REVIEW ARTICLE

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Tooth developmental biology: disruptions to enamel-matrix assembly and its impact on biomineralization

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Abstract

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Dental enamel is a composite bioceramic material that is the hardest tissue in the vertebrate body, containing long, thin crystallites of substituted hydroxyapatite (HAP). Over a lifetime of an organism, enamel functions under repeated and immense loads, generally without catastrophic failure. Enamel is a product of ectoderm-derived cells called ameloblasts. Recent investigations on the formation of enamel using cell and molecular approaches are now being coupled to biomechanical investigations at the nanoscale and mesoscale levels. For amelogenin, the principal structural protein for forming enamel, we have identified two domains that are required for its proper self-assembly into supramolecular structures referred to as nanospheres. Nanospheres are believed to control HAP crystal habit. Other structural proteins of the enamel matrix include ameloblastin and enamelin, but little is known about their biological importance. Transgenic animals have been prepared to investigate the effect of overexpression of wild-type or mutated enamel proteins on the developing enamel matrix. Amelogenin transgenes were engineered to contain deletions to either of the two self-assembly domains and these alterations produced significant defects in the enamel. Additional transgenic animal lines have been prepared and studied and each gives additional insights into the mechanisms for enamel biofabrication. This study summarizes the observed enamel phenotypes of recently derived transgenic animals. These data are being used to help define the role of each of the enamel structural proteins in enamel and study how each of these proteins impact on enamel biomineralization.

Key words: atomic force microscopy; dynamic light scattering; hydroxyapatite; leucine-rich amelogenin peptide; tyrosine-rich amelogenin peptide

Introduction

Enamel organic matrix assembly, and the subsequent processes of biomineralization occur outside the cell in the extracellular space. As is true for all extracellular biological matrices, the enamel organic matrix is assembled without direct contiguous cellular intervention. Enamel-matrix assembly follows the paradigm of basement membrane assembly. Like enamel, the basement membrane is a structure formed through the contributions of multiple protein members and is a structure that assembles solely by virtue of information contained within the protein constituents themselves. Some basement membrane proteins have been shown to contain multiple domains, with each domain contributing a unique interaction with another protein leading to assembly and the physiologic function is dependent upon the fully self-assembled ensemble of proteins (1,2). Unlike the basement membrane, neither does enamel remodel, nor does it remain in close contact with the cells that synthesize the enamel proteins. Rather, cells that produce enamel (ameloblasts) retract away from the forming matrix with concomitant mineral deposition (3). Once enamel has matured, the ameloblasts remain dormant until the tooth erupts and at this time these cells are shed from the enamel surface.

What remains hidden within the complex of enamel proteins is to discover the relationship between an enamel protein assembly interaction and the characteristic imposed by each of these interactions on the mineral crystallite. Protein–protein interactions may impose restraints on crystallite formation within the matrix, causing the preferred growth on a single crystallite face. Another protein–protein interaction may impose restrains on crystallite packing. These ideas have been (4), and continue to be explored by creating defects in the amelogenin assembly domains and ascertaining their effect in transgenic animals. That enamel prisms morphology reflects the morphology of ameloblasts in a species-specific manner is well appreciated (5). Unknown at present are the mechanisms by which the ameloblast remains in registry over a prescribed field of enamel organic matrix. Protein–protein interactions may also restrain the influence of each ameloblast to a specific field of the secreted enamel extracellular matrix. Our laboratory is actively pursuing proteomics studies aimed at identifying proteins that interact directly with the known enamel matrix proteins (6). Our methodologies include the yeast two-hybrid system (Y2H) (7) and other techniques that decipher protein–protein interactions.

Discovering protein–protein interactions of the enamel extracellular matrix

Since the discovery of a complementary DNA sequence for amelogenin in 1983 (8), our understanding of the enamel formation has been significantly aided by the subsequent discoveries of additional structural organic components of the enamel extracellular matrix, including ameloblastin (9,10) and enamelin (11). In addition, data from an animal model null for the biglycan gene (12,13) indicate that the biglycan protein, while not unique to the enamel matrix environment, plays a role in amelogenesis (14). Two enamel-specific proteases have also been recently characterized and discussed and these are kallikrein-4 (15) and matrix metalloproteinase-20 (MMP20) (16,17). The spatiotemporal expression of each of these enamel proteins have been, and continue to be, defined but what remains to be investigated is how each of these enamel matrix components interact with one another to form a self-assembled matrix competent to initiate and orchestrate the events of mineralization. These events of mineralization ultimately result in a mature enamel that is almost completely absent of any history of its protein origins. The important role that each individual protein plays toward the creation of enamel can be appreciated from the well-ordered hierarchical structure seen in mature enamel (18), but their roles in creating this elegant architecture has yet to be fully illuminated.

In this study, it is our intention to briefly introduce the nature of the known individual enamel proteins and summarize recent attempts at defining enamel-matrix assembly through identifying protein–protein interactions and protein assembly domains. Using the Y2H system (7) we have shown that amelogenin self-assembles and does not interact directly with ameloblastin (19). We were also able to demonstrate that ameloblastin does not self-assemble (19). With these data we and others believe that we can now define amelogenin nanosphere structures (20) as being mediated by definable self-assembly domains at the amino- and carboxyl-terminal regions of the amelogenin protein (4,21). Using the Y2H system we have data

(unpublished) showing that enamelin does not self-assemble, nor does it interact directly with either amelogenin or ameloblastin.

Animal studies may also shed light on the role each protein may play within the enamel matrix. For example, traces of a tyrosine-rich amelogenin peptide (TRAP) are present in mature enamel. The TRAP region of amelogenin includes the amino-terminal amelogenin self-assembly domain. In one study, we set out to answer whether changing the temporal expression TRAP to an earlier stage of enamel development would have a positive or negative impact on the resulting enamel architecture and its mechanical properties (22). These and other animal models are discussed.

Amelogenin

Amelogenin is the predominant protein in the developing enamel extracellular matrix. In humans, an amelogenin gene locus exists on both the X (AMELX; locus Xp22.3–p22.1) and Y (AMELY; locus Yp11) chromosomes (23) and both genes contain seven exons. In males, both the X and Y chromosomal-derived amelogenins are expressed, but the protein from the X-chromosome predominates (24). Functionally minor genomic differences exist between the AMELX and AMELY at the messenger RNA level (Fig. 1A) and at the protein level (Fig. 1B), but greater variation exists within the intron regions of these two genes (25) (Fig. 1C). These differences are often used in medicine and forensics to determine the sex of fetus or corpse whose sex is in question. For such investigational studies, the methodology of choice is the polymerase chain reaction (PCR) to distinguish between AMELX and AMELY based on the differences in size between well-defined genomic DNA regions (26,27). For example, using a forward PCR amplimer to an exon 3 sequence common to both genes and a reverse PCR amplimer to an exon 4 sequence common to both genes would produce an amelogenin-specific band differing in size for AMELX and AMELY. In this case, the size difference observed between exons 3 and 4 would be exactly 180 bp (Fig. 1C).

Several independent lines of investigation have showed an essential role for amelogenin during enamel organic extracellular matrix assembly. Humans affected by the inherited enamel defect *amelogenesis imperfecta* (AI) often exhibit alterations in the amelogenin X-chromosome gene locus which presumably reduces or eliminates amelogenin expression (28–30). Amelogenin knock-out mice also display an extremely severe AI phenotype (31). Enamel phenotypes resulting from gene mutations are broadly characterized as hypoplastic or hypomineralized (32,33) by clinicials, yet these terminologies imply that the condition is uniformly displayed throughout the enamel, and this is rarely the case.

Another informative feature of amelogenins is their highly conserved amino acid sequence. The molecular weight of the dominant isoform of secreted amelogenin from all species is approximately 20 kDa, which in humans equates to a 175 amino acid product, and in mice equates to a 180 amino acid product. Conservation is particularly obvious among the amino-terminal 50 residues and again in the carboxyl-terminal 20 residues (25,34). Conservation of the amino acid sequence often implies important physiologic relevance. The supramolecular assembly of amelogenin into 'nanospheres' has been assumed to be critical for the function of this structural protein during enamel formation. Two human pedigrees with an X-linked *amelogenesis imperfecta* (AIH1) phenotype (35,36) have point mutations in the amino-terminal, TRAP segment of amelogenin (*c.* amino acid residues 1–45). Both these documented AIH1 point mutations have been experimentally reproduced as recombinant proteins and, by comparing these mutated amelogenins with wild-type amelogenin, altered nanosphere dimensions (37) and altered amelogenin assembly kinetics (38) were observed.

The hydrophilic carboxyl-terminal of amelogenin binds hydroxyapatite (HAP) as demonstrated both *in vitro* and *in vivo*, and this suggests that the carboxyl-terminal region facilitates initial orientation of amelogenin along the forming enamel crystallites (39–42). Experimental support for self-assembly of enamel proteins comes from work on highly purified bacterial-expressed mouse amelogenin (M180) that was shown to assemble into nanospheres *in vitro*(4,43,44). Nanosphere assembly is also observed adjacent to HAP crystallites during *in vivo* enamel formation (45,46) suggesting that amelogenin self-assembly is an essential property required to direct the mineral phase.

Based upon this information, we initiated studies using the Y2H (21) to search for amelogenin-to-amelo-

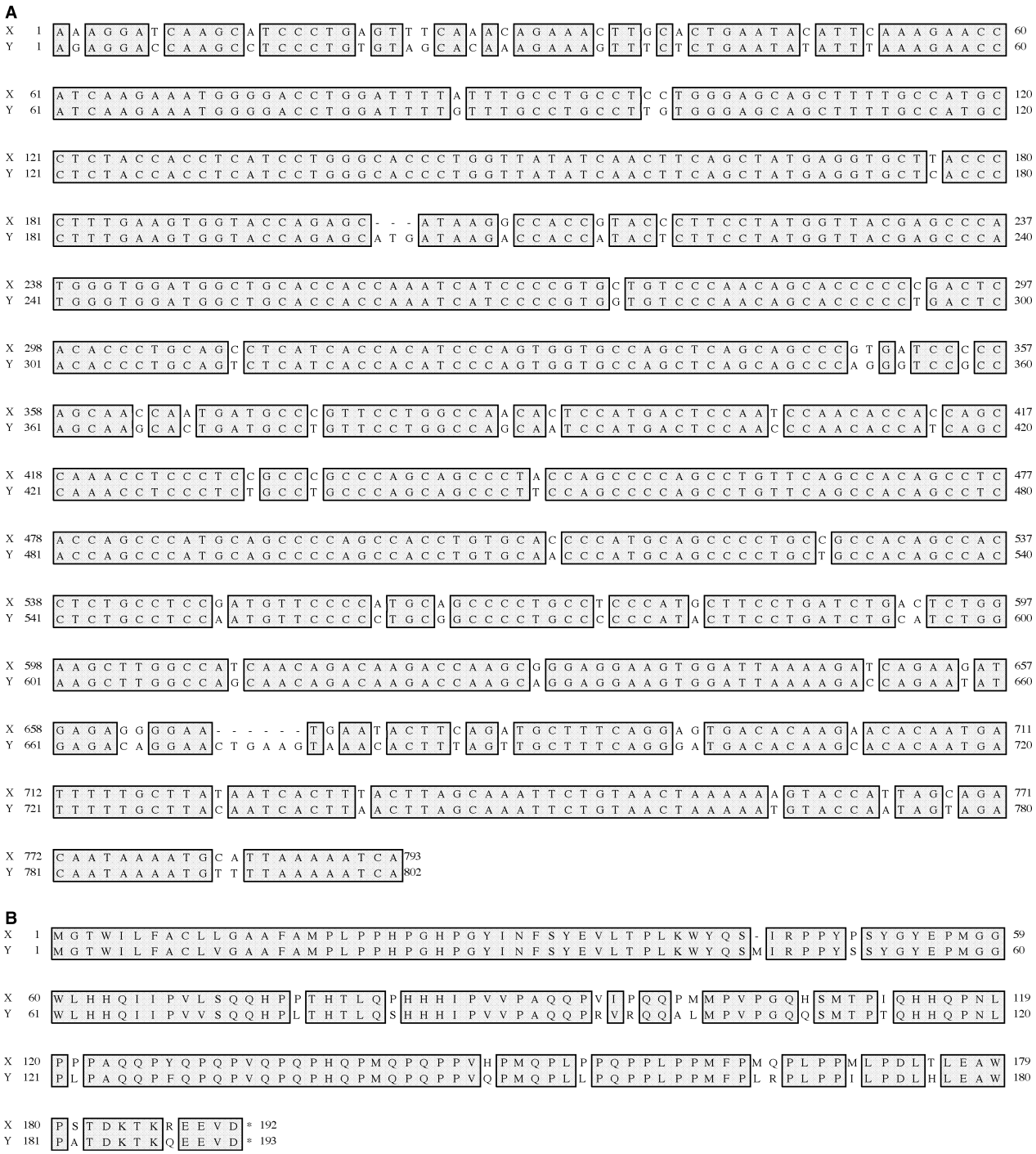

Fig. 1. ClustalW alignments for the human X-derived and Y-derived amelogenin gene and its mRNA and protein products. (A) Complementary DNA sequences for the human X (X) and human Y (Y) chromosome-derived amelogenin genes. (B) Protein sequences for the human X (X) and human Y (Y) chromosome-derived gene products. In males, the amelogenin protein is produced predominantly from the X chromosome. (C) Significant variation of the DNA sequence exists within the intron regions of these two amelogenin genes. The genomic sequence for AMELX exons 3–5 (AMELX Ex3–5) compared with AMELY exons 3–5 (AMELY Ex3–5) is shown. Exons 3–5 regions/domains are identified by the consensus line.

genin interacting peptide domains. This experimental strategy revealed that the mouse amelogenin self-assembly was dependent upon the amino-terminal residues 1–42 (domain A) and the carboxyl-terminal residues 157–173 (domain B)(21). Data suggest that the amelogenin self-assembly domains, as revealed by the

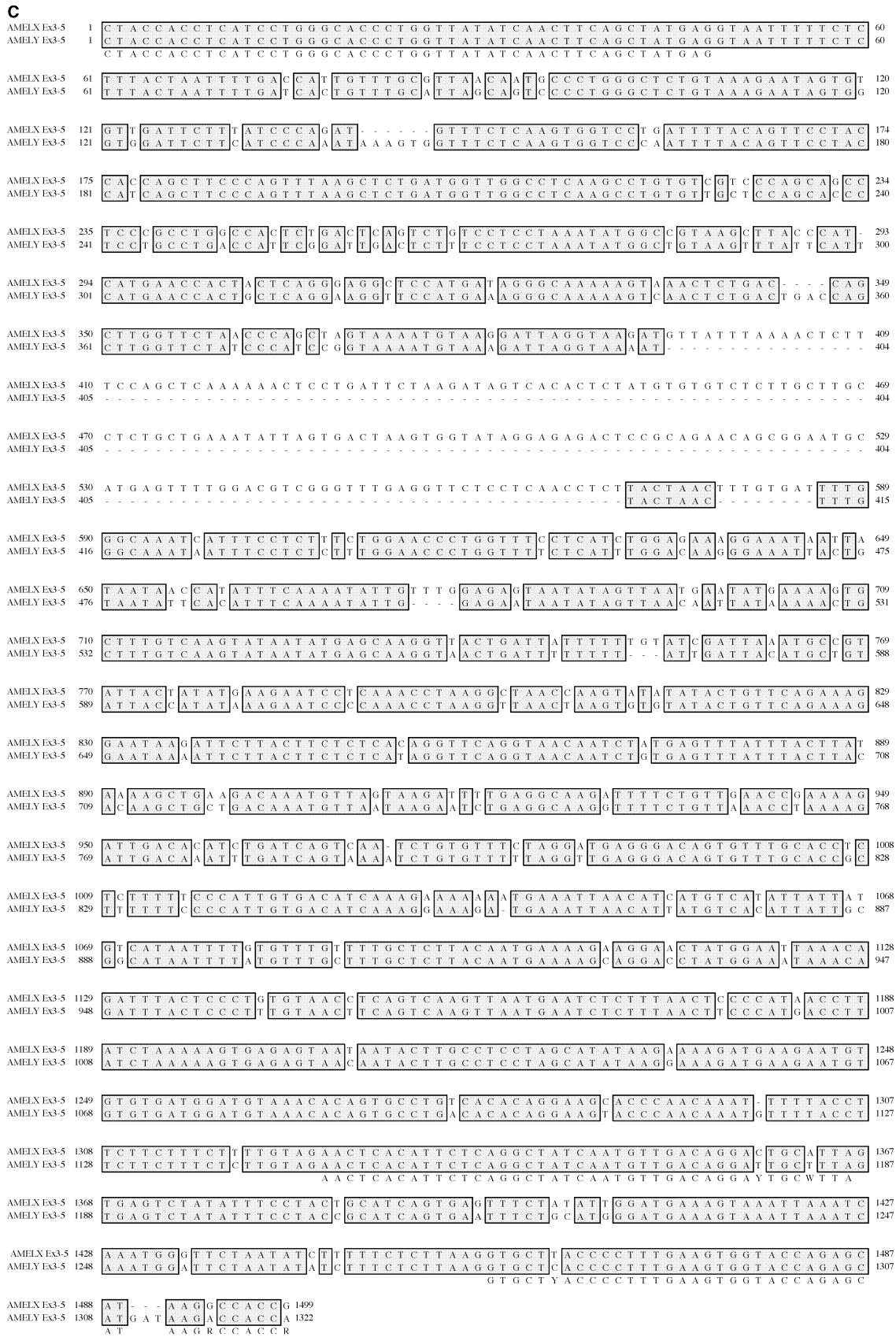

Fig. 1. (Continued)

Y2H system, are also relevant to the formation of normal enamel *in vivo*. It suggests further that amelogenin assembles though interactions between domains A and B and that the concept of amelogenin assembly into nanospheres is a valid model for enamel formation (38,47).

Leucine-rich amelogenin peptide

The leucine-rich amelogenin peptide (LRAP) is a result of alternate-splicing of the amelogenin mRNA (25,48). Alternative-splicing is possible because of the multiple exons contained in the transcribed amelogenin RNA. In the murine model, the secreted LRAP protein containing 59 amino acids composed of the 33 amino acid N′-terminal (not including the signal peptide) and the carboxyl-terminal 26 amino acids of mouse amelogenin M180 (49). Thus, the bulk of the internal region of M180 is absent, removed by alternative-splicing.

There is a growing body of literature suggesting that the LRAP isoform may act as a signaling molecule (48,50,51), thus altering the gene expression profile of cells that can receive the signal. This literature relates to animal models in which ectopic mineralized tissue results when enamel matrix proteins (including LRAP) are present (48), and also to the recent literature that relates to the clinical use of enamel matrix-derived proteins (EMDPs) for periodontal regeneration following destructive periodontal disease (51–53). Hence, discovering the physiologic role of LRAP is an area of enamel and periodontal regenerative biology that is currently attracting significant scientific and commercial attention.

Tyrosine-rich amelogenin peptide

During enamel mineralization, amelogenin is cleaved progressively from the carboxyl-terminus into smaller peptide fragments (54–56). Amelogenin fragments are generated by the actions of proteinases and result in the accumulation of the TRAP in the more mature enamel. In effect, TRAP is the amino-terminus of amelogenin. TRAP contains a lectin-binding motif defined previously as 'PYPSYGYEPMGGW' (57). It has been speculated that this lectin-binding property may serve to orient the amelogenin nanospheres by tethering them to the retracting Tomes' processes and the resulting interaction may serve to orientate the nanospheres to the retracting ameloblast (18). This would influence crystallite orientation and fits well with the previous observations that crystallites tend to form at an approximately perpendicular orientation to the secretory surfaces of the Tomes' processes (58). The TRAP segment of amelogenin contains all the self-assembly 'A' domains (amino acid residues 1–42)(21). Both *in vitro* and *in vivo* experimental data suggests that the removal of 'A' domain disrupts amelogenin-to-amelogenin self-assembly to favor the formation of amelogenin monomers (37).

Enamelin

In 1997, mRNAs for porcine enamelin were cloned and characterized (11), and in 2001, the human and mouse enamelin mRNAs were cloned and characterized (59). The human gene for enamelin (ENAM) maps to chromosome 4q21 (60,61), as does the ameloblastin gene (62,63). Human enamelin is a nine exon containing gene and is secreted as a 186-kD precursor protein which, once secreted, undergoes a series of proteolytic cleavages (11). Rajpar *et al.* (64) analyzed a family with an autosomal dominant, hypoplastic form of AI (AIH2) and found that the enamelin gene had a mutation in the splice donor site of intron 7. The position of this mutation appears to have an impact on mRNA splicing and the subsequent protein expression. Mardh *et al.* (65) have described a nonsense mutation in the enamelin gene causing AIH2.

Ameloblastin

In 1996 ameloblastin was simultaneously characterized by three different groups of investigators, two groups using rat incisors (9,62) and one group using porcine teeth (66). The human ameloblastin gene (AMBN) has been localized to chromosome 4q21 and contains 13 exons (62,63). As is true for amelogenin, multiple isoforms of ameloblastin exist in the developing enamel of all the species studied and each isoform may serve a unique physiologic role (67–69).

A physiologic role for the ameloblastin protein in tooth development remains unknown, however, there are

some observations that led to testable hypotheses. Immunologic identification of ameloblastin during secretory amelogenesis (enamel formation)(66,70) reveals an ameloblastin distribution within the enamel extracellular matrix that follows the ameloblast outline, resulting in a 'fish-net'-like partitioning (71). Ameloblastin can also be immunolocalized to Tomes' process, the highly specialized plasma membrane component of secretory ameloblast cells. The rat and mouse ameloblastin molecule has a 'DGEA' domain (72) that has been identified in collagen type I as a recognition site for alpha 2 beta 1 integrin (73,74), as well as a thrombospondin cell adhesion domain, 'VTXG' (1). These data previously prompted the suggestion that ameloblastin might serve as a part of the linkage between ameloblasts and the enamel extracellular matrix (71,72), however, neither of these peptide domains exist in the human or porcine ameloblastin molecules; thus, such a functional role appears unlikely. Ameloblastin has also been suggested to serve as a nucleator for crystallization (70,75); this is because it is expressed at mineralization initiation sites within the enamel (70,75). We have recently presented an animal model in which the upregulation of ameloblastin in the enamel organ resulted in a phenotype characteristic of AI (76).

Biglycan

The human biglycan gene (BGN) contains eight exons and is located on chromosome Xq28 (77,78). The protein encoded by this gene is a small cellular or pericellular matrix proteoglycan that is closely related in structure to two other small proteoglycans, decorin and fibromodulin (13,79). Decorin contains one attached glycosaminoglycan chain, while biglycan is thought to contain two glycosaminoglycan chains and it is for this reason that this protein is called biglycan. Biglycan is thought to function in the connective tissue metabolism by binding to collagen fibrils and transforming growth factor beta 1 (TGFB1) (80). High levels of TGFB1 mRNA and protein have been localized in developing cartilage, bone and skin suggesting that it plays a role in the growth and differentiation of these tissues (81).

Biglycan-deficient mice (gene knock-out animals) have been generated to study the role of biglycan (Bgn) *in vivo* (12). These transgenic animals appear normal at birth, but as they age they display a phenotype characterized by reduced growth rate and decreased bone mass. While this type of phenotype is commonly observed in specific collagen-deficient animals, it is rare to observe skeletal abnormalities in animals lacking non-collagenous proteins. Biglycan is also expressed in dentin (82). Goldberg *et al.* (14) studied these biglycan-null animals because they were interested in how dentin impacted such a phenotype. Significant changes in the enamel and dentin were noted in these animals. For example, the forming enamel was between threefold and fivefold thicker than that seen in normal control animals and this difference was explained by an enhanced level of amelogenin synthesis and secretion from secretory ameloblasts (14). The conclusion from this particular observation was that biglycan either directly or indirectly acts as a repressor of amelogenin expression (14).

Tuftelin

The human tuftelin gene (TUFT1) is located on chromosome 1q21. Circa 1987, a polyclonal antibody directed against a gel-purified 66 kDa non-amelogenin acidic enamel protein was used to screen a bovine, tooth-enriched gene expression library and from this screening a novel enamel cDNA was isolated and its corresponding protein was called tuftelin (83,84). The predicted amino acid sequence of tuftelin gave physical and chemical characteristics appropriate to the acidic non-amelogenin class of enamel proteins. Tuftelin has an amino acid composition similar to 'tuft' proteins of the mature enamel (84–86). The function of tuftelin remains unknown but one postulated function is as a nucleator for HAP crystal formation (86). This prediction is made based on its anionic character, its localization at dentine–enamel junction and its expression in ameloblast cells just prior to amelogenin (86,87). Tuftelin does not contain a recognizable signal peptide and, in addition it is also expressed in many non-mineralizing tissues (86,88–90), hence its role in enamel extracellular matrix biomineralization is currently being critically re-examined (91,92).

Kallikrein-4

The human gene for kallikrein-4 (KLK4) contains five exons and is located on chromosome 19q13.

Kallikrein-4 is one of the cluster of kallikrein genes located on chromosome 19q13. Kallikrein-4 (also known as prostase or enamel matrix serine proteinase 1 or serine proteinase 17) is a serine protease. In 1999, Nelson *et al.* (93) identified a gene they referred to as 'prostase' (now called kallikrein-4), which they believed demonstrated prostate-restricted expression. Kallikrein-4 encodes a 254 amino acid protein with a conserved and characteristic serine protease catalytic triad and an amino-terminal signal peptide. This arrangement of the kallikrein-4 protein indicates a secretory function. Today it is known that kallikrein-4 is expressed in limited tissues including prostate and developing teeth (15,93,94). In teeth, kallikrein-4 is produced by odontoblasts and also by secretory ameloblasts (15,94). Kallikrein-4 expression in the enamel matrix correlates with the disappearance of the enamel proteins (such as amelogenin) from the enamel matrix (15). The expression of proteolytic enzymes, including kallikrein-4, during enamel maturation appears to be necessary for the enamel to achieve its high degree of mineralization (54,95).

Matrix metalloproteinase-20

The human MMP20 gene is part of the cluster of matrix metalloproteinase genes that localize to human chromosome 11q22.3. The MMP20 gene contains 10 exon regions. Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of the extracellular matrix in normal physiologic processes, such as embryonic development and tissue remodeling (96,97), as well as in disease processes, such as arthritis and metastasis (98). Most MMPs are secreted as inactive proproteins, being activated after delivery to the extracellular environment.

The protein encoded by MMP20 degrades amelogenin and thus MMP20 is thought to play a critical role in tooth enamel formation. Under normal physiologic conditions, expression of MMP20 appears limited to teeth and in particular to the enamel organ (17). Only a single report exists in which MMP20 message is detected in cultured cell lines of non-dental origins (99). MMP20-deficient mice display an AI phenotype which includes disruptions to the enamel rod pattern and also hypoplastic enamel that delaminates from the dentin (17). With the exception of the changes noted in the dental tissue, these MMP20-null animals appear otherwise normal (17).

The amelogenin promoter, its spatiotemporal specificity and transgenic animal studies

A 3.5 kb bovine X chromosome-derived DNA region upstream of exon 1 of amelogenin has been used for promoter analysis and transgenic animal studies (100,101). This defined gene promoter region can be used to drive the expression of the selected gene products that are restricted to ameloblasts and the surrounding cells of the stratum intermedium. Thus this DNA contains the necessary regulatory elements required for the correct spatiotemporal amelogenin gene expression. This gene promoter can be used to change the enamel matrix composition by introducing the selected gene products through secretory ameloblast activities. Similarly, the 2.3 kb mouse X-chromosome amelogenin promoter encodes all the response elements to define the amelogenin gene activity (102–104). Detailed studies on the murine amelogenin promoter have been carried out and this confirmed that amelogenin gene expression was restricted to ameloblasts and the cells of the stratum intermedium in a developmentally staged expression pattern (102,103). This promoter is responsive to the CCAAT/enhancer-binding protein alpha (C/EBPalpha)(104). This mouse gene promoter has been used extensively to drive transgenes along the transgenic lines (4,22,76,105–107). Studies using this murine amelogenin promoter to drive expression of an introduced protein have included epitope tags within the expressed protein. Epitopes are used to confirm expression of the transgene and to track protein expression and for each epitope used, there are commercially available specific monoclonal antibodies. No ectopic expression has been noted till-date in any of the transgenic animal lines produced in our laboratories.

In addition to the bovine and murine amelogenin gene promoters, the murine ameloblastin promoter (2.5 kb upstream of intron 1) has also been cloned and partially characterized (75,108). This ameloblastin promoter is responsive to the transcription factor core-binding factor, alpha 1 subunit (CBF-alpha 1)(75) but to the authors knowledge, the ameloblastin promoter has not been used in transgenic animals.

The uniqueness of enamel may be defined by the sum of its individual components

As described above, forming enamel is a dynamic composite of a number of individual components and undoubtedly many more than those discussed. We have limited our focus to the organic extracellular components without including the mineral components. Many other genetic factors clearly have an impact on amelogenesis. The gene products briefly described above (with the exception of tuftelin) represent those whose expression remain relatively unique to the mineralized tooth structure, primarily to the enamel, but also in dentin during odontogenesis (69,70,109). It is apparent that the timing that these gene products are presented to the enamel matrix must be finely controlled and regulated. Understanding their gene transcription and the various transcriptional factors that govern RNA expression, are of major significance in our quest to understand amelogenesis. In the laboratory, this requires careful dissection of the gene promoter regions. This work has just begun for amelogenin, ameloblastin and also biglycan (110,111), but still awaits to be initiated for enamelin, kallikrein-4 and MMP20. Post-transcriptional modifications such as alternative splicing, and post-translational modifications such as phosphorylation (e.g. in amelogenin (112)) ensure that, even within the four structural proteins discussed (amelogenin, ameloblastin, enamelin and biglycan), functional diversity from a single gene is possible. Because of the limited tissue expression of these three enamel proteins, it appears that their removal from the enamel matrix during enamel maturation has required specific proteases whose spatiotemporal expression must also be exquisitely regulated. This need is met partially or fully with the serine protease kallikrein-4 and MMP20.

Alterations to amelogenin self-assembly *in vivo* result in enamel biomineralization defects

As stated above, the Y2H assay system for the detection of protein–protein interactions has been used to demonstrate that amelogenin contains two well-defined self-assembly domains, domain A and domain B (21). In addition, atomic force microscopy and dynamic light scattering have recently been used to study the assembly properties of recombinant amelogenin proteins that have been engineered with deletions of either the domain A or the domain B regions (37). By measuring the parameters of nanosphere size and assembly rates *in vitro*, it was concluded that domains A and domain B of amelogenin have significant and different roles to play in the nature and dynamics of the self-assembly of amelogenin nanospheres (37). Transgenic animals were then used to test the hypothesis that the self-assembly domains identified with *in vitro* model systems also operate *in vivo*. Transgenic animals were created using the murine amelogenin promoter and bearing either a domain A deleted or domain B deleted amelogenin transgene expressed in the altered amelogenin exclusively in ameloblasts (4). This altered amelogenin participates in the formation of an organic enamel extracellular matrix and, in turn, this matrix is defective in its ability to direct enamel mineralization (4). At the nanoscale level, the forming matrix adjacent to the secretory face of the ameloblast shows alteration in the size of the amelogenin nanospheres for the transgenic animal line, while at the mesoscale level of enamel structural hierarchy, 6-week-old enamel exhibits defects in enamel rod organization (Table 1). These changes are seen as being due to the perturbed organization of the precursor organic matrix (4). These animal studies reflect the critical dependency of amelogenin self-assembly to form a competent enamel organic matrix. Alterations to the matrix are reflected as defects in the structural organization of enamel.

Changes to the enamel matrix composition impacts on the final enamel structure as seen in transgenic animals

Recently our experimental work has included engineering transgenic animals that overexpress different enamel proteins, each in a spatiotemporal pattern mirroring that of amelogenin (102,103). Transgenic mice expressing and secreting mutated amelogenins into the enamel matrix have been briefly discussed above; and more detailed descriptions and discussions of these animals have been published (4,107). In addition, mice overexpressing the amino-terminal 45 amino acids of the mouse amelogenin, which is often referred to as the TRAP, have been engineered and

Table 1. **Transgenic animals showing an enamel phenotype**

Tg. animal and reference	Tomes' processes, nanoscale	Crystallite morphology, nanoscale/mesoscale	Prismatic enamel, mesoscale	Gross enamel phenotype, macroscale
Amelogenin deleted domain A (4)	Reduced nanosphere dimensions hypomineralization	NOD	Increased IR:R ratio	Hypomineralization
Amelogenin deleted domain B (4)	Heterogeneous nanosphere dimensions	NOD	Increased IR:R ratio	Mild hypomineralization
Amelogenin compound genetic defects (107)	Reduced nanosphere dimensions	NOD	Decreased level of prismatic organization and increased IR:R ratio	Hypomineralization
TRAP (22)	Reduced nanosphere dimensions	NOD	NOD	Mild hypomineralized
Ameloblastin (76)	Not investigated	Disruptions to the crystal-aspect ratio	Increased IR:R ratio	NOD
Tuftelin (92)	Not investigated	Disruptions to the crystal-aspect ratio	Decreased level of prismatic organization	NOD

Abbreviations: Tg., transgenic; NOD, no observed differences; IR, inter-rod; R, rod; TRAP, tyrosine-rich amelogenin peptide.

studied (22). Under normal circumstances, traces of TRAP remain as a part of the mature enamel and the reason that these animals were produced was to examine the impact such a hypermaturation (or accelerated maturation) had on enamel formation. There was little change in the phenotype of the enamel of TRAP transgenic animals (22). The other two transgenic animal lines which have been engineered and studied were for ameloblastin (76) and tuftelin (92). Overexpression of each of these enamel proteins had an impact on the enamel phenotype to some degree. Table 1 summarizes the observed changes for all these transgenic animal lines.

Clinical utility and applications

Dental caries has an adverse worldwide impact. Even if an individual is caries-free, he/she is encouraged to be continually monitored throughout their lifetime with the aim of preventing disease or recognizing incipient disease and preventing its progress. If dental caries are detected then the tooth is restored to a disease-free state. Despite the skill level of the dental practitioner, the mechanical removal of carious enamel and dentin also involves the removal of the unaffected tooth structure. As scientists, we are exploring enamel structure at the nanoscale (proteins and crystals) to the mesoscale (cell) through to the macroscale. For the better understanding of the enamel formation we are focusing on the biology of the organic and inorganic components of enamel. In a sense, we are looking at the individual 'building-blocks' that Nature has made available and uses to create the hardest biomineralized tissue known in vertebrates. Discovery through scientific investigations may ultimately dictate design codes and features for synthetically produced, but biologically inspired dental restorative materials. The authors believe that no currently available dental restorative material acts as an ideal enamel replacement, and that through a greater understanding of enamel formation (amelogenesis), better materials can be designed, created and/or crafted.

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References

1. Yamada Y, Kleinman HK. Functional domains of cell adhesion molecules. *Curr Opin Cell Biol* 1992;**4**:819–23.
2. Miner JH. Developmental biology of glomerular basement membrane components. *Curr Opin Nephrol Hypertens* 1998;**7**:13–19.
3. Simmer JP, Fincham AG. Molecular mechanisms of dental enamel formation. *Crit Rev Oral Biol Med* 1995;**6**:84–108.

4. Paine ML, Zhu DH, Luo W, Bringas Jr. P, Goldeberg M, White SN et al. Enamel biomineralization defects result from alterations to amelogenin self-assembly. *J Struct Biol* 2000;**132**:191–200.
5. Greenberg G, Bringas Jr. P, Slavkin HC. The epithelial genotype controls the pattern of extracellular enamel prism formation. *Differentiation* 1983;**25**:32–43.
6. Paine CT, Paine ML, Snead ML. Identification of tuftelin- and amelogenin-interacting proteins using the yeast two-hybrid system. *Connect Tissue Res* 1998;**38**:257–67.
7. Fields S, Song O. A novel genetic system to detect protein–protein interactions. *Nature* 1989;**340**:245–7.
8. Snead ML, Zeichner-David M, Chandra T, Robson KJ, Woo SL, Slavkin HC. Construction and identification of mouse amelogenin cDNA clones. *Proc Natl Acad Sci USA* 1983;**80**:7254–8.
9. Fong CD, Slaby I, Hammarstrom L. Amelin, an enamel related protein, transcribed in cells of the epithelial root sheath. *J Bone Miner Res* 1996;**11**:892–8.
10. Lee SK, Krebsbach PH, Matsuki Y, Nanci A, Yamada KM, Yamada Y. Ameloblastin expression in rat incisors and human tooth germs. *Int J Dev Biol* 1996;**40**:1141–50.
11. Hu C-C, Fukae M, Uchida T, Qian Q, Zhang Ch, Ryu OH et al. Cloning and characterization of porcine enamelin mRNAs. *J Dent Res* 1997;**76**:1720–9.
12. Xu T, Bianco P, Fisher LW, Longenecker G, Smith E, Goldstein S et al. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nat Genet* 1998;**20**:78–82.
13. Young MF, Bi Y, Ameye L, Chen XD. Biglycan knockout mice: new models for musculoskeletal diseases. *Glycoconj J* 2002;**19**:257–62.
14. Goldberg M, Septier D, Rapoport O, Young M, Ameye L. Biglycan is a repressor of amelogenin expression and enamel formation: an emerging hypothesis. *J Dent Res* 2002;**81**:520–4.
15. Hu JC, Zhang C, Sun X, Yang Y, Cao X, Ryu O et al. Characterization of the mouse and human PRSS17 genes, their relationship to other serine proteases, and the expression of PRSS17 in developing mouse incisors. *Gene* 2000;**251**:1–8.
16. Bartlett JD, Simmer JP, Xue J, Margolis HC, Moreno EC. Molecular cloning and mRNA tissue distribution of a novel matrix metalloproteinase isolated from porcine enamel organ. *Gene* 1996;**183**:123–8.
17. Caterina JJ, Skobe Z, Shi J, Ding Y, Simmer JP, Birkedal-Hansen H et al. Enamelysin (MMP-20) deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem* 2002;**277**:49598–604.
18. Paine ML, White SN, Luo W, Fong H, Sarikaya M, Snead ML. Regulated gene expression dictates enamel structure and tooth function. *Matrix Biol* 2001;**20**:273–92.
19. Paine ML, Krebsbach PH, Chen LS, Paine CT, Yamada Y, Deutch D et al. Protein-to-protein interactions: criteria defining the assembly of the enamel organic matrix. *J Dent Res* 1998;**77**:496–502.
20. Fincham AG, Moradian-Oldak J, Diekwisch TGH, Lyaruu DM, Wright JT, Bringas Jr. P et al. Evidence for amelogenin ''nanospheres'' as functional components of secretory-stage enamel matrix. *J Struct Biol* 1995;**115**:50–59.
21. Paine ML, Snead ML. Protein interactions during assembly of the enamel organic extracellular matrix. *J Bone Miner Res* 1997;**12**:221–7.
22. Paine ML, Zhu DH, Luo W, Snead ML. Over-expression of TRAP in the enamel matrix does not alter the enamel structural hierarchy. *Cells Tissues Organs* 2004;**176**:7–16.
23. Lau EC, Mohandas T, Shapiro LJ, Slavkin HC, Snead ML. Human and mouse amelogenin gene loci are on the sex chromosome. *Genomics* 1989;**4**:162–8.
24. Salido EC, Yen PH, Koprivnikar K, Yu LC, Shapiro LJ. The human enamel protein gene amelogenin is expressed from both the X and the Y chromosomes. *Am J Hum Genet* 1992;**50**:303–16.
25. Simmer JP, Snead ML. Molecular biology of the amelogenin gene. In: Robinson C, Kirkham J, Shore R, editors. *Dental Enamel: Formation to Destruction*. Boca Raton, FL: CRC Press; 1995. pp. 59–84.
26. Soares-Vieira JA, Billerbeck AE, Iwamura ES, Cardoso L, Romero Munoz D. Post-mortem forensic identity testing: application of PCR to the identification of fire victim. *Sao Paulo Med J* 2000;**118**:75–77.
27. Shinka T, Naroda T, Tamura T, Sasahara K, Nakahori Y. A rapid and simple method for sex identification by heteroduplex analysis, using denaturing high-performance liquid chromatography (DHPLC). *J Hum Genet* 2001;**46**:263–6.
28. Langerstrom M, Dahl N, Nakahori Y, Nakagime Y, Backman B Landegren U et al. A deletion in the amelogenin gene (AMG) causes X-linked amelogenesis imperfecta (AIH1). *Genomics* 1991;**10**:971–5.
29. Aldred MJ, Crawford PJM, Roberts E, Thomas NS. Identification of a nonsense mutation in the amelogenin gene (AMELX) in a family with X-linked amelogenesis imperfecta (AIH1). *Hum Genet* 1992;**90**:413–6.
30. Lagerstrom-Fermer M, Landegren U. Understanding enamel formation from mutations causing x-linked amelogenesis imperfecta. *Connect Tissue Res* 1995;**32**:241–6.
31. Gibson CW, Yuan ZA, Hall B, Longenecker G, Chen E, Thyagarajan T et al. Amelogenin-deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem* 2001;**276**:31871–5.
32. Hart PS, Hart TC, Gibson CW, Wright JT. Mutational analysis of X-linked amelogenesis imperfecta in multiple families. *Arch Oral Biol* 2000;**45**:79–86.
33. Wright JT, Hart PS, Aldred MJ, Seow K, Crawford PJ, Hong SP et al. Relationship of phenotype and genotype in X-linked amelogenesis imperfecta. *Connect Tissue Res* 2003;**44**(Suppl. 1):72–78.
34. Snead ML, Lau EC, Zeichner-David M, Fincham AG, Woo SL, Slavkin HC. DNA sequence for cloned cDNA for murine amelogenin reveal the amino acid sequence for enamel-specific protein. *Biochem Biophys Res Commun* 1985;**129**:812–8.
35. Lench NJ, Winter GB. Characterization of molecular defects in X-linked amelogenesis imperfecta (AIH1). *Hum Mutat* 1995;**5**:252–9.
36. Collier PM, Sauk JJ, Rosenbloom J, Yuan ZA, Gibson CW. An amelogenin gene defect associated with human x-linked amelogenesis imperfecta. *Arch Oral Biol* 1997;**42**:235–42.
37. Moradian-Oldak J, Paine ML, Lei YP, Fincham AG, Snead ML. Self-assembly properties of recombinant engineered amelogenin proteins analyzed by dynamic light scattering and atomic force microscopy. *J Struct Biol* 2000;**131**:27–37.
38. Paine ML, Lei YP, Dickerson K, Snead ML. Altered amelogenin self-assembly based upon mutations observed in human X-linked amelogenesis imperfecta (AIH1). *J Biol Chem* 2002;**277**:17112–6.
39. Aoba T, Moreno EC, Kresak M, Tanabe T. Possible roles of partial sequences at N- and C-termini of amelogenin in protein–enamel mineral interaction. *J Dent Res* 1989;**68**:1331–36.
40. Kirkham J, Zhang J, Brookes SJ, Shore RC, Wood SR, Smith DA et al. Evidence for charge domains on developing enamel crystal surfaces. *J Dent Res* 2000;**79**:1943–7.

41. Iijima M, Moriwaki Y, Wen HB, Fincham AG, Moradian-Oldak J. Elongated growth of octacalcium phosphate crystals in recombinant amelogenin gels under controlled ionic flow. *J Dent Res* 2002;**81**:69–73.
42. Shaw WJ, Campbell AA, Paine ML, Snead ML. The C-terminus of the amelogenin, LRAP, is orientated next to the hydroxyapatite surface. *J Biol Chem* 2004;**279**:40263–66.
43. Fincham AG, Moradian-Oldak J, Simmer JP, Sarte PE, Lau EC, Diekwisch TGH et al. Self-assembly of a recombinant amelogenin protein generates supramolecular structures. *J Struct Biol* 1994;**112**:103–9.
44. Moradian-Oldak J, Simmer PJ, Lau EC, Sarte PE, Slavkin HC, Fincham AG. Detection of monodisperse aggregates of a recombinant amelogenin by dynamic light scattering. *Biopolymers* 1994;**34**:1339–47.
45. Robinson C, Fuchs P, Weatherell JA. The appearance of developing rat incisor enamel using freeze-fracture technique. *J Cryst Growth* 1981;**53**:160–5.
46. Fincham AG, Moradian-Oldak J. Recent advances in amelogenin biochemistry. *Connect Tissue Res* 1995;**32**:119–24.
47. Fincham AG, Luo W, Moradian-Oldak J, Paine ML, Snead ML, Zeichner-David M. Enamel biomineralization: the assembly and dissassembly of the protein extracellular organic matrix. In: Teaford MF, Meredith-Smith M, Ferguson MWJ, editors. *Development, Function and Evolution of Teeth*. Cambridge: Cambridge University Press; 2000. pp. 303.
48. Veis A. Amelogenin gene splice products: potential signaling molecules. *Cell Mol Life Sci* 2003;**60**:38–55.
49. Lau EC, Simmer JP, Bringas Jr. P, Hsu DD, Hu C, Zeichner-David M et al. Alternative splicing of the mouse amelogenin primary RNA transcript contributes to amelogenin heterogeneity. *Biochem Biophys Res Commun* 1992;**188**:1253–60.
50. Veis A, Tompkins K, Alvares K, Wei K, Wang L, Wang XS et al. Specific amelogenin gene splice products have signaling effects on cells in culture and in implants in vivo. *J Biol Chem* 2000;**275**:41263–72.
51. Viswanathan HL, Berry JE, Foster BL, Gibson CW, Li Y, Kulkarni AB et al. Amelogenin: a potential regulator of cementum-associated genes. *J Periodontol* 2003;**74**:1423–31.
52. Zeichner-David M. Is there more to enamel matrix proteins than biomineralization? *Matrix Biol* 2001;**20**:307–16.
53. Diekwisch TG. The developmental biology of cementum. *Int J Dev Biol* 2001;**45**:695–706.
54. Bartlett JD, Simmer JP. Proteinases in developing dental enamel. *Crit Rev Oral Biol Med* 1999;**10**:425–41.
55. Fincham AG, Moradian-Oldak J. Comparative mass spectrometric analysis of enamel matrix proteins from five species suggest a common pathway of post-secretory proteolytic processing. *Connect Tissue Res* 1996;**35**:151–6.
56. Fincham AG, Moradian-Oldak J. Amelogenin post-translational modifications: carboxyterminal processing and the phosphorylation of bovine and porcine ''TRAP'' and ''LRAP'' amelogenins. *Biochem Biophys Res Commun* 1993;**197**:248–55.
57. Ravindranath RMH, Moradian-Oldak J, Fincham AG. Tyrosyl motif in amelogenins binds *N*-acetyl-D-glucosamine. *J Biol Chem* 1999;**274**:2464–71.
58. Nanci A, Warshawsky H. Characterization of putative secretory sites on ameloblasts of the rat incisor. *Am J Anat* 1984;**171**:163–89.
59. Hu JC, Zhang CH, Yang Y, Karrman-Mardh C, Forsman-Semb K, Simmer JP. Cloning and characterization of the mouse and human enamelin genes. *J Dent Res* 2001;**80**:898–902.
60. Hu CC, Hart TC, Dupont BR, Chen JJ, Sun X, Qian Q et al. Cloning human enamelin cDNA, chromosomal localization, and analysis of expression during tooth development. *J Dent Res* 2000;**79**:912–9.
61. Dong J, Gu TTDS, MacDougall M. Enamelin maps to human chromosome 4q21 within the autosomal dominant amelogenesis imperfecta locus. *Eur J Oral Sci* 2000;**108**:353–8.
62. Krebsbach PH, Lee SK, Matsuki Y, Kozac C, Yamada KM, Yamada Y. Full-length sequence, localization, and chromosome mapping of ameloblastin: a novel tooth-specific gene. *J Biol Chem* 1996;**271**:4431–5.
63. Mardh CK, Backman B, Simmons D, Golovlev I, Gu TT, Holmgren G et al. Human ameloblastin gene: genomic organization and mutation analysis in amelogenesis imperfecta patients. *Eur J Oral Sci* 2001;**109**:8–13.
64. Rajpar MH, Harley K, Laing C, Davies RM, Dixon MJ. Mutation of the gene encoding the enamel-specific protein, enamelin, causes autosomal-dominant amelogenesis imperfecta. *Hum Mol Genet* 2001;**10**:1673–7.
65. Mardh CK, Backman B, Holmgren G, Hu JC, Simmer JP, Forsman-Semb K. A nonsense mutation in the enamelin gene causes local hypoplastic autosomal dominant amelogenesis imperfecta (AIH2). *Hum Mol Genet* 2002;**11**:1069–74.
66. Hu C-C, Fukae M, Uchida T, Qian Q, Zhang Ch, Ryu OH et al. Sheathlin: cloning, cDNA/polypeptide sequences, and immunolocalization of porcine enamel sheath proteins. *J Dent Res* 1997;**76**:648–57.
67. Simmons D, Gu TT, Krebsbach PH, Yamada Y, MacDougall M. Identification and characterization of a cDNA for mouse ameloblastin. *Connect Tissue Res* 1998;**39**:3–12.
68. MacDougall M, Simmons D, Gu TT, Forsman-semb K, Mardh CK, Mesbah M et al. Cloning, characterization and immunolocalization of human ameloblastin. *Eur J Oral Sci* 2000;**108**:303–10.
69. Lee SK, Kim SM, Lee YJ, Yamada KM, Yamada Y, Chi JG. The structure of the rat ameloblastin gene and its expression in amelogenesis. *Mol Cells* 2003;**15**:216–25.
70. Nanci A, Zalzal S, Lavoie P, Kunikata M, Chen W, Krebsbach PH et al. Comparative immunochemical analyses of the developmental expression and distribution of ameloblastin and amelogenin in rat incisors. *J Histochem Cytochem* 1998;**46**:911–34.
71. Snead ML. Enamel biology logodaedaly: getting to the root of the problem, or ''Who's on first…''. *J Bone Miner Res* 1996;**11**:899–904.
72. Cerny R, Slaby I, Hammarstrom L, Wurtz T. A novel gene expressed in rat ameloblasts codes for proteins with cell binding domains. *J Bone Miner Res* 1996;**11**:883–91.
73. Staatz WD, Fok KF, Zutter MM, Adams SP, Rodriguez BA, Santoro SA. Identification of a tetrapeptide recognition sequence for the alpha2 beta1 integrin in collagen. *J Biol Chem* 1991;**266**:7363–7.
74. McCarthy AD, Uemura T, Etcheverry SB, Cortizo AM. Advanced glycation endproducts interfere with integrin-mediated osteoblastic attachment to a type-I collagen matrix. *Int J Biochem Cell Biol* 2004;**36**:840–8.
75. Dhamija S, Krebsbach PH. Role of Cbfa1 in ameloblastin gene transcription. *J Biol Chem* 2001;**276**:35159–64.
76. Paine ML, Wang HJ, Luo W, Krebsbach PH, Snead ML. A transgenic animal model resembling amelogenesis imperfecta related to ameloblastin over-expression. *J Biol Chem* 2003;**278**:19447–52.

77. Fisher LW, Heegaard AM, Vetter U, Vogel W, Just W, Termine JD et al. Human biglycan gene: putative promoter, intron–exon junctions, and chromosomal localization. *J Biol Chem* 1991;**266**:14371–7.
78. Traupe H, van den Ouweland AMW, van Oost BA, Vogel W, Vetter U, Warren ST et al. Fine mapping of the human biglycan (BGN) gene within the Xq28 region employing a hybrid cell panel. *Genomics* 1992;**13**:481–3.
79. Hardingham TE, Fosang AJ. Proteoglycans: many forms and many functions. *FASEB J* 1992;**6**:861–70.
80. Redini F. Structure and regulation of articular cartilage proteoglycan expression. *Pathol Biol (Paris)* 2001;**49**:364–75.
81. Dickinson ME, Kobrin MS, Silan CM, Kingsley Dm, Justice MJ, Miller DJ et al. Chromosomal localization of seven members of the murine TGF-beta superfamily suggests close linkage to several morphogenetic mutant loci. *Genomics* 1990;**6**:505–20.
82. Septier D, Hall RC, Embery G, Goldberg M. Immunoelectron microscopic visualization of pro- and secreted forms of decorin and biglycan in the predentin and during dentin formation in the rat incisor. *Calcif Tissue Int* 2001;**69**:38–45.
83. Deutsch D, Palmon A, Catalano-Sherman J, Laskov R. Production of monoclonal antibodies against enamelin and against amelogenin proteins of developing enamel matrix. *Adv Dent Res* 1987;**1**:282–8.
84. Deutsch D, Palmon A, Fisher LW, Kolodny N, Termine JD, Young MF. Sequencing of bovine enamelin (''tuftelin'') a novel acidic enamel protein. *J Biol Chem* 1991;**266**:16021–8.
85. Deutsch D. Structure and function of enamel gene products. *Anat Rec* 1989;**224**:189–210.
86. Mao Z, Shay B, Hekmati M, Fermon E, Taylor A, Dafni L et al. The human tuftelin gene: cloning and characterization. *Gene* 2001;**279**:181–96.
87. Zeichner-David M, Vo H, Tan H, Diekwisch T, Berman B, Theimann F et al. Timing of the expression of enamel gene products during mouse tooth development. *Int J Dev Biol* 1997;**41**:27–38.
88. Deutsch D, Palmon A, Dafni L, Mao Z, Leytin V, Young M et al. Tuftelin–aspects of protein and gene structure. *Eur J Oral Sci* 1998;**106**:315–23.
89. MacDougall M, Simmons D, Dodds A, Knight C, Luan X, Zeichner-David M et al. Cloning, characterization, and tissue expression pattern of mouse tuftelin cDNA. *J Dent Res* 1998;**77**:1970–8.
90. Saarikoski ST, Rivera SP, Hankinson O. Mitogen-inducible gene 6 (MIG-6), adipophilin and tuftelin are inducible by hypoxia. *FEBS Lett* 2002;**530**:186–90.
91. Mao Z, Shay B, Hekmati M, Fermon E, Taylor A, Dafni L et al. The human tuftelin gene: cloning and characterization. *Gene* 2001;**279**:181–96.
92. Luo W, Wen X, Wang HJ, MacDougall M, Snead ML, Paine ML. The in vivo over-expression of tuftelin in the enamel organic matrix. *Cells Tissues Organs* 2004;**177(4)**:212–20.
93. Nelson PS, Gan L, Ferguson C, Moss P, Gelinas R, Hood L et al. Molecular cloning and characterization of prostase, an androgen-regulated serine protease with prostate-restricted expression. *Proc Natl Acad Sci USA* 1999;**96**:3114–9.
94. Ryu O, Hu JC, Yamakoshi Y, Villemain JL, Cao X, Zhang C et al. Porcine kallikrein-4 activation, glycosylation, activity, and expression in prokaryotic and eukaryotic hosts. *Eur J Oral Sci* 2002;**110**:358–65.
95. Smith C. Cellular and chemical events during enamel maturation. *Crit Rev Oral Biol Med* 1998;**9**:128–61.
96. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000;**14**:2123–33.
97. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol* 2002;**3**:207–14.
98. Lynch CC, Matrisian LM. Matrix metalloproteinases in tumor-host cell communication. *Differentiation* 2002;**70**:561–73.
99. Vaananen A, Srinivas R, Parikka M, Palosaari H, Bartlett JD, Iwata K et al. Expression and regulation of MMP-20 in human tongue carcinoma cells. *J Dent Res* 2001;**80**:1884–9.
100. Chen E, Piddington R, Decker S, Park J, Yuan Z, Abrams W et al. Regulation of amelogenin gene expression during tooth development. *Dev Dyn* 1994;**199**:189–98.
101. Adeleke-Stainback P, Chen E, Collier P, Yaun ZA, Piddington R, Decker S et al. Analysis of the regulatory region of the bovine X-chromosomal amelogenin gene. *Connect Tissue Res* 1995;**32**:115–8.
102. Snead ML, Paine ML, Chen LS, Yoshida B, Luo W, Zhu D-H et al. The murine amelogenin promoter: developmentally regulated expression in transgenic animals. *Connect Tissue Res* 1996;**35**:41–47.
103. Snead ML, Paine ML, Luo W, Zhu D-H, Yoshida B, Lei Y-P et al. Transgene animal model for protein expression and accumulation into forming enamel. *Connect Tissue Res* 1998;**38**:279–86.
104. Zhou YL, Snead ML. Identification of CCAAT/enhancer-binding protein alpha as a transactivator of the mouse amelogenin gene. *J Biol Chem* 2000;**275**:12273–80.
105. Dunglas C, Septier D, Paine ML, Zhu DH, Snead ML, Goldberg M. Ultrastructure of forming enamel in mouse bearing a transgene that disrupts the amelogenin self-assembly domains. *Calcif Tissue Int* 2002;**71**:156–66.
106. Fong H, Heidel D, Sarikaya M, White SN, Paine ML, Luo W et al. Enamel structure-properties controlled by engineered proteins in transgenic animals. *J Bone Miner Res* 2003;**18**:2052–9.
107. Paine ML, Luo W, Zhu DH, Bringas P, Snead ML. Functional domains for amelogenin revealed by compound genetic defects. *J Bone Miner Res* 2003;**18**:466–72.
108. Dhamija S, Liu Y, Yamada Y, Snead ML, Krebsbach PH. Cloning and characterization of the murine ameloblastin promoter. *J Biol Chem* 1999;**274**:20738–43.
109. Begue-Kirn C, Krebsbach PH, Bartlett JD, Butler WT. Dentin sialoprotein, dentin phosphoprotein, enamelysin and ameloblastin: tooth-specific molecules that are distinctively expressed during murine dental differentiation. *Eur J Oral Sci* 1998;**106**:963–70.
110. Ungefroren H, Gellersen B, Krull NB, Kalthoff H. Biglycan gene expression in the human leiomyosarcoma cell line SK-UT-1. Basal and protein kinase A-induced transcription involves binding of Sp1-like/Sp3 proteins in the proximal promoter region. *J Biol Chem* 1998;**273**:29230–40.
111. Ungefroren H, Lenschow W, Chen WB, Faendrich FHK. Regulation of biglycan gene expression by transforming growth factor-beta requires MKK6-p38 mitogen-activated protein Kinase signaling downstream of Smad signaling. *J Biol Chem* 2003;**278**:11041–9.
112. Fincham AG, Moradian-Oldak J, Sarte PE. Mass-spectrographic analysis of a porcine amelogenin identifies a single phosphorylated locus. *Calcif Tissue Int* 1994;**55**:398–400.