Vertebrate mineralized tissues are vital to the adaptive evolution of various traits. Among these traits is the tooth, which consists of two characteristic mineralized tissues, a highly mineralized surface layer (enamel in tetrapods and enameloid in fish) and a softer body (dentin), both supported by basal bone. However, enamel and enameloid are significantly different in development, and dentin shows many histological variations; hence their evolution has been intensively studied. Nevertheless, their genetic basis has been revealed only in tetrapods. We previously reported that many genes involved in tetrapod tissue mineralization arose from a common ancestor and constitute the secretory calcium-binding phosphoprotein (SCPP) gene family. Now we show that teleost fish also use many SCPPs for enameldent and dentin mineralization, but none of these directly corresponds to tetrapod SCPPs. This finding suggests that teleost and tetrapod SCPP genes have experienced independent parallel duplication histories. Thus, through phenogenetic drift, the tooth has remained a stable trait in jawed vertebrates, while evolving distinct genetic bases in teleosts and tetrapods. The characteristics of teleost SCPP genes and their expression domains in tooth development suggest the possibility that enameloid arose from dentin and enamel from enameldent more than once in vertebrate evolution. In fugu (puffer fish), expression of SCPP genes is also detected in an unusual beak-like structure that shelters numerous teeth. Their expression pattern suggests that the jaw consists of the dentin beak and supportive bone. These findings illustrate the complexity of the homology concept in understanding evolution, particularly the evolution of mineralized tissues.

Phenogenetic drift in evolution: The changing genetic basis of vertebrate teeth

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Vertebrate mineralized tissues are vital to the adaptive evolution of various traits. Among these traits is the tooth, which consists of two characteristic mineralized tissues, a highly mineralized surface layer (enamel in tetrapods and enameloid in fish) and a softer body (dentin), both supported by basal bone. However, enamel and enameloid are significantly different in development, and dentin shows many histological variations; hence their evolution has been intensively studied. Nevertheless, their genetic basis has been revealed only in tetrapods. We previously reported that many genes involved in tetrapod tissue mineralization arose from a common ancestor and constitute the secretory calcium-binding phosphoprotein (SCPP) gene family. Now we show that teleost fish also use many SCPPs for enameldent and dentin mineralization, but none of these directly corresponds to tetrapod SCPPs. This finding suggests that teleost and tetrapod SCPP genes have experienced independent parallel duplication histories. Thus, through phenogenetic drift, the tooth has remained a stable trait in jawed vertebrates, while evolving distinct genetic bases in teleosts and tetrapods. The characteristics of teleost SCPP genes and their expression domains in tooth development suggest the possibility that enameloid arose from dentin and enamel from enameldent more than once in vertebrate evolution. In fugu (puffer fish), expression of SCPP genes is also detected in an unusual beak-like structure that shelters numerous teeth. Their expression pattern suggests that the jaw consists of the dentin beak and supportive bone. These findings illustrate the complexity of the homology concept in understanding evolution, particularly the evolution of mineralized tissues.

Mineralized tissues consisting of vertebrate teeth are closely related to those in the tubercles ornamenting the dermal skeleton of Paleozoic agnathans (Fig. 1) (1, 2). Both of these elements are characterized by two principal mineralized tissues: a highly mineralized surface layer (enameloid or enamel) and a softer body (dentin), both of which are supported by basal bone (2, 3). However, the highly mineralized surface tissue is not consistently present in teeth and only occasionally exists in dermal skeleton (Fig. 1), suggesting multiple evolutionary origins of this tissue (4, 5). In contrast, dentin is more stable during evolution (Fig. 1), yet this tissue has many histological variations (1, 3). Despite critical roles of these mineralized tissues in adaptive vertebrate evolution, the underlying genetic basis has been revealed only in tetrapods and is unknown for non-tetrapods (6, 7).

In tetrapods, many secretory calcium-binding phosphoproteins (SCPPs) cooperatively regulate extracellular calcium phosphate concentrations, and this regulation is crucial to the development of elaborated mineralized skeletal system (7, 8). These SCPPs include extracellular matrix (ECM) proteins for (i) enamel [ameloblastin (ABMN), enamelin (ENAM), and amelogenin (AMEL)]; (ii) both dentin and bone [dentin sialoprotein (DSP), dentin matrix acidic phosphoprotein 1 (DMP1), integrin-binding sialoprotein (IBSP), matrix extracellular phosphoglycoprotein (MEPE), and secreted phosphoprotein 1 (SPP1) also called osteopontin]; (iii) eggshell matrix protein [ovocleidin 116 (OC116)] in birds; and (iv) milk caseins and salivary proteins in mammals. These SCPP genes all have a structure common to the 5′ region of SPARC (secreted protein, acidic, cysteine-rich, or osteonectin) and SPARCL1 (SPARC-like 1). Although human SPARC resides on chromosome 5, SPARCL1 and all other SCPP genes form a cluster on 4q13–q21, with the exception of AMEL, a copy of which is located on the sex chromosomes, the sole exception to the tandem cluster array of SCPP genes in all tetrapods studied so far (7, 8). Thus, the SCPP gene family originated from the 5′ region of SPARCL1, which initially arose from SPARC coding an abundant dentin and bone ECM protein (7). The phylogenetic distribution of these SCPPs in various vertebrates would elucidate the evolutionary history of mineralized tissues. However, efforts to identify fish SCPPs have been largely unsuccessful. To date, only SPP1 has been found in zebrafish and trout, but no SCPP genes have been identified in the genome sequences of two puffer fish species, Takifugu rubripes (fugu) and Tetraodon nigroviridis (spotted green puffer). This result suggests that many tetrapod SCPPs arose during the evolution of sarcopterygians (7).

The SCPPs are secreted from cells differentiated through epithelial–mesenchymal interactions (8). In tooth development, oral epithelium initially forms a dental lamina, which regionally invaginates into the neural-crest-derived mesenchyme, developing into dental epithelium, while mesenchymal cells condense underneath, forming the dental papilla (9, 10). Eventually, the initial ECM proteins for dental tissues are deposited between the inner layer of dental epithelium (IDE, which corresponds to ameloblasts for enamel formation) and mesenchymal cells (odontoblasts) (3). However, the process of tissue mineralization is significantly different between tetrapods and teleosts (4, 9, 11). In tetrapods, odontoblasts initially deposit dentin ECM proteins, and then ameloblasts secrete enamel proteins. Dentin, as well as bone, forms on type-I collagen (COL1), and many dentin/bone SCPPs modulate mineralization, whereas enamel mineralization is regulated by enamel SCPPs, including the enamel scaffold protein, amelogenin (6). By contrast, in teleosts and also non-teleost actinopterygians and chondrichthians (Fig. 1), both IDE and odontoblasts initially organize enameldent and then odontoblasts form dentin. Enameldent forms on COL1, similar to dentin and bone. During enamel and enameldent mineralization, ameloblasts and IDE secrete proteasomes that degrade ECM proteins and remove them from the matrix; hence these two tissues mature into highly mineralized inorganic tissues (12–16). The function of teleost IDE with regard to ECM protein secretion into enameldent is important to reveal the evolutionary conflict of interest statement: No conflict declared.

Abbreviations: COL1, type-I collagen; n-dpf, n day postfertilization; ECM, extracellular matrix; IDE, inner layer of dental epithelium; ISH, in situ hybridization; IPE, inner layer of pharyngeal epithelium; SCPP, secretory calcium-binding phosphoprotein.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ066520–DQ066526).

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Hagfishes/Lampreys
Pteraspidomorphs
Cephalaspidomorphs
Placoderms
Chondrichthians
Acanthodians
Actinopterygians
Bichir teleosts
Sarcopterygians
Lungfish
Mammals

Fig. 1. Phylogeny and distribution of mineralized tissues. Nodes 1–3 represent agnathans (jawless fish). Cephalaspidomorphs form a monophyletic group but are represented here as a single branch (5). Jaws appeared in node 4; thus, nodes 4–9 represent gnathostomes (jawed vertebrates). Teeth arose in node 5, although some placoderms may have independently evolved teeth (48). Nodes 7–9 represent osteichthians (bony fish). Dashed lines show extinct clades. The phylogeny and distribution of the surface and body mineralized tissues (separated by a slash) are based on previous reports (5, 32). D, dentin; E, enamel; Ed, enameloid; G, ganoin; N, not detected.

Results
Teleost SCPPs. Many dentin/bone and enamel SCPP genes form a cluster in various tetrapod genomes including frogs (data not shown), chickens (7), and mammals (8, 30, 31). Hence, we searched the fugu genome sequence adjacent to their ancestral SPARCL1 and identified seven SCPP genes (Fig. 2A; SCPP1–SCPP5, with three distinct but closely related SCPP3 genes). These fugu SCPP genes share a unique structure with all of the tetrapod SCPP genes and the 5’ regions of their ancestral SPARCL1 and SPARC (7, 8). That is, the fugu SCPP genes consist of all phase 0 introns (which lie between the adjacent codons rather than interrupting a triplet) and a characteristic exon-2 that codes an untranslated region at the 5’ end, the entire signal peptide (which localizes the protein in ECM), and typically two amino acids of the mature protein at the 3’ end (Fig. 2B).

Histology and in Situ Hybridization (ISH). Fugu embryos, obtained as described in ref. 28, were fixed with Bouin’s solution (Sigma) or 4% paraformaldehyde (Sigma), embedded in Paraplast plus (SPI Supplies, West Chester, PA), and sectioned to 5–7 μm in thickness. Tissues were stained with alcan blue (Sigma), Mayer’s hematoxylin (Sigma), and Papanicolaou stain (Gill’s modified EA formula, Ricca Chemical, Arlington, TX). We used the entire body (20- to 22-dpf embryos) and whole head (24- to 43-dpf embryos) for ISH analysis (29). The probes were made by using the plasmid clones containing the 3’ end (SCPP2, SCPP3A, SCPP3B, SCPP3C, SCPP4, and SCPP5) or the 5’ end (SCPP1) of cDNA fragments obtained by PCR as described above. For the COL1A1 (collagen type-I α1), COL1A1A and SPARC probes, the cDNA fragments were amplified by using the following primers: 5’-CCCTACCGCATGTTCCGTGCTGAT-3’ and 5’-GAGGTTTAGGCGCGGAGACGCGTCCAT-3’ for COL1A1A; and 5’-CCTGGACCTACGTTCCGTGCTGAT-3’ and 5’-CCTGGAGTGTGATAGT-3’ for SPARC. The methods for probe labeling, hybridization, and signal detection were described in refs. 28 and 29. Hybridization signal was developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega).

Methods
Identification of SCPP Genes. The fugu genomic sequence was retrieved from the U.S. Department of Energy Joint Genome Institute web site (http://genome.jgi-psf.org). The nucleotide sequence was analyzed by using a gene prediction program, GenScan (http://genes.mit.eduGENSCAN.html). Methods for other bioinformatic analyses were described in refs. 7 and 8. PCR primers were designed for the predicted genes and used to screen cDNA libraries constructed from 18-day or 29-day postfertilization (dpf) whole fugu embryos. The methods for PCR construction and the isolation of the 5’ and 3’ ends of cDNA fragments were described in ref. 28. The sequences of PCR primers used for the initial screening are as follows: 5’-TTGCTCTAGATGAGCCCTTTT-3’, 5’-GGCGGTCTGTCTGACGATGTTG-3’ for SCPP1; 5’-GGAGCTCCGACGCTATCTT-3’, 5’-GTGCTTGGAGGCCCTATTAGCA-3’ for SCPP2; 5’-TGGCTCCTGCTGCTGCTGAT-3’, 5’-GAGTTCCGCCGCACTGTGAATGTT-3’ for SCPP3A; 5’-AGACGCTTGTGTCTTGGAGATGTTG-3’, 5’-GGAGCGAGCTGTTGGATGAGTA-3’ for SCPP3B; 5’-GCAATTGTTAGCCTGAGATGTTGTT-3’, 5’-GGGCTTGCGTCTCCGCTATCGAC-3’ for SCPP3C; 5’-AACGTCGCTGTCTGCTGTTA-3’, 5’-GGGCTGCTGACAGGACGAA-3’ for SCPP4; and 5’-CGGCCGACGCGCCCTCAG-3’, 5’-GCGGCTTACACCCACCTACA-3’ for SCPP5. Some of these primers, together with the 5’ or 3’ end primers specific to the SMART cDNA library (Clontech), were also used for PCR to obtain the 5’ or 3’ end of cDNA fragments (SCPP3A, SCPP3B, SCPP3C, and SCPP5) (7). In addition, the following primers were used to amplify the 5’ or 3’ end of DNA fragments: 5’-TCTCCGACATTCTCCTACCTCC-3’, 5’-ACACCTGGCGAGGAGAGACGAC-3’ for SCPP1; 5’-TTCCGAGTCCGACGCTGCTGATGTTG-3’, 5’-CCACAGCTGCTGGCTCCTTCAAT-3’ for SCPP2; and 5’-GGAGGTCCGTCGGAGATGTTGATGTTG-3’ for SCPP3. These PCR products were cloned into pCR1-TOPO (Invitrogen) and used to determine the nucleotide sequences (GenBank accession nos. DQ006652–DQ006652) (7).
SCPP5 are expressed in odontoblasts, whereas contact the IPE (Fig. 4).

Dental epithelium regresses, and mineralizing teeth directly

COL1 and SCPP3B are in the IPE associated with erupting teeth (Fig. 4).

Fugu SCPPs in Tooth Mineralization. Fugu has teeth in both the jaws (34, 35) and the pharyngeal cavity (Fig. 3A). Pharyngeal teeth are common in teleosts and are histogenetically similar to oral teeth (36). In the initial secretory stage after early tooth development (Fig. 4A and B), ISH analysis revealed the expression of SCPP genes as well as COL1 and SPARC (Fig. 4G–N); SCPP2, SCPP4, and SCPP5 in the IDE; SCPP1 and SCPP5 in odontoblasts; and SCPP3A and SCPP3B in the inner layer of pharyngeal epithelium (IPE) overlying tooth germs (Fig. 4C).

Subsequently, dental epithelium regresses, and mineralizing teeth directly contact the IPE (Fig. 4D and E). At these stages, SCPP1 and SCPP5 are expressed in odontoblasts, whereas SCPP3A and SCPP3B are in the IPE associated with erupting teeth (Fig. 4F).

COL1 and SPARC are expressed in early and late secretory stages in both IDE and odontoblasts as well as in the dental papilla and surrounding mesenchyme (Fig. 4C and F).

These observations clearly show that the IDE secretes both collagen and noncollagenous proteins. Notably, expression of the SCPP genes was detectable only in dental tissues and jaws (described in Unusual Fugu Jaw) with no significant signal detected in any soft tissues, cartilage, or bone, suggesting that many SCPPs cooperatively facilitate tooth mineralization in teleosts as well. The IDE expresses ECM protein genes early in the secretory stage, whereas odontoblasts continuously express these genes throughout this stage. These results suggest that SCPP2 and SCPP4, exclusively expressed by the IDE, are crucial to cap enameloïd, whereas SCPP5 is important for both enameloïd and dentin mineralization. In addition, SCPP1 is involved in dentin mineralization. Expression of SCPP3A and SCPP3B begins before the IPE directly contacts mineralizing teeth (Fig. 4C). Because of their small sizes (81 or 90 aa) these proteins seem to diffuse to the tooth surface and are involved in enameloïd mineralization. These two genes are continuously expressed in the IPE, which directly contacts mineralizing dentin (Fig. 4E).

Although our ISH analysis did not detect the expression of SCPP3C or SPARC1, the immediate common ancestor of SCPP genes was detectable only in dental tissues and jaws.
This structure is actually the jaw itself (Fig. 3), consisting of a beak-like structure but, as Georges Cuvier large tooth in each jaw quadrant (upper and lower, right and left) may suggest, the cartilage was stained with alcian blue, hematoxylin, and eosin. Left, labial side; right, lingual side. Meckel’s cartilage is stained with dark blue. (Scale bars: 10 μm.) ISH analysis revealed the expression of SCPP3A (F) and SCPP5 (G) at the initial secretory stage and SCPP1 (H), SCPP3A (I), SCPP3B (J), SCPP5 (K), COL1 (L), and SPARC (M) at the early jaw-wall completion stage, the latter of which are summarized in E. Arrowheads indicate the protein matrix (A–D) and the outer surface of the jaw-wall matrix (H–M). The oral epithelium detached from the jaw-wall surface is artifactual (D and H–M). IOE, inner oral epithelium; Ms, mesenchyme forming jaw wall; OOE, outer oral epithelium; PRM, protein matrix.

Unusual Fugu Jaw. Superficially, fugu (Tetraodontidae) has one large tooth in each jaw quadrant (upper and lower, right and left) consisting of a beak-like structure but, as Georges Cuvier described in 1805, this structure is actually the jaw itself (Fig. 3B) (34). Since then, the material of this structure has been described several times as bone, although its shiny ivory- or enamel-like appearance was also appreciated by the same authors (34, 35, 37–41). In the lower jaw, the early matrix for tissue mineralization develops between Meckel’s cartilage and the oral epithelium as a thin vertical plate, which appears to form within a mesenchymal condensation similar to bone (Fig. 5 A and B). This matrix associates with the lingual side of Meckel’s cartilage, whereas the jaw bone (dentary) itself forms on the labial side (Fig. 5C). At the apex, the wall bends toward the labial side and erupts into the oral cavity, although its surface remains covered by oral epithelium (Fig. 5D). The cells inside the jaw walls extend processes into the matrix in which the cell bodies are never embedded. In addition, the cuboidal or columnar inner epithelial and flat outer epithelial cell layers overlay the jaw walls. These characteristics are similar to those of the pharyngeal tooth (Fig. 4E) and are generally seen in teleost tooth development (18).

The similarity of the development of the jaw wall and teeth is corroborated by gene expression patterns (Fig. 5 E–M): SCPP3A and SCPP3B are expressed in the inner oral epithelium covering the jaw; SCPP1 and SCPP5 in the mesenchyme inside and at the bottom of the jaw wall where this structure grows; and COL1 and SPARC in two distinct mesenchymal cell populations, forming the jaw wall and the underlying dentary bone. This expression pattern is similar to that in the late secretory stage of pharyngeal tooth development (Fig. 4F). Thus, we conclude that the fugu lower jaw consists of beak-like dentin walls and the supportive dentary bone. The distribution of cells expressing SCPP3B and SCPP5 in early secretory stages is consistent with our histological observation that the initial ECM proteins accumulate within a mesenchymal condensation (Fig. 5 F and G). At these early stages, the true teeth have not yet grown. Later, a stack of horizontally oriented lamellar teeth consisting almost entirely of enameloid grow between the labial and lingual jaw walls (35, 38). When the occlusal surface of the wall is worn down, these teeth erupt through vertically, and they become worn and then are sequentially replaced from below. These tooth plates sheltered within beak-like jaws are also present in other teleosts such as parrot fish (34, 42).

Discussion

Teleost and Tetrapod SCPPs. The teeth in teleosts and tetrapods arose from a common ancestor, but the underlying genetic basis for tissue mineralization has evolved independently in these two lineages. Nevertheless, the SCPPs involved in tooth development show similar biochemical features among functionally corresponding proteins. Tetrapod enamel is a uniquely specialized hard tissue that forms on distinctive SCPPs secreted by ameloblasts, whereas teleost enamoid develops on COL1, the most abundant protein in the body, secreted by both IDE and odontoblasts (11). Despite this critical difference, SCPP2, SCPP4, and SCPP5, involved in enamoid mineralization, are all rich in Pro and Gln, similar to tetrapod enamel SCPPs. In addition, SCPP1 resembles dentin matrix acidic phosphoprotein 1, an abundant dentin ECM protein in tetrapods, in that both are highly acidic and have many Ser-Xaa-Glu motifs. Tetrapod SCPPs are rich in Pro, Gln, Ser, and/or Glu, a cluster of which forms an intrinsically disordered region that does not fold into rigid three-dimensional structures (31, 43). These regions bind to ions and crystals at low affinities and phosphorylation within these regions regulates activity of these proteins. Thus, the teleost and tetrapod SCPPs with similar amino acid compositions seem to share common mechanisms for dental tissue mineralization. However, the expression of SCPP3A and SCPP3B in the IPE overlying tooth germs was totally unexpected. In tetrapods, oral epithelium does not seem to secrete ECM proteins for tooth mineralization because their teeth develop deep in the jaws. In contrast, the teeth of many teleosts grow close to the oral or pharyngeal epithelium (e.g., zebrafish) (44), suggesting that these tissues are also involved in dental tissue mineralization in other teleosts.

Dentin in Jaw. We assume that the fugu jaw walls consist of dentin, as proposed by a previous study (38). However, many other studies have reported that the jaw walls are bone; later studies...
confirmed earlier macroscopic analysis (33, 34, 36, 38–40). Although the reasoning is not clearly described, the idea probably developed because true teeth grow inside this structure, hence the casing is the tooth and, even though it appears similar to ivory or enamel, the material of jaws cannot be considered as dentin or enameloid because these tissues have been found only in teeth and dermal skeletons. In fact, the structure of the fugu jaw is even more complicated. Diodontidae (such as porcupine fish) and some Tetraodontidae species have coalesced teeth embedded (ankylosed) in lingual jaw walls (34, 39, 45). Although these teeth had been undetectable in fugu (41), we found small teeth embedded in a part of the upper lingual jaw wall in fugu embryos (data not shown). These teeth, however, become completely incorporated into the jaw wall and are invisible in adults. No such small teeth were detected in other parts of the jaws.

In the lower jaw, the dentin wall is supported by the dentary bone, and these structures together constitute the jaw (Fig. 3B). This interpretation is consistent with our microcomputerized tomography analysis of adult fugu, which suggests that the mineral density of the shiny jaw wall is higher than that of the dentary bone (data not shown). However, we have not been able to identify any apparent dental lamina in the initial development of jaw wall, corroborating previous findings that the dentin walls are not teeth but constitute part of the “jaw” (34, 46). Moreover, the initial ECM proteins for the jaw wall appear to accumulate within mesenchymal condensation. This process is distinct from the development of previously known dentinous elements that form adjacent to the basal lamina between epithelium and mesenchyme, with the exception of fossil batoids (chondrichthians), some of which have dentin within the basal plate of a large scale distant from the basal lamina (3). Furthermore, the dentin wall extends at the bottom, at the boundary with the supportive bone (Fig. 5), whereas dentin usually grows only inward or centripetally (1, 3). Thus, we assume that the fugu jaw contains unique elements consisting of dentin.

The existence of this unique structural element in fugu jaws implies flexible constraints on the development of dental tissues (22) and suggests the possibility that other vertebrates may also develop unique structures through a process similar to that of the fugu jaw wall. The jaws of arthrodires, a group of placoderms (Fig. 1), have dentin columns on the lingual side of their specialized jawbone (47). The dentin column grows at the boundary between this structure and the adjoining bone in the basal part, similar to fugu jaw walls. The growth of the dentin column has been explained either by the transformation of the basal bone tissue into this dentin column (47) or by the coalescence of small teeth (48). Now, we suggest another possibility, that the dentin column grew within a mesenchymal condensation similar to the fugu jaw wall. The transformation theory is based on the histological similarity between bone and this particular type of dentin (semdentin). Thus semidentin, as well as some other types of ancient dentin with distinct histological characteristics, is considered as an intermediate tissue between bone and the typical dentin (orthodentin) of modern gnathostomes (1, 49). In contrast, beak dentin is genetically similar to tooth dentin, but the developmental process appears to be more like bone.

**Phenogenetic Drift in Tooth Evolution.** During the evolution of gnathostomes, strong and persistent adaptive natural selection for feeding and predation seems to have favored mineralized teeth (1). This stable phenotype is, however, sustained by independently evolved SCPPs in teleosts and tetrapods. Thus, this phenomenon appears to be an instance of phenogenetic drift; that is, a shift in the genotype that underlies the stable phenotype (26, 27). In osteichthians (Fig. 1), individual SCPPs have flexible functions, as illustrated by disordered proteins regulating extracellular calcium phosphate concentrations, and although the mineralized tooth is maintained by natural selection, independent histories of gene duplication allowed new SCPPs to become similarly specialized in the teleost and tetrapod lineages. Below, we will argue the evolution of teeth from the phenogenetic point of view.

Enameloid is closely related to dentin, a more stable tissue during vertebrate evolution (1, 11). Indeed, enameloid is always formed together with dentin in a cooperative process, but dentin can form without an enameloid cover (3). In fugu teeth, both IDE and odontoblasts express common genes, COL1, SPARC, and SCPP5 in enameloid and dentin formation, implying a similar function of these two tissues in ECM protein secretion. This similarity suggests that SCPP2 and SCPP4, expressed only in the IDE, could have been created by a duplication of ancient dentin SCPP genes and the subsequent loss of expression in odontoblasts. Thus, enameloid originally formed by ECM proteins common to dentin, and enameloid ECM proteins could have been easily co-opted from dentin proteins. Although we have suggested the possibility that neither agnathans nor chondrichthians had SCPPs (7), this argument is relevant to any enameloid ECM proteins. In fact, enameloid is present in pteraspidomorphs (Fig. 1), chondrichthians, and osteichthians (5), each of which is phylogenetically separated by clade(s) without enameloid; hence enameloid was innovated more than once in vertebrate evolution (4, 5). Our analysis supports this scenario and further suggests that dentin ECM proteins were co-opted as enameloid proteins at their origins, which allowed a gradual specialization from dentin to enameloid.

We have observed remarkable similarities in the function of the IDE in enameloid and ameloblasts in enamel formation. The IDE expresses genes for mineralization scaffold (COL1) and regulators specific to enameloid (SCPP2 and SCPP4). Moreover, the IDE secretes proteinases and removes ECM proteins (12, 14, 15). These functions are both common to ameloblasts during enamel mineralization (13, 16). However, enamel is more different from dentin than enameloid is from dentin, in that ameloblasts solely deposit distinctive enamel ECM proteins. Thus, we assume that enamel arose from enameloid, not vice versa, which is consistent with previous studies (4, 11, 19), and we suggest a scenario for the origin of enamel. Early in the evolution of sarcopterygians or osteichthians, enamel arose from an ancient enameloid, which was close to current teleost enameloid in terms of development and protein composition. During this evolution, an ancient enameloid SCPP was co-opted to serve as a scaffold and eventually replaced COL1. The duplication of enameloid SCPP genes may have facilitated this process.

Although enameloid was absent in the teeth of acanthodians (Fig. 1) or some extinct basal actinopterygians (50), the close relationship between teleost enameloid and tetrapod enamel supports the common origin of these two tissues. We thus assume that the highly mineralized surface tooth layer had been continuously present in osteichthians but that this layer was secondarily lost in these early actinopterygians. Alternatively, ancient enameloid SCPPs might have been used for scales of basal actinopterygians; highly mineralized tissue was absent in their teeth but present on scales (50). This tissue, called ganion, develops on noncollagenous ECM proteins deposited solely by epithelium (36); hence, ganion is similar to tetrapod enamel, but their evolutionary relationship has not been well resolved. The ancient enameloid SCPPs might have been used for ganion, whereas early actinopterygians secondarily lost tooth enameloid. Later, when enameloid covered their teeth again, ganion SCPPs were resurrected as enameloid proteins. This latter hypothesis, however, requires more intermediate steps. Two modern actinopterygians, bichirs (*Polypterus*) and gar pikes (*Lepisosteus*), also have scales with ganion, although their teeth are covered with cap enameloid (51). The identification of their ganion and
enameloid ECM proteins will further elucidate the evolutionary relationship among these highly mineralized tissues.

Dentin has many histological variations, some of which are considered as intermediates between bone and odontin (1, 2). Odontin is present in modern gnathostomes and some pteraspidomorphs (Fig. 1) but absent in many other agnathans (1, 5), suggesting that this tissue evolved more than once in vertebrates. In fact, some cephalaspidomorphs completely lack dentin (52); hence, some other dentinous tissues in agnathans may also have independent evolutionary origins. Similarly, enamloid arose from dentin more than once and, possibly enamel and ganoin may have independently originated from enamloid (4, 53). Thus, it is likely that at least some highly mineralized tissues and softer dentinous tissues are the result of convergent evolution. In addition, our analysis suggests that mineralization of teleost and tetrapod dentin has a distinct genetic basis in these lineages, generated by parallel gene duplications. Furthermore, in frog, tooth dentin and beak dentin are similar genetically but are produced by different developmental processes. This overall picture illustrates the subtle nature of the homology concept in evolution: tissues that are apparently similar at one level are not always similar at all levels; thus, homology can be partial and can be applicable to only some aspects of a trait, developmental process, or underlying genetic basis (21–27). We assume that, during vertebrate evolution, strong and persistent natural selection has sustained mineralized tissues, whereas the genes for tissue mineralization have extensively drifted because of relatively flexible biochemical constraints for mineral crystallization. Reiterative gene duplications may have facilitated this process. Vertebrate mineralized tissues, vital skeletal elements innovated by Paleozoic agnathans and continuously present in all major vertebrate clades, have evolved through phenogenetic drift.

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