenchyme. This epithelial structure precedes all following stages and was called the successional dental lamina (or, briefly, the successional lamina). This epithelial structure buds off from the epithelial fold that surrounds the erupted part of a functional tooth (called crypt, cf. Huysseune and Sire, 2004), close to the crypt base and at the posterior side of the erupted tooth (Figs. 2D,E, 3). Thus, on transverse sections, given that functional teeth point backwards, this epithelial structure can only be observed together with the tip of the predecessor. During morphogenesis stage the distal end of the successional lamina invaginates and forms a two-layered, bell-shaped enamel organ (Fig. 2F); during early cytodifferentiation the ameloblasts and odontoblasts differentiate and during late cytodifferentiation enameloid and dentine are deposited (cf. Laurenti *et al.*, 2004). However, given the considerable size of the replacement teeth and the progressive differentiation from tip to base of the tooth, I included teeth with a small amount of matrix (enameloid) into the early differentiation stage as defined by

Laurenti *et al.* (2004) (who worked mainly on first-generation teeth).

Relationship between state of functionality of the functional teeth and state of differentiation of the replacement teeth

All functional teeth appeared to be associated either with a successional lamina, or with a replacement tooth developing from it (Table 1, Fig. 4). A successional lamina was not present when teeth were not erupted.

Erupted functional teeth that were still in the process of ankylosis (i.e., attaching functional teeth) were always associated with a successional lamina as such (i.e., a tooth bud was not yet forming at its distal end). Thus, of the 17 loci with attaching teeth (whether from the ventral, mediadorsal or dorsal row), all (100%) showed the presence of a successional lamina (Table 1, Fig. 4).

Despite the fact that, percentage-wise, the number of young replacement teeth decreased and the number of more advanced replacement teeth increased with ever more mature functional teeth (Fig. 4) there was no 1:1 correspondence between the state of functionality of the functional tooth and the state of development of its successor.

Thus, well attached but still young functional teeth appeared to be associated almost exclusively with replacement teeth at an early stage of development: with a successional lamina in 2/3 of the cases and with morphogenesis or early cytodifferentiation stage in another approximately 25%. In two cases (repre-

senting 4.4%) the replacement tooth was well advanced with a considerable amount of matrix deposited (late cytodifferentiation stage).

In contrast, older functional teeth (both the categories 'old' and 'in resorption') were not necessarily associated with replacement teeth in an advanced stage of development. E.g., in 13 cases, old functional teeth were associated with a successional lamina only; one functional tooth in resorption was associated with a morphogenesis stage successor.

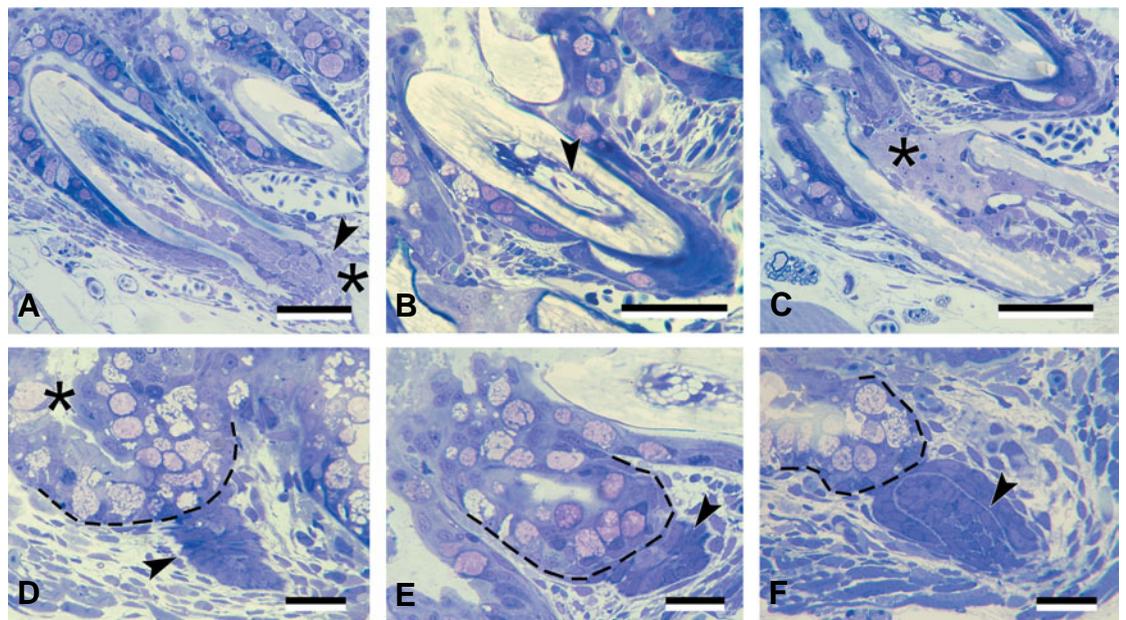
Finally, where the functional tooth was absent, the locus housed a replacement tooth either in early (2 cases, 7.7%) or, mostly, in late cytodifferentiation stage (24 cases, 92.3%) (Table 1, Fig. 4).

When the data on the three smallest and the three largest specimens were pooled separately and compared, old functional teeth appeared to be associated with a successional lamina only more often in larger specimens than in smaller specimens (38.9% versus 8.3%, compare with the average of 28.9% for all specimens together).

Discussion

In contrast to the data gathered from cleared and stained material (Van der heyden *et al.*, 2001), it is clear that every functional tooth in the zebrafish is associated *ab initio* with an epithelial downgrowth, which I have called a successional dental lamina (or, briefly, a successional lamina), the distal end of which will subsequently develop into a replacement tooth

Fig. 2. One μm thick sections through the pharyngeal dentition of a zebrafish of 13 mm SL. Functional teeth are shown in (A-C), their associated successional lamina or replacement tooth developing from it in (D-F). In (D-F) a dashed line indicates the boundary between the crypt epithelium and the successional lamina (D,E, arrowheads) or the developing replacement tooth bud (see arrowhead in (F)). Note that in the latter, the dental lamina has been taken up into the expanding enamel organ of the tooth bud. (A) Tooth in position 4V in a phase of attachment (arrowhead) to the dentigerous bone (asterisk). Tall, polarized odontoblasts are found in the tip of the pulp cavity, more plump cells line the dentine surface. The tooth tip pierces the epithelium at another level of sectioning. Scale bar, 50 μm . (B) Mature functional tooth in position 3MD. Note the empty-looking pulp cavity and flattened cells lining the dentine (arrowhead). Scale bar, 50 μm . (C) Functional tooth in resorption (position 4MD). Part of the tooth wall is broken away. The pulp cavity is largely filled with a multinucleated osteoclast (asterisk). Scale bar, 50 μm . (D) Successional dental lamina (arrowhead) associated with the attaching functional tooth shown in (A). An asterisk marks the crypt surrounding the exposed part of the functional predecessor. Given that functional teeth in the animal point backwards and that the successional lamina buds off from the crypt surrounding the tip, only the tip of the predecessor can sometimes be observed at the same cross-sectional level as the successional lamina (not in D-F). Scale bar, 20 μm . (E) Successional lamina (arrowhead) associated with the mature functional tooth shown in (B). Scale bar, 20 μm . (F) Replacement tooth germ, stage of morphogenesis (arrowhead), associated with the functional tooth in resorption shown in (C). Scale bar, 20 μm .



(Fig. 3). I also show that there can be a time difference between formation of the successional lamina and initiation of the tooth bud itself. Once the tooth bud starts to differentiate, the successional lamina is soon taken up into the enamel organ of the growing bud. This, together with the small size of the successional lamina and the fact that its tip usually goes on differentiating immediately into a tooth bud, is likely to make the successional lamina easily overlooked. The successional lamina was previously included in the early phases of morphogenesis by Van der heyden *et al.* (2000) because its possible temporary persistence as such remained unnoticed at the time. The successional lamina bears considerable resemblance to a dental lamina as described by Reif (1982) for actinopterygians: the successional lamina is discontinuous (i.e., there is a separate downgrowth for each tooth family) and it is also non-permanent (i.e., it is formed anew for every replacement tooth). Importantly, the enamel organ of the first-generation teeth develops directly from the oral or pharyngeal epithelium, without the involvement of a lamina, not only in zebrafish (Huysseune *et al.*, 1998; Laurenti *et al.*, 2004), but also, e.g. in trout (Fraser *et al.*, 2004) and in cichlids (Huysseune and Sire, 1997) and this may be a general feature for teleosts and non-mammalian tetrapods (cf. Huysseune and Sire, 1998; Sire *et al.*, 2002).

Although the data suggest that together with an increase of functional life time of a predecessor, the developmental stage of its successor is, on average, ever more advanced, there is no strict 1:1 correspondence between the state of functionality of a tooth and the developmental stage of its successor. Such would only be the case if one would find that, e.g., all young functional teeth are associated with morphogenesis stage replacement teeth, all old functional teeth with late cytodifferentiation stage replacement teeth, etc. Rather, the data presented here reveal two important new findings regarding the mechanisms that control replacement tooth formation in zebrafish: (i)

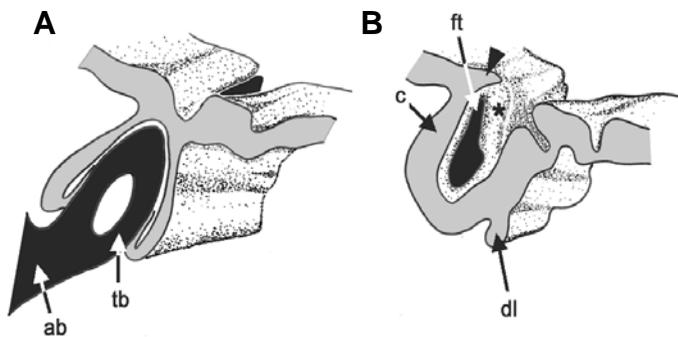


Fig. 3. Schematic representation of an erupted functional tooth and its associated successional dental lamina. Schematic representation, based on a 3D-reconstruction of tooth 1V in a zebrafish of 11 mm SL, of the successional dental lamina (dl) and its relationship to the crypt (c) surrounding the tip of the erupted functional tooth 1V (ft). The reconstruction is artificially divided in two parts to show two levels of sectioning of tooth 1V: anteriorly (A), where the tooth base (tb) is continuous with the attachment bone (ab) and posteriorly (B), where only the tooth tip (ft) is left. Note that the tooth tip, despite lying freely in the crypt, is still hidden by a fold of the epithelial surface (arrowhead). The epithelial crypt closes behind this tooth tip (asterisk). Grey, sectioned epithelium; black, tooth 1V.

there appears to be a developmental link between the eruption of a tooth and the development of a successional dental lamina (see below for a definition of tooth eruption in zebrafish) and (ii) the successional dental lamina can persist for a while as such, without giving rise at its distal end to a replacement tooth bud and this appears to be more often the case in larger (older) compared to smaller (younger) animals.

(i) The developmental link between tooth eruption and replacement tooth formation was already noticed in organotypic cultures: first-generation teeth which develop up to attachment stage fail to erupt and do not produce a replacement tooth germ (Van der heyden *et al.*, 2005). Eruption of a tooth in zebrafish is not the result of tooth movement or of a final growth spurt of the tooth, but results from epithelial remodeling around the tip of the tooth (Huysseune and Sire, 2004). Through detachment of the epithelial cells covering the tooth tip and retraction of the epithelial layers, the tooth tip becomes exposed. This retraction displaces the epithelium, widens the crypt surrounding the exposed tooth tip and likely brings the epithelium into contact with a new mesenchymal environment. Possibly, such a newly established contact could activate the epithelium and lead to the production of a new downgrowth, irrespective of whether adult epithelial stem cells are involved (cf. Huysseune and Thesleff, 2004), progenitor cells of some sort, or even dedifferentiated epithelial cells. In this scenario, the developmental link between eruption and the formation of a successional lamina, as the anlage of the replacement tooth, would be a causal one. Alternatively, the two processes (eruption and formation of the successional lamina) may be two independent outcomes of the same (molecular) event. The search for candidate genes potentially involved in such an event can benefit from a comparison with hair formation. Despite being non-homologous features, hairs and teeth show many similarities in terms of being cycling structures, developing as a result of epithelial-mesenchymal interactions, etc. They can be considered an example of so-called generative homoplasia (Wake, 2003). Hairs and teeth are at least partly controlled by similar molecular cascades and many mutations affecting hairs also affect teeth (cf. Huysseune and Thesleff, 2004). In hairs, downregulation of E-cadherin through activation of Wnt signaling reduces cell adhesion in the stem cell niche, required to rearrange the epithelial cells to make a new bud (Barrandon, 2003; Jamora *et al.*, 2003). Earlier, we have suggested that E-cadherin could also be involved in the remodeling of the epithelial layers covering the tooth tip prior to eruption (that is, inner and outer dental epithelium, possibly also a superficial pharyngeal epithelial cell layer), leading to cell detachment and exposure of the tooth tip (Huysseune and Sire, 2004; Huysseune and Thesleff, 2004). If E-cadherin is at the same time downregulated in other parts of the enamel organ, notably the area of the outer dental epithelium from which the successional lamina will develop, this could lead to a loosening of cell contacts enabling the formation of a bud, similar to what has been suggested for hairs (Barrandon, 2003; Jamora *et al.*, 2003). In this scenario, eruption and formation of the successional lamina would be independent outcomes of the same molecular event (such as downregulation of E-cadherin).

At this point, it is relevant to compare the situation in zebrafish with other vertebrates which also show continuous

replacement. Both in amphibians (Wistuba *et al.*, 2002; Davit-Béal *et al.*, pers. comm.) and in lizards (Edmund, 1969; Sire *et al.*, 2002; Delgado *et al.*, 2005) the successional lamina forms well before the predecessor has erupted. This makes a causal link between eruption and successional lamina formation unlikely but it does not preclude that successional lamina formation in zebrafish and tetrapods could be under a similar control. Like in zebrafish the successional lamina in amphibians and lizards is non-permanent (i.e. it disappears when the replacement tooth erupts) and discontinuous (i.e. there is a separate downgrowth for each replacement tooth). However, unlike in zebrafish, the successional lamina is quite long and therefore remains distinctive over a longer period of replacement tooth formation, before it is finally taken up into the growing enamel organ of the replacement tooth germ. The difference therefore seems to be quantitative, rather than qualitative. Several findings nevertheless caution for a careless extrapolation of zebrafish data to crownward osteichthyans (like tetrapods). Indeed, earlier studies on the molecular control of tooth formation in zebrafish have already revealed fundamental differences with that in mice (e.g. Laurenti *et al.*, 2004; Jackman *et al.*, 2004; Borday-Birraux *et al.*, 2006).

(ii) Assuming that the state of functionality of a tooth (whether young, mature, old, or in resorption) reflects the time the tooth has been functional, the frequent presence of successional dental laminae associated with old functional teeth, with no indication of differentiation of their distal end into a tooth germ, suggests that the successional lamina can remain quiescent for some time. Some trigger, other than the state of the predecessor, appears to be responsible for starting the further development (i.e. the true initiation) of the replacement tooth in the zebrafish. In teleosts which display intramedullary (intraosseous) replacement (cf. Trapani, 2001), an often very long epithelial downgrowth develops from the crypt epithelium and penetrates the bone, before its distal end finally differentiates into a tooth germ within the medullary cavity of the tooth-bearing bone. This too suggests that a separate mechanism triggers the start of further differentiation of the tooth germ. The nature of this trigger is of high interest, given some remarkable parallels to the human dentition. In humans, a successional lamina appears on the lingual side of each enamel organ. In deciduous teeth, these lingual downgrowths give rise to the tooth germs of the permanent successors (incisors, canines, premolars) (Mjör and Fejerskov, 1979; Melfi, 1982; Bhaskar, 1990). Remarkably, such downgrowths are also said to be produced from the enamel organs of permanent teeth, but reported to eventually disappear (Berkovitz *et al.*, 1992). Thus, like in zebrafish, there seems to be an uncoupling between the development of a successional dental lamina and the eventual proliferation of the cells at its distal end to give rise to a tooth germ. Moreover, in humans, the three permanent molars - which do not have deciduous predecessors - arise from a distal extension of the primary dental lamina in a period spread over several years. Mjör and Fejerskov (1979) correctly highlighted this discrepancy between the formation of the dental lamina and the formation of teeth developing from it, by stating "Although the overall activity of the dental lamina thus covers a considerable period of time (4-5 years) any particular portion of it only functions for a very short period before differentiation and

desintegration." Recently, Wang *et al.* (2005) suggested that the transcription factor Runx2 might inhibit secondary tooth formation in mice by preventing the formation of an extension of the dental lamina for successional teeth. Humans affected from cleidocranial dysplasia (a disorder caused by the lack of expression of one allele of *Runx2*) have supernumerary teeth, that develop from secondary teeth and sometimes form an almost complete third dentition (Jensen and Kreiborg, 1990).

The intimate relationship which appears to exist between eruption of a tooth and formation of the successional lamina strongly suggests that there is a local control over replacement tooth formation, rather than a general type of control that orchestrates the formation of replacement teeth in the entire dentition. Such a general type of control, under the form of field substances, or morphogens, travelling over the jaw and eliciting replacement tooth formation in successive loci, has formerly been suggested to underlie the orderly and predictable

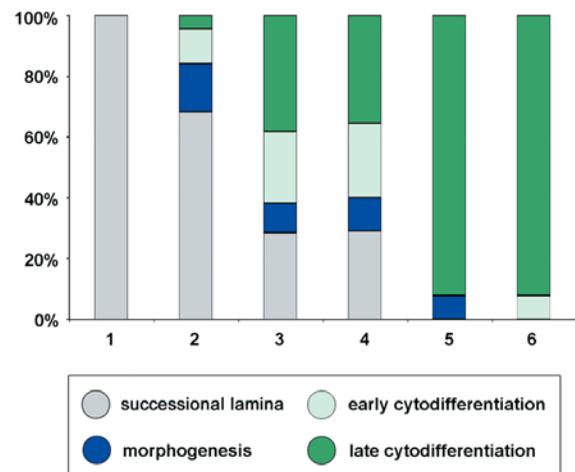


Fig. 4. Graphic representation of relationship between state of functionality of functional teeth and state of differentiation of their successor. Percentage of functional teeth (1, attaching functional teeth (FT); 2, young FT; 3, mature FT; 4, old FT; 5, FT in resorption) associated with specific stages of development of the replacement teeth; 6, replacement teeth with predecessor absent. The data concern all 205 tooth positions examined.

patterns of tooth replacement often observed in non-mammalian vertebrates (see reviews by Berkovitz, 2000; Smith, 2003; Huysseune and Witten, 2006). Van der heyden *et al.* (2001) examined the order of replacement in the dentition of juvenile and adult zebrafish, based on around 90 cleared and stained specimens. Although one pattern emerged as the most common, these authors also showed the existence of considerable variation in this formula (up to 30% of the jaws examined). If replacement were to be under the control of some general field effect, one would not expect such a variation in the order of replacement.

Taken together, the detailed observations on serially sectioned zebrafish pharyngeal dentitions presented here suggest that there are two phases in the process of securing the formation of a replacement tooth, that are likely acting independently: the development of a successional lamina, linked to the

eruption of the predecessor and the start of morphogenesis and differentiation of the tooth germ proper, which can, but not necessarily does, immediately follow the formation of the successional lamina. Epithelial stem cells, as a local control mechanism, could be involved in the first of these processes, but others need to be considered as well: a pool of pluripotent progenitor cells, dedifferentiation of epithelial cells, etc. The factor(s) triggering the further differentiation of the distal end of the successional lamina into a tooth germ remain to be elucidated. In particular, the question needs to be addressed whether the molecular cascade that controls this further differentiation is similar to that controlling initiation of first-generation teeth (cf. Laurenti *et al.*, 2004; Jackman *et al.*, 2004; Borday-Birraux *et al.*, 2006) and to what extent principles of budding morphogenesis, involving interactions between activator and inhibitor substances, are applicable (cf. Peterková *et al.*, 2000, 2002).

Materials and Methods

Zebrafish of 9, 11, 11.5, 13, 15, 16.5, 20, 25 and 32 mm SL (a total of ten specimens) were anaesthetized using an overdose of MS222, fixed in a paraformaldehyde-glutaraldehyde solution, dehydrated and embedded in epon according to procedures described previously (cf. Huysseune and Sire, 1992). Serial, semithin (1 or 2 μm) transverse sections were prepared of the head and stained with toluidine blue.

First, the position (locus) of every functional tooth was identified by screening all sections in the pharyngeal region (i.e., positions 1V to 5V in the ventral tooth row, positions 1MD to 4MD in the mediodorsal row and 1D to 2D in the dorsal row, cf. Van der heyden and Huysseune, 2000). Next, for every position, the functional tooth and its successor were scored. Using histological characteristics, functional teeth (i.e., erupted and attached to the underlying bone) were assigned to one of the following categories: attaching, young, mature, old, or in resorption. Replacement teeth were assigned to one of the following stages: successional lamina stage, morphogenesis, early cytodifferentiation, late cytodifferentiation, the latter three according to features described previously (Laurenti *et al.*, 2004) (for a definition of the successional lamina stage, see the results section) (Fig. 1). Since Van der heyden *et al.* (2001) showed that there is no relationship between the state of differentiation/replacement of the right and the left halve of the dentition, both sides of each animal were scored separately. Thus, a total of 205 loci was scored, i.e. 15 less than the expected 220 – 11 loci on each jaw – because of loss of some sections, or absence of functional teeth in some loci.

Finally, all observations for the three tooth rows were pooled and for each category of functional teeth percentages were calculated of the different stages of tooth germs associated with these teeth.

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