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The SCPP gene repertoire in bony vertebrates and graded differences in mineralized tissues

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Abstract

The vertebrate tooth is covered with enamel in most sarcopterygians or enameloid in chondrichthyans and actinopterygians. The evolutionary relationship among these two tissues, the hardest tissue in the body, and other mineralized tissues has long been controversial. We have recently reported that specific combinations of secretory calcium-binding phosphoprotein (SCPP) genes are involved in the mineralization of bone, dentin, enameloid, and enamel. Thus, the early repertoire of SCPP genes would elucidate the evolutionary relationship across these tissues. However, the diversity of SCPP genes in teleosts and tetrapods and the roles of these genes in distinct tissues have remained unclear, mainly because many SCPP genes are lineage-specific. In this study, I show that the repertoire of SCPP genes in the zebrafish, frog, and humans includes many lineage-specific genes and some widely conserved genes that originated in stem osteichthyans or earlier. Expression analysis demonstrates that some frog and zebrafish SCPP genes are used primarily in bone, but also in dentin, while the reverse is true of other genes, similar to some mammalian SCPP genes. Dentin and enameloid initially use shared genes in the matrix, but enameloid is subsequently hypermineralized. Notably, enameloid and enamel use an orthologous SCPP gene in the hyper-mineralization process. Thus, the hypermineralization machinery ancestral to both enameloid and enamel arose before the actinopterygian–sarcopterygian divergence. However, enamel employs specialized SCPPs as structuring proteins, not used in enameloid, reflecting the divergence of enamel from enameloid. These results show graded differences in mineralized dental tissues and reinforce the hypothesis that bone–dentin–enameloid–enamel constitutes an evolutionary continuum.

Keywords

Vertebrate evolution; Biomineralization; Amelogenesis; Odontogenesis; Extracellular matrix protein

Introduction

Vertebrate teeth consist of three primary tissues: basal bone (attachment bone or alveolar bone), body dentin, and a surface tissue. In most sarcopterygians, the surface tissue is enamel, whereas that of chondrichthyans, actinopterygians, and larval urodele is called enameloid (Poole 1967). Enamel and enameloid are both highly mineralized, wear-resistant

tissues and thus thought to be functionally equivalent. These mineralized dental tissues form on extracellular matrix (ECM) proteins, deposited by cells that develop through epithelial–mesenchymal interactions (Nanci 2003; Hall 2005). During mammalian tooth development, predentin is initially deposited by odontoblasts of mesenchymal origin (Linde and Goldberg 1993). Then, ameloblasts, differentiated from inner dental epithelial (IDE) cells, deposit mineralizing enamel matrix consisting of specialized enamel-structuring proteins (Fincham et al. 1999; Paine and Snead 2005; Bartlett et al. 2006). These proteins are subsequently degraded by proteinases secreted from ameloblasts and removed from maturing enamel (Simmer and Hu 2002). This process facilitates subsequent growth of enamel crystallites; enamel thus grows into a hypermineralized tissue (Simmer and Fincham 1995; Smith 1998).

In contrast, the initial ECM proteins secreted during teleost tooth development are pre-enameloid, instead of predentin (Sasagawa 1997; Van der heyden et al. 2000). Pre-enameloid primarily consists of type I collagen (COL1), similar to the ECM of bone and dentin, and is deposited by both mesenchyme-derived cells (odontoblasts) and the IDE cells. After pre-enameloid begins mineralization, the IDE cells continue to secrete proteins into enameloid for a short period of time, while odontoblasts deposit ECM proteins for dentin (Shellis and Miles 1974). Enameloid is thus different from the enamel in ECM proteins and early mineralization processes. Yet, enameloid ECM proteins are also degraded by proteinases secreted from the IDE cells, and enameloid matures into a hyper-mineralized tissue, similar to enamel (Kawasaki et al. 1987). Hence, it has been thought that enamel was derived from enameloid through a shift in the timing of ECM protein secretion (Poole 1971; Shellis and Miles 1974). This view has been, however, challenged by the analysis of tissue distribution in the fossil record; that is, enamel is primitive and enameloid is derived (Smith 1992; Smith 1995). Thus, there has been controversy over the evolutionary relationship among enamel, enameloid, and other mineralized tissues (Kawasaki and Weiss 2008).

Many genes involved in dental tissue mineralization comprise the secretory calcium-binding phosphoprotein (SCPP) gene family (Kawasaki and Weiss 2003; Kawasaki and Weiss 2006; Kawasaki and Weiss 2007). The SCPP genes arose by gene duplication initially from the common ancestor, the secreted protein, acidic, cysteine-rich like 1 (*SPARCL1*) gene (Kawasaki et al. 2004). The SCPPs are classified into two subclasses: acidic SCPPs containing >25% of Glu, Asp, and phospho-Ser residues and Pro/Gln (P/Q)-rich SCPPs consisting of >20% of Pro and Gln. In mammals, acidic SCPPs are primarily involved in bone and dentin mineralization, whereas P/Q-rich SCPPs enable enamel mineralization (Kawasaki and Weiss 2008). In fugu, on the other hand, both acidic and P/Q-rich SCPP genes are used in dentin, and many distinct P/Q-rich SCPP genes are expressed in enameloid (Kawasaki et al. 2005). However, the diversity of SCPP genes in tetrapods and teleosts and their roles in distinct mineralized tissues have remained unclear. This is mainly because many SCPP genes are lineage-specific and also because only limited species and developmental stages have been studied intensively.

In this study, I report the repertoire of SCPP genes in the frog and zebrafish and investigate their spatiotemporal expression domains. The results reveal genetic similarities between bone and dentin, dentin and enameloid, and enameloid and enamel. In particular, enameloid and enamel both use the odontogenic, ameloblast associate (*ODAM*) gene for tissue hypermineralization.

Materials and methods

Cloning of SCPP genes by RT-PCR

Mouse, frog (*Xenopus tropicalis*), and zebrafish SCPP genes were sought from their genome sequence. I designed polymerase chain reaction (PCR) primers for the genomic regions

predicted to code SCPP genes and used these primers for screening these genes by reverse transcription PCR (RT-PCR) as previously described (Kawasaki et al. 2005). RNA molecules were purified from the jaws of a 4-day neonate mouse, the upper and lower jaws of an adult frog, and from the pharyngeal jaw of an adult zebrafish using the RNeasy mini kit (Qiagen).

For the mouse SCPP-Pro/Gln-rich 1 (*SCPP-PQ1*) gene, the following PCR primers (primer sequences are all shown in supplementary data) were used for rapid amplification of cDNA ends (RACE): SCPPPQ1dwn1, SCPPPQ1dwn2, and SCPPPQ1up2 (nested PCR primers were not required for all genes).

For frog SCPP genes, amelogenin (*AMEL*), ameloblastin (*AMBN*), enamelin (*ENAM*), and *ODAM* were cloned from the tooth-bearing upper jaw (with teeth at different developmental stages) and dentin matrix acidic phosphoprotein 1 (*DMP1*) and integrin-binding sialoprotein (*IBSP*) from the toothless lower jaw. The following PCR primers were used for RACE: for the SCPP acidic 1 gene (*SCPPA1*), XSCPP1dwn1, XSCPP1dwn2, XSCPP1up2, and XSCPP1up3; for *DMP1*, XDMP1dwn2, XDMP1dwn4, XDMP1up2, and XDMP1up5; for the SCPP acidic 2 gene (*SCPPA2*), XSCPP2dwn1, XSCPP2dwn2, and XSCPP2up1; for *IBSP*, XIBSPdwn1, XIBSPdwn2, and XIBSPup2; for *ODAM*, XSCPP3dwn1, XSCPP3dwn2, and XSCPP3up1; for *ENAM*, XENAMdwn1, XENAMdwn2, XENAMup1, and XENAMup2; for the 3'-end of *AMBN*, XAMBNup1 and XAMBNup2; and for the 3'-end of *AMEL*, XAMELup1 and XAMELup2.

The following PCR primers were also used to amplify intermediate regions of frog *SCPP1* and *DMP1*: XSCPP1up1 used with XSCPP1dwn1, XDMP1up1 and XDMP1dwn3, and XDMP1dwn1 used with XDMP1up2.

For zebrafish SCPP genes, the following primers were used for RACE: for *SCPP1*, dSCPP1dwn1, dSCPP1dwn2, dSCPP1up1, and dSCPP1up2; for *SCPP6*, dSCPP6dwn1, dSCPP6dwn2, dSCPP6up1, and dSCPP6up2; for *SCPP8*, dSCPP8dwn1, dSCPP8dwn2, dSCPP8up1, and dSCPP8up2; and for *SCPP9*, dSCPP9up1, dSCPP9up2, dSCPP9dwn1, and dSCPP9dwn2.

Nucleotide sequences used in this study

GenBank accession numbers obtained in this study are as follows: mouse *SCPPPQ1* (EU642618), frog *IBSP* (EU642607), *DMP1* (EU642605), *ODAM* (EU642609), *ENAM* (EU642606), *SCPPA1* (EU642616), and *SCPPA2* (EU642617), and zebrafish *ODAM* (EU642608), *SCPP1* (EU642610), *SCPP5* (EU642611), *SCPP6* (EU642612), *SCPP7* (EU642613), *SCPP8* (EU642614), and *SCPP9* (EU642615). The 5'-regions of *X. tropicalis* *AMEL* and *AMBN* were reconstructed from the genome sequence using the nucleotide sequences of the *Xenopus laevis* orthologs (Toyosawa et al. 1998; Shintani et al. 2003).

Histological analysis and in situ hybridization

The adult frog was obtained from Prof. Martin Flajnik and Dr. Yuko Ohta (University of Maryland), and tadpoles (Daudin's stage 59; Nieuwkoop and Faber 1967) were purchased from Xenopus I Inc. (Dexter, MI, USA). Adult and 14-day post-fertilization (dpf) zebrafish were obtained from Prof. Keith Cheng and Ms. Peggy Hubley (Penn State University). Both frog tadpoles and zebrafish were fixed with Bouin's solution (Sigma) and used for in situ hybridization (ISH) analysis as previously described (Kawasaki et al. 2005).

For ISH analysis of frog SCPP genes, probes were prepared from plasmid clones containing the entire 3'-RACE products with the exception of *SCPPA2*, for which the 689-nucleotide (nt) fragment between XSCPP2up1 to the downstream *HindIII* site was subcloned. For

zebrafish gene analysis, plasmid clones [FDR306-P00046_I05 for *ODAM*, FDR306-P00007_L05 for *SCPP5*, FDR103-P00052_M23 for *SCPP7*, FDR103-P00030_A10 for secreted phosphoprotein 1 (*SPP1*), and FDR306-P00041_P14 for *SPARC*], constructed at the Genome Institute of Singapore (Ng et al. 2005), were recloned or subcloned and used for probing. The insert of these plasmid clones was amplified by PCR using M13seq and M13revseq primers, digested with *Bam*HI that has restriction sites at both termini of the vector, and recloned into the *Bam*HI site of pBluescript II SK(+). For *SPP1*, dSPP1dwn1 (containing a *Bam*HI recognition site) primer was used with M13seq for subcloning the 1,121-nt *Bam*HI fragment. Similarly for *SPARC*, dSPARCdwn1 (containing a *Bam*HI site) and M13seq primers were used for subcloning the 912-nt fragment. Other zebrafish probes were derived from the PCR products obtained using the following primer pairs: dSCPP1up1-dSCPP1dwn1, dSCPP6up1-dSCPP6dwn1, dSCPP8up1-dSCPP8dwn1, dSCPP9up1-dSCPP9dwn1, and dCOL1up1-dCOL1dwn1 for *COL1a1*.

Results

SCPPPQ1 and the SCPP gene cluster of stem amniotes

In the current study, I discovered *SCPPPQ1* in mammalian and lizard (*Anolis carolinensis*, version anoCar1) genomes and cloned this gene from the mouse. *SCPPPQ1* is clustered in an array with *SPARCL1* on the other side of the acidic SCPP genes, hence separated from the other P/Q-rich SCPP genes in the human genome (Fig. 1a). Notably, the last exon of *SCPPPQ1* is totally untranslated; that is, the termination codon sits in the penultimate exon. This untranslated last exon is a characteristic shared by all P/Q-rich SCPP genes located between the α_{S1} -casein (*CSN1S1*) and κ -casein (*CSN3*) genes (Fig. 1a), indicating their close evolutionary relationship to *SCPPPQ1*. These findings suggest that the originally contiguous P/Q-rich SCPP gene cluster was split by an intrachromosomal rearrangement before the divergence of mammals and the lizard (Fig. 2).

Frog SCPP gene repertoire and gene expression in teeth and bone

I identified four P/Q-rich SCPP genes, including all three major enamel-structuring protein genes, *AMEL*, *AMBN*, and *ENAM*, and four acidic SCPP genes in the frog (*X. tropicalis*) genome (Fig. 1b). Among these, *ENAM* and *ODAM* have not been previously identified in the frog, and *SCPPA1* and *SCPPA2* never been reported in any vertebrates. Expression of these genes was detected by RT-PCR either in the tooth-bearing upper jaw alone (*AMEL*, *AMBN*, *ENAM*, *ODAM*, and *SCPPA1*) or in both the upper and the toothless lower jaws (*DMP1*, *IBSP*, and *SCPPA2*) of an adult frog (data not shown). Then, by ISH using tadpoles, I confirmed expression of *AMEL* and *ODAM* in ameloblasts and *DMP1* in odontoblasts (Fig. 3f–h and Table 1). These expression domains are similar to those of their mammalian orthologs. Expression of *SCPPA2* was weak in odontoblasts and strong in both osteoblasts surrounding bone and osteocytes embedded in bone (Fig. 3i, j and Table 1), indicating the use of *SCPPA2* primarily in bone but also more weakly in dentin. Expression of *SCPPA1* was not confirmed by ISH.

SCPP gene cluster of early frogs and stem tetrapods

In the frog genome, P/Q-rich *ODAM* is only 33-kb from acidic *SCPPA1* (Fig. 1b, not to scale). In contrast, *ENAM* resides 112-kb away from *ODAM* in the opposite transcriptional direction. The order and transcriptional directions of *AMBN*, *ENAM*, and *ODAM* in the human and frog genomes suggest that a small inversion has split the originally contiguous *ENAM-AMBN-ODAM* array in an ancestral frog after the divergence from the amniote lineage (Fig. 2), corroborating our earlier study (Kawasaki et al. 2007). The reconstructed genomic orders of SCPP genes in stem amniotes and ancestral frogs are consistent, which further suggests the order of SCPP genes in stem tetrapods (Fig. 2). The location of *AMEL*

is unclear but, before translocation to a different chromosome, ancient *AMEL* was most likely located adjacent to a closely related enamel gene, *AMBN* or *ENAM*.

Teleost SCPP gene repertoire and gene cluster

In the zebrafish genome, I identified eight SCPP genes. Among these genes, five orthologs were identified in the fugu genome based on amino acid sequence similarity, but three were not found (*SCPP6*, *SCPP8*, and *SCPP9* in Fig. 1c, d). I have now identified *SCPP2* as the teleost ortholog of *ODAM*, based on their similarities in the coded amino acid sequence, size distribution of exons, totally untranslated last exon, original location within the cluster (Figs. 1 and 2, see below), and common expression pattern in the IDE cells as described below.

Among these, all five P/Q-rich SCPP genes (*SCPP5*, *SCPP6*, *SCPP7*, *SCPP9*, and *ODAM*) are clustered 10-megabases away from acidic *SCPP1* on the same chromosome (Fig. 1c). These P/Q-rich SCPP genes appear to have been recently separated from *SCPP1* in the ancestral zebrafish (arrow in Fig. 1c) because all these SCPP genes are clustered in the fugu (Fig. 1d), medaka, and stickleback genomes. In contrast, two other acidic SCPP genes, *SPP1* and *SCPP8*, are clustered together but on a different chromosome from the other SCPP genes in the zebrafish (Fig. 1c) and stickleback. Similarly, no chromosomal linkage between *SPARCL1* and *SPP1* has been detected in fugu (Fig. 1d) or medaka, suggesting that these two loci separated before the divergence of these teleosts.

Zebrafish SCPP gene, SPARC, and COL1 expression in teeth and the jaw

Expression of all these eight zebrafish SCPP genes, *COL1*, and *SPARC* was detected in the tooth-bearing pharyngeal jaw by RT-PCR (data not shown). ISH analysis confirmed expression of five zebrafish SCPP genes (*SCPP1*, *SPP1*, *SCPP5*, *ODAM*, and *SCPP9*) in adult dental tissues (Fig. 4f–r), whereas expression of the other three genes (*SCPP6*, *SCPP7*, and *SCPP8*) has not been detected in teeth or other parts of the jaws. Table 1 summarizes the results of ISH analysis.

Initially weak expression of *SCPP1* by odontoblasts at the secretory stage of enameloid is upregulated during dentin formation (Fig. 4f, g), which is consistent with our previous study in fugu (Kawasaki et al. 2005). In this study, weak expression of *SCPP1* was also detected in the IDE cells and osteocytes located within the jaw bone and the attachment bone of an adult (Fig. 4g, s). These observations suggest the use of *SCPP1* primarily in dentin but also weakly in bone and probably in enameloid as well.

Strong expression of *SPP1* was detected in cells present on the surface of the attachment bone and the jaw bone and weak expression was also detected in odontoblasts (Fig. 4n, t). Because the distribution of *SPP1*- and *COL1*-expressing cells on these bones are well correlated, this *SPP1*-expressing cell population appears to contain osteoblasts (see also below). This expression pattern demonstrates the use of *SPP1* primarily in bone and slightly in dentin, similar to the mammalian ortholog (Fujisawa et al. 1993; Qin et al. 2001).

Expression of both *SCPP1* and *SPP1* was further analyzed in 14-dpf larvae. At this stage, three functional teeth and two successive teeth are supported by each pharyngeal jaw (fifth ceratobranchial; CBV) via the attachment bone (Fig. 5a; Van der heyden and Huysseune 2000). Expression of *SCPP1* was detected in the dental pulp, where odontoblasts reside (Fig. 5b). However, significant expression was not observed within or on the surface of the attachment bone, although some cells are located within bone matrix. These results in both adults and larvae suggest that *SCPP1* is important for later stages of bone formation, probably for homeostasis of bone, which is reminiscent of the function of tetrapod DMP1 in bone (Toyosawa et al. 2001).

In 14-dpf larvae, remarkably strong expression of *SPP1* was detected in teeth and the jaw, the bottom of the attachment bone (Fig. 5c), a perichondrial region of the CBV (Fig. 5d), and the membrane bone growing from the CBV (Fig. 5e). The *SPP1*-expressing cells located on the newly deposited mineralizing membrane bone are probably largely osteoblasts. This observation is consistent with a recent study showing that *SPP1* expression is strong in active osteoblasts at early developmental stages in the zebrafish (Laue et al. 2008). In contrast, the bottom of the attachment bone, where a functional tooth and its successive tooth are associated (Fig. 5a, c), is under remodeling by both osteoblasts and osteoclasts (Huyseune et al. 1998). Expression of *SPP1* in osteoclasts is known in mammals (Sodek et al. 2000) but remains to be confirmed in teleosts.

The expression pattern of *SCPP5* (Fig. 4l, m) and *SPARC* (Fig. 4o, p) is similar, strong in both the IDE cells and odontoblasts during deposition of enameloid and dentin matrix. These results are consistent with our previous study using fugu and suggest that *SCPP5* and *SPARC* are included in both the enameloid and dentin matrix.

Strong expression of *COL1* was detected in both the IDE cells and odontoblasts during deposition of enameloid matrix (Fig. 4q) and only in odontoblasts during dentin formation (Fig. 4r). The results of this analysis are consistent with our previous study but reveal the expression pattern in more detail. The strong expression of *COL1* in the IDE cells is immediately downregulated at the maturation stage of cap enameloid covering the apex of teeth. At this stage, weak *COL1* expression is detected in the IDE cells adjacent to the nascent matrix for the tooth body (Fig. 4r). A distinct thin hypermineralized tissue, called collar enamel or enameloid, is known to surround the teleost tooth body (Sasagawa and Ishiyama 1988; Smith 1995). These results suggest that collar enameloid is also produced by both the IDE cells and odontoblasts, similar to cap enameloid.

Strong expression of *SCPP9* and *ODAM* was detected only in the IDE cells at the maturation stage of enameloid after the underlying enameloid is fully mineralized (Fig. 4h, j). This late expression was not identified previously due to the limited developmental stages studied (Kawasaki et al. 2005), but now, this result suggests that neither *SCPP9* nor *ODAM* is included in enameloid matrix, but instead, these proteins are both involved in the hypermineralization process, which I will discuss below. Subsequently, the IDE cells overlying the fully mineralized tooth body also express *SCPP9* and *ODAM* (Fig. 4i, k), suggesting that *SCPP9* and *ODAM* are also involved in hypermineralization of collar enameloid, in addition to cap enameloid.

Discussion

Major bone SCPP genes in tetrapods and teleosts

SPP1 is the major bone SCPP in mammals, strongly expressed in both osteoblasts and osteocytes (Sodek et al. 2000). *SPP1* has also been detected in dentin extracts at a level less than 1/10 or 1/70 of bone extracts (Fujisawa et al. 1993; Qin et al. 2001). Similarly, expression of zebrafish *SPP1* is strong in osteoblasts and weak in odontoblasts as described above.

In the frog, expression of *SCPPA2* is strong in both osteoblasts and osteocytes, similar to mammalian *SPP1*. However, *SCPPA2* does not show any significant sequence homology to *SPP1* even in the region around their integrin-binding Arg-Gly-Asp sequence that is well conserved in *SPP1* from mammals to teleosts (Bobe and Goetz 2001). Instead, *SCPPA2* shows the highest amino acid sequence identity to mammalian DMP1 in the N-terminal region coded by exon 2. Moreover, the location of *SCPPA2* is not equivalent to that of *SPP1* within the gene cluster (Fig. 2). To date, no *SPP1* ortholog has been identified in the frog

genome sequence or expressed sequence tag (EST; *X. tropicalis* and *X. laevis*) databases, even though *SPPI* is abundant in EST databases of other vertebrates, including zebrafish and stickleback. These results suggest that frog *SCPPA2* is not orthologous to mammalian or teleost *SPPI*, although these genes may have adopted similar functions in bone mineralization.

Major dentin SCPP genes in tetrapods and teleosts

In mammals, expression of *DSPP* is strong in dentin and weak in bone (Qin et al. 2002). This gene has not been identified in the frog, however. Although *SCPPA1* is located upstream of *DMP1* at the position equivalent to mammalian *DSPP* (Fig. 1a, b), expression of *SCPPA1* was not detected in dentin or bone in the tadpole. In mammals, *DMP1* is expressed in both odontoblasts and osteocytes (Toyosawa et al. 2001; Qin et al. 2007). In this study, RT-PCR demonstrated expression of frog *DMP1* in the toothless lower jaw, possibly in bone; yet, ISH analysis failed to detect expression of *DMP1* in osteocytes or osteoblasts. These results show that expression of frog *DMP1* in bone is, if any, considerably weaker than that in dentin. Thus, *DMP1* is the major dentin SCPP gene in the frog.

Expression of zebrafish *SCPP1* is strong in odontoblasts and weak in osteocytes (Fig. 4g, s). The strong expression of teleost *SCPP1* in odontoblasts is similar to that of both mammalian *DSPP* and frog *DMP1*, and the expression in both odontoblasts and osteocytes is common to that of mammalian *DMP1*. In addition, the location of *SCPP1* within the cluster is similar to that of both *DSPP* and *DMP1*, upstream of the major bone SCPP genes, *SPPI* and *SCPPA2* (Fig. 2). Despite these similarities, *SCPP1* does not show any significant amino acid sequence similarity to *DSPP* or *DMP1* (with the exception of the signal peptide relatively well conserved across distinct SCPPs). Although orthology between *SCPP1* and *DMP1* or *DSPP* remains to be confirmed, the similar expression profiles and phylogenetic distributions of these genes suggest that either *SCPP1* or *DMP1*, or perhaps a closely related acidic SCPP gene primarily used in dentin, was already present in stem osteichthyans (Fig. 2).

Common and different SCPP genes used in teleost enameloid

ODAM was initially purified as a short C-terminal product, coded by exons 6–11, from amyloid associated with human calcified epithelial odontogenic tumor (Solomon et al. 2003). The N-terminal of a longer product was subsequently identified from the rat enamel organ (Moffatt et al. 2006a). Because *SCPP2* (teleost ODA) shows highest sequence similarity to mammalian and frog ODA at the N-terminal region coded by exons 2–4, fugu *SCPP2* was not identified as the ortholog of *ODAM* in our earlier study (Kawasaki et al. 2005).

Strong expression of zebrafish *ODAM* and *SCPP9* was detected only in the IDE cells, which is similar to *ODAM* and *SCPP4* in fugu (Kawasaki et al. 2005). This similar expression pattern suggests a close relationship between zebrafish *SCPP9* and fugu *SCPP4*. However, while zebrafish, medaka, and stickleback *SCPP9* all show high sequence similarity to one another, none of these *SCPP9* sequences has significant similarity to puffer fish (fugu and Tetraodon) *SCPP4*. Furthermore, the location of zebrafish *SCPP9* and fugu *SCPP4* is not equivalent within the gene cluster (Fig. 1c, d). Thus, *SCPP9* does not appear to be orthologous to *SCPP4*, although these two genes may have common functions.

During the formation of fugu enameloid and dentin, strong expression of two closely related *SCPP3* genes (*SCPP3A* and *SCPP3B*) was detected in the inner pharyngeal epithelium overlying tooth buds (Kawasaki et al. 2005). Similar *SCPP3* genes are also present in the medaka and stickleback genomes but have not been identified in the zebrafish genome.

Thus, among SCPP genes used in mineralization, only four SCPP genes, *SCPP5*, *ODAM*, *SCPP1*, and *SPPI*, have been confirmed in all these teleosts (Fig. 2).

Common hypermineralization machinery in enamel and enameloid

At the onset of the enamel maturation stage, a specialized basal lamina is formed between ameloblasts and the enamel surface (Takano 1979; Nanci et al. 1993; Al Kwas and Warshawsky 2008). During the maturation stage, ameloblasts actively transport substantial calcium ions to enamel, while water and enzymatically degraded ECM proteins are removed from enamel (Smith 1998; Simmer and Hu 2002). In rodents, expression of both *AMTN* and *ODAM* is detected in ameloblasts at this stage (Iwasaki et al. 2005; Park et al. 2007). While *AMTN* is localized precisely to the basal lamina, *ODAM* is distributed more diffusely along apical surfaces of ameloblasts abutting the basal lamina (Moffatt et al. 2006b; Moffatt et al. 2008).

In contrast to enamel formation, the basal lamina is absent at the maturation stage of enameloid (Sasagawa 1997), implying that *AMTN*-like basal lamina proteins are not used in enameloid. However, the expression of zebrafish *ODAM* is detected in the IDE cells at this stage. The similar expression pattern of *ODAM* in both tetrapods and teleosts strongly suggests that ancient hypermineralization machinery, which arose at least before the divergence of actinopterygians and sarcopterygians, has descended to both enamel and enameloid. On the other hand, specialized scaffold proteins are not necessarily required for hyper-mineralization as shown by teleost enameloid. Thus, I hypothesize that *ODAM* or its closely related P/Q-rich SCPP genes evolved early and were used for tissue hypermineralization, and one of these genes was later coopted for use in more specialized enamel-structuring proteins.

Phylogenetic distributions of the P/Q-rich SCPP genes possessing the totally untranslated last exon (Fig. 1) suggest that *ODAM* is probably the last common ancestor of the casein genes, whose products make milk supersaturated with calcium phosphate and prevent its precipitation in the mammary glands (Little and Holt 2004; Smyth et al. 2004). In fact, expression of *ODAM* itself has been detected in lactating mammary glands (Rijnkels et al. 2003). Thus, I suggest that, during enamel hypermineralization, *ODAM* creates a specialized microenvironment preventing spontaneous calcium phosphate precipitation and facilitating its transport between the hypermineralizing tooth surface and the overlying dental epithelium. This function appears to be cardinal to the hypermineralization process in general and is not opposed to but rather complements the previous proposition that *ODAM* mediates adhesion of dental epithelium to the tooth surface (Moffatt et al. 2008).

Genetic similarities and differences among bone, dentin, enameloid, and enamel

I have shown that in both the frog and zebrafish, some acidic SCPP genes are strongly expressed in dentin and weakly expressed in bone or vice versa (although expression of frog *DMP1* has not been detected in bone). These findings are consistent with the idea that dentin and bone use common SCPPs, and their essential differences lie in their composition and post-translational modifications, as suggested by studies of mammalian acidic SCPPs (Qin et al. 2001; Butler et al. 2003; Qin et al. 2004). The use of common SCPPs with different compositions in bone and dentin, in both tetrapods and teleosts, suggests a close evolutionary relationship between these two tissues.

ODAM and *SCPP9* are not included in the enameloid ECM. Therefore, the shared expression of *COL1*, *SPARC*, *SCPP5*, and *SCPP1* in odontoblasts and the IDE cells indicates the use of common ECM proteins in dentin and enameloid, although their

composition and post-translational modifications may be different. Pre-enameloid is thus similar to predentin, but enameloid is hypermineralized after initial mineralization.

Both teleost enameloid and tetrapod enamel use similar ODAM-based hypermineralization machinery descended from their common ancestral tissue. Thus, the significant difference between these two tissues lies in the use of specialized P/Q-rich SCPPs as the mineralization scaffold in enamel (Fincham et al. 1999), demonstrating the divergence of enamel from enameloid, dentin, and bone.

It has been proposed that the enamel–enameloid–dentin system forms a continuum of tissues that have diverged from one another (Meinke 1982; Meinke and Thomson 1983). Recent biomechanical, paleontological, and genetic studies integrate bone (Currey 2002; Kawasaki and Weiss 2008) and also cartilage in the continuum (Donoghue et al. 2006). The results of this study illustrate graded differences among the four principal dental tissues, which reinforces the hypothesis that enamel–enameloid–dentin–bone constitutes an evolutionary continuum. It is possible that distinct SCPP genes were independently co-opted for different mineralized tissues. However, it is more likely that an early SCPP gene was employed by the initial mineralized tissue, and new duplicated SCPP genes were used for subsequently diversified mineralized tissues. The reconstructed genomic array of SCPP genes in the stem osteichthyan genome is arranged as *ENAM/AMBN-ODAM-(SCPP1 or DMP1)-SPPI*, although the enamel structuring genes may have arisen later in sarcopterygians (Fig. 2). Interestingly, this arrangement is reminiscent of the tissue grades in the mineralization continuum. Thus, I assume that early tandem duplications of SCPP genes were correlated with incremental diversification of mineralized tissues. This hypothesis could be tested by studying the distribution of mineralized tissues early in vertebrate phylogeny and SCPP genes in various additional vertebrates, especially chondrichthyans.

Supplementary Material

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References

- Al Kaws S, Warshawsky H. Ultrastructure and composition of basement membrane separating mature ameloblasts from enamel. *Arch Oral Biol.* 2008; 53:310–317. [PubMed: 18054891]
- Bartlett JD, Ganss B, Goldberg M, Moradian-Oldak J, Paine ML, Snead ML, Wen X, White SN, Zhou YL. Protein–protein interactions of the developing enamel matrix. *Curr Top Dev Biol.* 2006; 74:57–115. [PubMed: 16860665]
- Bobe J, Goetz FW. A novel osteopontin-like protein is expressed in the trout ovary during ovulation. *FEBS Lett.* 2001; 489:119–124. [PubMed: 11165234]
- Butler WT, Brunn JC, Qin C. Dentin extracellular matrix (ECM) proteins: comparison to bone ECM and contribution to dynamics of dentinogenesis. *Connect Tissue Res.* 2003; 44(Suppl 1):171–178. [PubMed: 12952193]
- Currey, JD. *Bones: structure and mechanics.* Princeton University Press; Princeton: 2002.

- Donoghue PC, Sansom IJ, Downs JP. Early evolution of vertebrate skeletal tissues and cellular interactions, and the canalization of skeletal development. *J Exp Zool B Mol Dev Evol.* 2006; 306B:278–294.
- Fincham AG, Moradian-Oldak J, Simmer JP. The structural biology of the developing dental enamel matrix. *J Struct Biol.* 1999; 126:270–299. [PubMed: 10441532]
- Fujisawa R, Butler WT, Brunn JC, Zhou HY, Kuboki Y. Differences in composition of cell-attachment sialoproteins between dentin and bone. *J Dent Res.* 1993; 72:1222–1226. [PubMed: 8360366]
- Hall, BK. *Bones and cartilage: developmental and evolutionary skeletal biology.* Elsevier; San Diego: 2005.
- Huysseune A, Van der heyden C, Sire JY. Early development of the zebrafish (*Danio rerio*) pharyngeal dentition (Teleostei, Cyprinidae). *Anat Embryol (Berl).* 1998; 198:289–305. [PubMed: 9764543]
- Iwasaki K, Bajenova E, Somogyi-Ganss E, Miller M, Nguyen V, Nourkeyhani H, Gao Y, Wendel M, Ganss B. Amelotin—a novel secreted, ameloblast-specific protein. *J Dent Res.* 2005; 84:1127–1132. [PubMed: 16304441]
- Kawasaki K, Weiss KM. Mineralized tissue and vertebrate evolution: the secretory calcium-binding phosphoprotein gene cluster. *Proc Natl Acad Sci U S A.* 2003; 100:4060–4065. [PubMed: 12646701]
- Kawasaki K, Weiss KM. Evolutionary genetics of vertebrate tissue mineralization: the origin and evolution of the secretory calcium-binding phosphoprotein family. *J Exp Zool B Mol Dev Evol.* 2006; 306B:295–316.
- Kawasaki K.; Weiss, KM. Genetic basis for the evolution of vertebrate mineralized tissue. In: Bäuerlein, E., editor. *Handbook of biomineralization.* Wiley-VCH; Weinheim: 2007. p. 331–347.
- Kawasaki K, Weiss KM. SCPP gene evolution and the dental mineralization continuum. *J Dent Res.* 2008; 87:520–531. [PubMed: 18502959]
- Kawasaki K, Shimoda S, Fukae M. Histological and biochemical observations of developing enameloid of the sea bream. *Adv Dent Res.* 1987; 1:191–195. [PubMed: 3332771]
- Kawasaki K, Suzuki T, Weiss KM. Genetic basis for the evolution of vertebrate mineralized tissue. *Proc Natl Acad Sci U S A.* 2004; 101:11356–11361. [PubMed: 15272073]
- Kawasaki K, Suzuki T, Weiss KM. Phenogenetic drift in evolution: the changing genetic basis of vertebrate teeth. *Proc Natl Acad Sci U S A.* 2005; 102:18063–18068. [PubMed: 16332957]
- Kawasaki K, Buchanan AV, Weiss KM. Gene duplication and the evolution of vertebrate skeletal mineralization. *Cells Tissues Organs.* 2007; 186:7–24. [PubMed: 17627116]
- Laue K, Jänicke M, Plaster N, Sonntag C, Hammerschmidt M. Restriction of retinoic acid activity by Cyp26b1 is required for proper timing and patterning of osteogenesis during zebrafish development. *Development.* 2008; 135:3775–3787. [PubMed: 18927157]
- Linde A, Goldberg M. Dentinogenesis. *Crit Rev Oral Biol Med.* 1993; 4:679–728. [PubMed: 8292714]
- Little EM, Holt C. An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein phosphopeptides. *Eur Biophys J.* 2004; 33:435–447. [PubMed: 14735250]
- Loh YH, Brenner S, Venkatesh B. Investigation of loss and gain of introns in the compact genomes of pufferfishes (fugu and tetraodon). *Mol Biol Evol.* 2008; 25:526–535. [PubMed: 18089580]
- Meinke DK. A histological and histochemical study of developing teeth in *Polypterus* (Pisces, Actinopterygii). *Arch Oral Biol.* 1982; 27:197–206. [PubMed: 6953936]
- Meinke DK, Thomson KS. The distribution and significance of enamel and enameloid in the dermal skeleton of osteolepiform. *Paleobiology.* 1983; 9:138–149.
- Moffatt P, Smith CE, Sooknanan R, St-Arnaud R, Nanci A. Identification of secreted and membrane proteins in the rat incisor enamel organ using a signal-trap screening approach. *Eur J Oral Sci.* 2006a; 114(Suppl 1):139–146. [PubMed: 16674676]
- Moffatt P, Smith CE, St-Arnaud R, Simmons D, Wright JT, Nanci A. Cloning of rat amelotin and localization of the protein to the basal lamina of maturation stage ameloblasts and junctional epithelium. *Biochem J.* 2006b; 399:37–46. [PubMed: 16787391]
- Moffatt P, Smith CE, St-Arnaud R, Nanci A. Characterization of Apin, a secreted protein highly expressed in tooth-associated epithelia. *J Cell Biochem.* 2008; 103:941–956. [PubMed: 17647262]

- Nanci A, Zalzal S, Kogaya Y. Cytochemical characterization of basement membranes in the enamel organ of the rat incisor. *Histochemistry*. 1993; 99:321–331. [PubMed: 8500995]
- Nanci, A. Ten Cate's oral histology. 6. Mosby; St. Louis: 2003.
- Ng P, Wei CL, Sung WK, Chiu KP, Lipovich L, Ang CC, Gupta S, Shahab A, Ridwan A, Wong CH, Liu ET, Ruan Y. Gene identification signature (GIS) analysis for transcriptome characterization and genome annotation. *Nat Methods*. 2005; 2:105–111. [PubMed: 15782207]
- Nieuwkoop, PD.; Faber, J. Normal table of *Xenopus laevis* (Daudin). 2. North-Holland; Amsterdam: 1967.
- Paine ML, Snead ML. Tooth developmental biology: disruptions to enamel–matrix assembly and its impact on biomineralization. *Orthod Craniofac Res*. 2005; 8:239–251. [PubMed: 16238604]
- Park JC, Park JT, Son HH, Kim HJ, Jeong MJ, Lee CS, Dey R, Cho MI. The amyloid protein APin is highly expressed during enamel mineralization and maturation in rat incisors. *Eur J Oral Sci*. 2007; 115:153–160. [PubMed: 17451507]
- Poole, DFG. Phylogeny of tooth tissues: Enameloid and enamel in recent vertebrates with a note in the history of cementum. In: Miles, AEW., editor. Structural and chemical organization of teeth. Academic; New York: 1967. p. 111-149.
- Poole, DFG. An introduction to the phylogeny of calcified tissues. In: Dahlberg, AA., editor. Dental morphology and evolution. University of Chicago Press; Chicago: 1971. p. 65-79.
- Qin C, Brunn JC, Jones J, George A, Ramachandran A, Gorski JP, Butler WT. A comparative study of sialic acid-rich proteins in rat bone and dentin. *Eur J Oral Sci*. 2001; 109:133–141. [PubMed: 11347657]
- Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, Nagai N, Butler WT. The expression of dentin sialophosphoprotein gene in bone. *J Dent Res*. 2002; 81:392–394. [PubMed: 12097430]
- Qin C, Baba O, Butler WT. Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Crit Rev Oral Biol Med*. 2004; 15:126–136. [PubMed: 15187031]
- Qin C, D'Souza R, Feng JQ. Dentin matrix protein 1 (DMP1): new and important roles for biomineralization and phosphate homeostasis. *J Dent Res*. 2007; 86:1134–1141. [PubMed: 18037646]
- Rijkels M, Elnitski L, Miller W, Rosen JM. Multispecies comparative analysis of a mammalian-specific genomic domain encoding secretory proteins. *Genomics*. 2003; 82:417–432. [PubMed: 13679022]
- Sasagawa I. Fine structure of the cap enameloid and of the dental epithelial cells during enameloid mineralisation and early maturation stages in the tilapia, a teleost. *J Anat*. 1997; 190(Pt 4):589–600. [PubMed: 9183681]
- Sasagawa I, Ishiyama M. The structure and development of the collar enameloid in two teleost fishes, *Halichoeres poecilopterus* and *Pagrus major*. *Anat Embryol (Berl)*. 1988; 178:499–511. [PubMed: 3223608]
- Shellis RP, Miles AEW. Autoradiographic study of the formation of enameloid and dentine matrices in teleost fishes using tritiated amino acids. *Proc R Soc Lond B*. 1974; 185:51–72.
- Shintani S, Kobata M, Toyosawa S, Ooshima T. Identification and characterization of ameloblastin gene in an amphibian, *Xenopus laevis*. *Gene*. 2003; 318:125–136. [PubMed: 14585505]
- Simmer JP, Fincham AG. Molecular mechanisms of dental enamel formation. *Crit Rev Oral Biol Med*. 1995; 6:84–108. [PubMed: 7548623]
- Simmer JP, Hu JC. Expression, structure, and function of enamel proteinases. *Connect Tissue Res*. 2002; 43:441–449. [PubMed: 12489196]
- Smith CE. Cellular and chemical events during enamel maturation. *Crit Rev Oral Biol Med*. 1998; 9:128–161. [PubMed: 9603233]
- Smith, MM. Microstructure and evolution of enamel amongst osteichthyan and early tetrapods. In: Smith, P.; Tchernov, E., editors. Structure, function and evolution of teeth. Freund; Tel Aviv: 1992. p. 73-101.
- Smith, MM. Heterochrony in the evolution of enamel in vertebrates. In: McNamara, KJ., editor. Evolutionary change and heterochrony. Wiley; Chichester: 1995. p. 125-150.

- Smyth E, Clegg RA, Holt C. A biological perspective on the structure and function of caseins and casein micelles. *Int J Dairy Technol.* 2004; 57:121–126.
- Sodek J, Ganss B, McKee MD. Osteopontin. *Crit Rev Oral Biol Med.* 2000; 11:279–303. [PubMed: 11021631]
- Solomon A, Murphy CL, Weaver K, Weiss DT, Hrnčić R, Eulitz M, Donnell RL, Sletten K, Westermarck G, Westermarck P. Calcifying epithelial odontogenic (Pindborg) tumor-associated amyloid consists of a novel human protein. *J Lab Clin Med.* 2003; 142:348–355. [PubMed: 14647039]
- Stock DW. Zebrafish dentition in comparative context. *J Exp Zool B Mol Dev Evol.* 2007; 308:523–549.
- Takano Y. Cytochemical studies of ameloblasts and the surface layer of enamel of the rat incisor at the maturation stage. *Arch Histol Jpn.* 1979; 42:11–32. [PubMed: 219803]
- Toyosawa S, O’Hugin C, Figueroa F, Tichy H, Klein J. Identification and characterization of amelogenin genes in monotremes, reptiles, and amphibians. *Proc Natl Acad Sci U S A.* 1998; 95:13056–13061. [PubMed: 9789040]
- Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, Ijuhin N, Komori T. Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. *J Bone Miner Res.* 2001; 16:2017–2026. [PubMed: 11697797]
- Van der heyden C, Huysseune A. Dynamics of tooth formation and replacement in the zebrafish (*Danio rerio*) (Teleostei, Cyprinidae). *Dev Dyn.* 2000; 219:486–496. [PubMed: 11084648]
- Van der heyden C, Huysseune A, Sire JY. Development and fine structure of pharyngeal replacement teeth in juvenile zebrafish (*Danio rerio*) (Teleostei, Cyprinidae). *Cell Tissue Res.* 2000; 302:205–219. [PubMed: 11131132]

Fig. 1.

SCPP gene clusters in the human (a), frog (b), zebrafish (c), and fugu (d) genomes. These maps illustrate location and transcriptional orientation of *SPARCL1* (*open pentagon*), acidic SCPP genes (*shaded pentagon*), P/Q-rich SCPP genes with the termination codon present in the last exon (*closed pentagon*), and P/Q-rich SCPP genes possessing the totally untranslated last exon (*closed box with open tail*). **a** In the human genome, two large SCPP gene clusters are 17 Mb apart. Gene symbols not described in the text were summarized previously (Kawasaki et al. 2007). **b** Frog *SPARCL1* has not been identified in the genome sequence or EST databases. In the human and frog genomes, *AMEL* and the other SCPP genes reside on different chromosomes. **c** In the zebrafish genome, *SPP1* and *SCPP8* reside on a chromosome different from the other SCPP genes. The P/Q-rich SCPP gene (*SCPP5–SCPP9*) cluster was originally located adjacent to the *SPARCL1–SCPP1* cluster as shown here, but an intrachromosomal rearrangement has separated these two clusters 10 Mb away (arrow). **d** No linkage between *SPP1* and the other SCPP genes has been detected in the fugu genome. Note that zebrafish *SCPP5*, *SCPP7*, and *ODAM* have a totally untranslated last exon, whereas no fugu SCPP genes have such an exon. The untranslated last exons were probably lost from fugu SCPP genes during the evolution of the small genome size (Loh et al. 2008). These maps were based on the following versions of the genome sequences: hg18 (human), xenTro2 (frog), fr2 (fugu), and danRer5 (zebrafish)

Fig. 2.

Reconstruction of ancient SPP gene clusters. SPP genes involved in tissue mineralization and *SCPPPQ1* are shown. The SPP gene cluster was rearranged independently in a stem amniote and an ancestral frog (rearrangements shown by *arrows*). Because enamel structuring genes have been identified only in tetrapods, the presence or absence of enamel SPP genes in the stem bony fish (osteichthyans) is not clear. See text for *SCPP1* or *DMP1* in the stem bony fish

Fig. 3.

Expression of frog *AMEL*, *ODAM*, *DMP1*, and *SCPPA2*. The upper jaw of frog tadpoles was sagittally dissected (**a**) and stained (**b**, **c**, **e**) or used for ISH analysis (**f–j**). Tooth buds and the maxillary bone are indicated by *rectangles* (**b**) and separately shown in (**c**) and (**e**). The histological section in **c** was illustrated in **d**, showing tall columnar ameloblasts (*Am*, *closed arrowhead*; these cells correspond to the IDE cells in zebrafish teeth) lining outside the tooth enamel (*E*) and odontoblasts (*Od*, *open arrowhead*) extending cell processes into dentin (*D*) from inside. Enamel was dissolved by the acidic fixative and a white gap remains in the enamel space (**c**, **d**). *Closed and open arrowheads* in **f–i** also indicate ameloblasts and odontoblasts, respectively. The *orange and blue areas* between ameloblasts and odontoblasts in **c** and **d** represent mineralized dentin and not-yet mineralized predentin, respectively, and those between mesenchymal cells in **e** indicate mineralized bone (*B*) and unmineralized collagenous ECM, respectively. ISH analysis reveals expression of *AMEL* (**f**) and *ODAM* (**g**), *DMP1* (**h**), and *SCPPA2* (**i**) in tooth buds, and *SCPPA2* (**j**) in bone at an early developmental stage. Probes used in ISH analysis are shown at the *bottom* (**f–j**)

Fig. 4.

Expression of zebrafish *SCPP*, *SPARC*, and *COL1* genes. The pharyngeal jaw was coronally dissected (a) and stained (b–e) or used for ISH analysis (f–t). A tooth bud and the attachment bone (fused with the jaw bone) of an erupted tooth are indicated by *rectangles* in the left half of the pharyngeal jaw (b). Adult zebrafish have teeth at various developmental stages in the pharyngeal jaw (Van der heyden and Huysseune 2000; Stock 2007). Three representative developmental stages of tooth buds are shown: the secretory stage of enameloid (c) and early (d) and late (e) secretory stages of dentin (maturation stages of enameloid). *Closed* and *open arrowheads* indicate the IDE cells (*IDE*) and odontoblasts (*Od*), respectively (c–r). See Fig. 3d for distributions of the IDE cells and odontoblasts. The *blue area* between the IDE cells and odontoblasts in c represents not-yet mineralized pre-enameloid. The white gap (*Ed*) in d and e represents the space of enameloid, dissolved by the acidic fixative. The orange areas (*D*) underlying the white gap represent mineralized dentin (d, e). ISH analysis reveals expression of *SCPP1* (f, g), *SCPP9* (h, i), *ODAM* (j, k), *SCPP5* (l, m), *SPP1* (n), *SPARC* (o, p), and *COL1* (q, r) in tooth buds, and *SCPP1* (s) and *SPP1* (t) in the attachment bone and the jawbone. Probes and developmental stages of tooth buds (c, d, or e) in each ISH analysis (f–t) are shown at the bottom

Fig. 5.

Expression of zebrafish *SCPP1* and *SPP1* in 14-dpf larvae. The whole body was sagittally sectioned (**a**, *l*) and used for ISH (**b–e**; *upper half*) and histological (**b–e**; *lower half*) analyses. At 14 dpf, three functional teeth (*FT*) and two successive teeth (*ST*) are attached to CBV (**a**, *3*, *5*), located posterior to ceratohyal (*CH*) and the first four ceratobranchials (*CBI–IV*) in **a**, *2*), via the attachment bone. For these teeth, the border between dentin (*D*) and bone (*B*) matrices is indistinguishable (**a**, *4*). In addition to these five teeth, tooth matrix is deposited in four tooth buds (*TB* in **b**). Strong hybridization signals are shown by an *open arrowhead* (**b–e**). Probes used in this analysis are indicated at the *bottom* of the upper half (**b–e**). Note that bone matrix in **c–e** is largely blue, demonstrating that these portions are not yet mineralized or the degree of mineralization is considerably low. *T*tooth, *MB* membrane bone

Table 1

Summary of ISH analysis

Gene	Ameloblast/IDE	Odontoblast	Osteoblast/Osteocyte
Frog			
AMEL	++	ND	ND
ODAM	++	ND	ND
DMP1	ND	++	ND
SCPPA2	ND	+	++
Zebrafish			
SCPP1	+	++	+
SCPP9	++	ND	ND
ODAM	++	ND	ND
SCPP5	++	++	ND
SPP1	ND	+	++
SPARC	++	++	++
COL1	++/+	++	++

ND not detected, (++) strong expression, (++) strong or weak expression, depending on the developmental stage (see text), (+) weak expression