Partial Rescue of the Amelogenin Null Dental Enamel Phenotype*

Yong Li‡, Cynthia Suggs‡, J. Timothy Wright§, Zhi-an Yuan‡, Melissa Aragon§, Hanson Fong¶, Darrin Simmons‡, Bill Daly§, Ellis E. Golub¶, Gerald Harrison‡, Ashok B. Kulkarni**, and Carolyn W. Gibson††

From the Departments of Anatomy and Cell Biology and Biochemistry, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania 19104-6030, the Department of Pediatric Dentistry, University of North Carolina, School of Dentistry, Chapel Hill, North Carolina 27599, the Department of Materials Science and Engineering, University of Washington, Seattle, Washington 98195, and the Functional Genomics Section, Laboratory of Cell and Developmental Biology, NIDCR, National Institutes of Health, Bethesda, Maryland 20892

The amelogenins are the most abundant secreted proteins in developing dental enamel. Enamel from amelogenin (Amelx) null mice is hypoplastic and disorganized, similar to that observed in X-linked forms of the human enamel defect amelogenesis imperfecta resulting from amelogenin gene mutations. Both transgenic strains that express the most abundant amelogenin (TgM180) have relatively normal enamel, but strains of mice that express a mutated amelogenin (TgP70T), which leads to amelogenesis imperfecta in humans, have heterogeneous enamel structures. When Amelx null (KO) mice were mated with transgenic mice that produce M180 (TgM180), the resultant TgM180KO offspring showed evidence of rescue in enamel thickness, mineral density, and volume in molar teeth. Rescue was not observed in the molars from the TgP70TKO mice. It was concluded that a single amelogenin protein was able to significantly rescue the KO phenotype and that one amino acid change abrogated this function during development.

Vertebrate tooth formation within the developing mandible or maxilla requires a series of intercellular interactions between ectomesenchymal and ectodermal cellular layers (1, 2). Reciprocal interactions lead to differentiation of a layer of ectomesenchymal cells in the internal dental papilla, which become odontoblast cells that secrete the dentin layer. The inner layer of the overlying enamel organ differentiates into ameloblast cells, which secrete a mineralized enamel layer over the dentin and is joined to it at the dentin-enamel junction. The two mineralized layers thicken during secretion of the respective mineralizing matrices, thereby separating the odontoblasts and the ameloblasts by the growing dentin and enamel layers. The dental root composed of underlying dentin and an outer layer of cementum develops later, while the tooth erupts into the oral cavity. After eruption, the odontoblasts in the dental pulp have the ability to repair the dentin, but the ameloblast layer originally on the surface of the crown is no longer present, and therefore biological repair of enamel is not possible.

In dentin, the mineral forms within a type I collagen matrix, in some ways similar to that found in bone, but a quite different mechanism is encountered in enamel. Non-collagenous proteins comprise the secreted enamel organic matrix forming a scaffold, which begins to mineralize immediately after deposition (3). During and after the secretory stage, programmed and extensive proteolysis of the enamel proteins is associated with mineral crystal growth (4, 5). Enamel crystals are arranged in bundles known as rods or prisms, which can extend from the junction with dentin to the enamel surface (6).

Approximately 90% of the enamel extracellular matrix is comprised of the highly conserved amelogenin proteins (7, 8). The biochemistry and structural biology of the amelogenins have been extensively studied (9–13), but problems are encountered in functional characterization because at least 15 amelogenin messages are produced due to extensive alternative splicing of the primary transcript (14–16). In addition, the translated amelogenins, as well as other enamel proteins, undergo organized proteolytic cleavages, which orchestrate the growth and development of the mineral crystals within the matrix (4). Eventually proteolysis is completed, most of the peptides are withdrawn from the enamel, and large enamel crystals have continued to grow, resulting in mature enamel, which is the hardest tissue in the body (13). Enamel of erupted teeth contains about 95% mineral by weight (4).

The amelogenin gene (AMELX in humans; Amelx in mice) has seven exons, although exons 8 and 9 have been reported in some species (17, 18). The amelogenin proteins range from 203 to 44 amino acids before proteolytic processing, not including the signal sequence. An active Y-chromosomal amelogenin gene has been identified in humans but is not present in mice (19–21).
The heterogeneous dental enamel defects known as amelogenesis imperfecta (AI;\textsuperscript{2} MIM (Mendelian Inheritance in Man) 300391, 301200) are the result of inherited conditions characterized by enamel that is hypoplastic or poorly mineralized and disorganized (for review, see Ref. 22). Mutations that account for some of the phenotypic variability have been described in several enamel protein or protease genes including amelogenin, enamelin, KLK4, and EMSP1, but there are other cases where the causative mutations have not yet been identified, and it is assumed that other gene mutations will be discovered (23). Fourteen gene mutations have been reported in the human AMELX gene, all leading to some form of AI. These individuals have hypoplastic enamel where the layer is either abnormally thin enamel with defective structure or an intermediate clinical presentation with characteristics of both hypoplasia and hypomineralization (for a review of the nomenclature, see Ref. 24). Different mutations within the AMELX gene are associated with different phenotypic appearance, with N-terminal mutations being associated with a mineral defect termed hypomaturation (soft with too much organic material), whereas C-terminal mutations or mutations in the signal sequence are associated with hypoplastic enamel (25, 26). Due to the extensive alternative splicing, a point mutation could affect several different amelogenin proteins but have no effect on others.

Because of complexities related to the number of amelogenin proteins, additional peptides resulting from proteolysis (which could then have new functions), and phenotypic differences between AI kindreds with X-linked mutations, it has been difficult to assign functions to individual amelogenin proteins. We began to address this question by generation of a murine model for AI that has an Amelx null mutation (KO), leading to hypoplastic and disorganized enamel (27). These KO mice have a deletion in the secretion signal sequence and the first 2 amino acids of the mature protein and have no detectable amelogenin proteins by Western blot using three anti-amelogenin antibodies.

In this report, we describe “rescue” experiments whereby transgenic mice that secrete a single amelogenin protein (28) are mated with KO mice to evaluate the effect of the individual amelogenin during enamel development, to gain insight into amelogenin function and the causes behind heterogeneity in X-linked AI. A mutation associated with AI in humans, a single amino acid change at codon 70, abrogates the rescue of the KO phenotype.

**Experimental Procedures**

**Animal Models**—Generation of the Amelx KO, TgM180 and TgP70T mice was described previously (27,28) (Table 1). Mice were housed in an AAALAC accredited facility, and procedures for some of the phenotypic variability have been described in several enamel protein or protease genes including amelogenin, enamelin, KLK4, and EMSP1, but there are other cases where the causative mutations have not yet been identified, and it is assumed that other gene mutations will be discovered (23). Fourteen gene mutations have been reported in the human AMELX gene, all leading to some form of AI. These individuals have hypoplastic enamel where the layer is either abnormally thin enamel with defective structure or an intermediate clinical presentation with characteristics of both hypoplasia and hypomineralization (for a review of the nomenclature, see Ref. 24). Different mutations within the AMELX gene are associated with different phenotypic appearance, with N-terminal mutations being associated with a mineral defect termed hypomaturation (soft with too much organic material), whereas C-terminal mutations or mutations in the signal sequence are associated with hypoplastic enamel (25, 26). Due to the extensive alternative splicing, a point mutation could affect several different amelogenin proteins but have no effect on others.

Because of complexities related to the number of amelogenin proteins, additional peptides resulting from proteolysis (which could then have new functions), and phenotypic differences between AI kindreds with X-linked mutations, it has been difficult to assign functions to individual amelogenin proteins. We began to address this question by generation of a murine model for AI that has an Amelx null mutation (KO), leading to hypoplastic and disorganized enamel (27). These KO mice have a deletion in the secretion signal sequence and the first 2 amino acids of the mature protein and have no detectable amelogenin proteins by Western blot using three anti-amelogenin antibodies.

In this report, we describe “rescue” experiments whereby transgenic mice that secrete a single amelogenin protein (28) are mated with KO mice to evaluate the effect of the individual amelogenin during enamel development, to gain insight into amelogenin function and the causes behind heterogeneity in X-linked AI. A mutation associated with AI in humans, a single amino acid change (29, 30), abrogates the rescue of the KO phenotype.

**Microscopic Analysis of Teeth**—Mandibles were fixed in 4% paraformaldehyde overnight, and light microscopy was used for initial characterization of all samples. Scanning electron microscopy (SEM) analysis of tooth surfaces and fractured internal enamel and dentin surfaces of incisors and molars was completed at 20 kV (JEOL JSM T330A, JEOL, Inc., Peabody, MA).

**MicroCT**—MicroCT scans were performed using a Skyscan portable x-ray microtomograph (MicroPhotonics, Allentown, PA). Samples were scanned under maximum voltage and power under saline solution in a 600-μl microcentrifuge tube, through 180° of rotation, with an exposure time of 420 ms. The specimens were positioned with the body of the mandible perpendicular to the sectioning plane with the condyle positioned apically and the incisor tip pointing up. The resulting images were processed by three-dimensional reconstruction software and analyzed to determine enamel density, dentin density, whole tooth density, and volumes for each in relation to the whole tooth. Hydroxyapatite standards were used for instrument calibration. Analysis of each incisor was localized to two 1-mm sections. The first region started where the incisor exited the bone and ended 1 mm toward the posterior, and the second region started at the first root canal of the first mandibular molar and proceeded 1 mm posteriorly. The mandibular first molar was analyzed at the position of the mesial root apex to determine enamel thickness and density.

**Nanoindentation**—Lower left mandibles were dissected from mouse heads, and the incisor was separated from each mandible, resulting in an intact set of molars and an incisor.
The set of molars or incisor was mounted in room temperature cure epoxy (Allied High Tech Products, Inc., Rancho Dominguez, CA). The mounted set of molars was ground from the mesial side using a 400-grit silicon carbide paper until the interior of the first molar was exposed, i.e. pulp, dentin, and enamel were simultaneously visible. Similarly, incisors were ground from the mesial side to remove 1 mm of the mature end using a 400-grit silicon carbide paper. The exposed interior of first molars or incisors was further polished by 1500-grit silicon paper followed by ultramicrotoming with a 2.5-mm-wide and 45° angle diamond knife (Diatome, Inc., Hatfield, PA) fitted on a MT 6000-XL ultramicrotome (Bal-Tec RMC, Inc., Tucson, AZ) in air. Regions that qualified for indentation measurements exhibited root-mean-square roughness <10 nm as measured by the nanoindenter (Berkovich diamond indenter with an 80-nm-tip radius). On every tooth, measurements were made on enamel and intertubular dentin for each region, at a contact depth of 10 µm. The nanoindentation unit on the Triboscope™nanoindentation unit (Hysitron, Inc., Minneapolis, MN) was adapted to nanoindentation using a 80-nm-tip radius. On every tooth, measurements were made on enamel and intertubular dentin for each region, at a contact depth of 10 µm. The magnification bar on SEM is 10 µm.

**RESULTS**

**Generation of Mouse Models to Evaluate Phenotypic Rescue of Amelogenesis Imperfecta in Amelogenin Null Mice**—Amelx KO female mice were mated with transgenic males that express the most abundant 180-amino-acid amelogenin (TgM180). Because Amelx is X-chromosomal without a Y-associated locus in mice, male pups from this cross would be Amelx KO and transgene-positive (TgM180KO; potential rescue) or transgene-negative (Amelx KO). Tail DNA was analyzed by PCR for genetic background and for the presence of the transgene (not shown).

**Analysis of Phenotype by MicroCT and Scanning Electron Microscopy**—MicroCT analysis of the mandible was used to simultaneously analyze mandibular incisors and molars of an individual mouse. Wild-type mice had elevated density in the enamel layer of molar and incisor when compared with dentin (Fig. 1A), whereas KO mice did not have any obvious enamel layer by MicroCT (Fig. 1B). Increased enamel opacity for molars of TgM180KO mice when compared with KO indicated potential rescue in these mice (Fig. 1C, arrowhead), whereas elevated opacity in the enamel of incisors in TgM180KO mice was not obvious (Fig. 1C, arrow). Scanning electron microscopy of molars from the TgM180KO mice (Fig. 1F) indicated that a thicker enamel layer was deposited in the molars of rescued mice, intermediate between wild-type (Fig. 1D) and KO (Fig. 1E), whereas the incisor (at higher magnification in Fig. 1I) showed little improvement in thickness or structure. An enamel layer was evident in both TgM180 and TgP70T molars and incisors (Fig. 1, G and H).
whereas TgM180KO mice developed up to 85% of the wild-type enamel volume, indicating that rescue had occurred when this normal amelogenin was expressed on the KO background. Enamel volume of TgM180KO mice was significantly different from that of KO and of TgP70TKO mice, which lacked evidence of rescue ($p < 0.001$; Fig. 2A).

Enamel volumes were similar in wild-type and TgM180 mice and in some of the TgP70T transgenic mice. The other TgP70T mice (8 out of 24) had no detectable enamel layer; this enamel phenotypic heterogeneity did not correlate with age, gender, or strain in the four TgP70T strains analyzed.

The enamel density from TgM180KO mice was similar to that of wild type, verifying that the 180-amino-acid amelogenin is able to orchestrate considerable rescue of enamel properties. Again, a significant difference between TgM180KO and KO enamel densities was observed ($p < 0.001$; Fig. 2B). Dentin volumes were similar for all mouse models analyzed (Fig. 2C), and many dentin densities (Fig. 2D) were similar, but dentin from TgM180 mice was elevated significantly over the dentin from the other mice ($p < 0.001$).

Analysis of Hardness and Elastic Modulus—Enamel hardness and elastic modulus measurements were compared using nanoindentation for wild-type and KO incisors and molars and for molars from the transgenic and transgenic/KO mice. Values for enamel were lower for KO incisors and molars when compared with wild-type (Table 2), complementing the microCT measurements described above. Enamel from the transgenic strains was similar to wild-type, but enamel from both of the transgenic/KO mice was similar to KO for both hardness and modulus, indicating minimal rescue of these mechanical properties by either transgene. Dentin hardness and modulus values were less than those of enamel as expected but similar when comparing molars to incisors of wild-type or of KO mice.

Comparison of Incisors and Molars—Because phenotypic rescue by TgM180 was observed in molars but not incisors (Fig. 1, C, F, and I), extracts were made from teeth from wild-type and transgenic/KO mice for protein analysis by Western blot (Figs. 3A and 4A). When identical amounts of wild-

![FIGURE 2. Scatter plot graphs indicate results from microCT reconstruction analysis for each of the mouse models. A and B, molar enamel volume (A) and density (B). C and D, molar dentin volume (C) and density (D).](image)

### TABLE 2

Enamel and dentin: hardness and elastic modulus (GPa)

|        | Enamel  | Dentin  |
|--------|---------|---------|
|        | Hardness| Elastic modulus | Hardness| Elastic modulus |
| Incisor |         |         |         |         |
| 4 week  | WT      | $n = 1$ | 2.9 ± 0.3 | 84 ± 4 | 0.7 ± 0.1 | 25 ± 2 |
|         | KO      | $n = 2$ | 1.7 ± 0.2 | 46 ± 2 | 0.7 ± 0.2 | 23 ± 3 |
| 6 week  | WT      | $n = 2$ | 2.8 ± 0.5 | 87 ± 4 | 0.8 ± 0.2 | 24 ± 3 |
|         | KO      | $n = 2$ | 1.8 ± 0.2 | 45 ± 2 | 0.7 ± 0.2 | 23 ± 3 |
| Molar   |         |         |         |         |         |
| 4 week  | WT      | $n = 1$ | 2.7 ± 0.3 | 83 ± 4 | 0.7 ± 0.1 | 23 ± 2 |
|         | KO      | $n = 1$ | 1.7 ± 0.2 | 46 ± 2 | 0.6 ± 0.2 | 22 ± 3 |
| 6 week  | WT      | $n = 1$ | 2.7 ± 0.4 | 84 ± 3 | 0.7 ± 0.2 | 24 ± 3 |
|         | KO      | $n = 1$ | 1.6 ± 0.3 | 45 ± 3 | 0.7 ± 0.2 | 22 ± 3 |
| Molar   | TgM180  | $n = 9$ | 2.9 ± 0.3 | 84 ± 4 | 0.7 ± 0.1 | 24 ± 3 |
|         | TgP70T  | $n = 4$ | 2.8 ± 0.4 | 84 ± 4 | 0.8 ± 0.1 | 22 ± 3 |
| Molar   | TgM180KO| $n = 3^*$| 1.7 ± 0.5 | 41 ± 5 | 0.7 ± 0.2 | 23 ± 4 |
|         | TgP70TKO| $n = 2^*$| 1.6 ± 0.5 | 41 ± 4 | 0.7 ± 0.3 | 22 ± 3 |

*For both dentin measurements, $n = 4$. 

MAY 30, 2008 • VOLUME 283 • NUMBER 22
Amelogenin Null Rescue

Western analysis of amelogenin in incisors and molars. A, extracts from incisors or M1 molars from 4-day-old mice were separated by gel electrophoresis and transferred to a membrane, which was probed with anti-amelogenin antibody. Lanes 1 and 2 (incisors) from TgM180KO, 10 μg; lanes 3 (molars) and 4 (incisors) from WT, 5 μg; lanes 5 (molars) and 6 (incisors) from WT, 10 μg; and lanes 7 (incisors) and 8 (molars) from TgM180KO, 20 μg. The arrow indicates a 26-kDa amelogenin. B, dot blot analysis indicated that similar amounts of total protein were used for each sample. The numbers refer to the lanes in the Western blot.

DISCUSSION

Transgenic mice that overexpress TgM180 (28) had relatively normal enamel structure, indicating that excess normal amelogenin was tolerated well. This was not surprising as mice that overexpress other amelogenins such as TgLRAP (leucine-rich amelogenin peptide) or TgTRAP (tyrosine-rich amelogenin peptide) also do not have enamel anomalies (31, 35). When the proline to threonine mutation was introduced at amino acid 70 (TgP70T), transgenic mice developed an enamel defect (28) similar to that seen in human patients with this mutation (36).

type molar or incisor protein extract were loaded on a gel, the amount of amelogenin detectable by anti-amelogenin antibody is approximately the same by Western blot. However, when the same experiment was done with transgenic/KO molars and incisors, we invariably found more transgenic amelogenin detectable in molar extracts when compared with extracts from incisors. Although the wild-type samples have several amelogenin bands as expected, primarily the TgM180 or TgP70T amelogenins are seen in extracts from TgM180KO or TgP70TKO mice due to the KO background. This analysis was repeated using additional TgM180KO or TgP70TKO mice, and Western blot band intensities were analyzed by ImageJ. The ratio of molar to incisor band intensities was relatively constant for wild-type amelogenins but varied considerably for all TgM180KO and TgP70TKO mice examined, with molars invariably having more intense amelogenin bands (p < 0.05). Protein concentrations by dot blot analyses did not vary significantly (Figs. 3B and 4B) when molars and incisor total protein concentrations from transgenic/KO mice were compared by t test.

The M180 amelogenins assemble in vitro into structures referred to as nanospheres, which are 15–20-nm spherical structures found between the growing ribbon-like crystals early in enamel development (38–40). Nanospheres organized along the developing crystals are thought to provide a scaffold to guide crystal growth as the amelogenin proteins are hydrolyzed in an orchestrated manner. It has been proposed that the nanospheres could provide the environment for the initiation of mineral crystals in normal enamel or have a required interactive relationship between protein assembly and mineral growth (13, 41). Although similar micro-ribbon-like structures are observed by transmission electron microscopy in vivo as can be reproduced in vitro with Escherichia coli-expressed 179-amino-acid amelogenins (see review by Ref. 42), the actual composition in vivo may include other enamel proteins, or other amelogenins.

A single residue alteration in the 180-amino-acid amelogenin protein abrogated any rescue in the TgP70TKO mice, and several theories have emerged as to how this amelogenin mutation could lead to defective enamel. Nanospheres assembled in vitro from M180 with a P70T mutation are larger with hydrodynamic radii of 45.5 nm, leading to the idea that protein–protein interactions are altered during assembly when this mutation is present (43). The P70T mutation is adjacent to a normal amelogenin cleavage site, and in vitro the mutation causes delay in the proteolytic cleavage time frame, which could lead to retention of excess protein in enamel (44, 45). This region has also been identified as a binding domain for sugars or sugar mimicking peptides and could have a role in assembly or binding to other enamel or dentin proteins (46, 47). Since cell binding activity has been described for M180, this function may also be disrupted by this mutation (48).

Because amelogenin expression has been detected at a low level in dental pulp cells from several species (36, 49–51), the
properties of dentin were compared in wild-type and KO mice. The absence of amelogenin does not appear to alter histological appearance, hardness, elastic modulus, or volume of dentin in the KO mice, but a slight increase in enamel density and a significant increase (p < 0.001) in dentin density were seen in TgM180 mice. Overexpression of TgM180 on this wild-type background could lead to this increase as it has been shown that overexpression in enamel of a dentin sialophosphoprotein protein involved in dentin formation positively impacted the physical properties of enamel in the transgenic mice (52).

Rescue by TgM180 is detected in molars where expression of the transgene is higher, whereas rescue is not obvious in incisors by microCT of TgM180KO mice. In vitro crystal growth experiments have shown that large mineral crystals grew in the presence of 1–2% amelogenins but not at 0.5% or less (53). The higher expression of the transgene in molars when compared with incisors could be due to the use of bovine regulatory regions for these transgenes, as rodent incisors are continuously erupting, whereas bovine upper incisors do not develop and lower incisors do not have a continuous eruption pattern. An advantage to this strategy is that it may provide an in vivo model where the transgene has two levels of activity in two different tooth types, allowing direct comparison of degree of rescue in a single animal. It may, in future work, be possible to correlate the amount of TgM180 present during development with level of rescue.

CONCLUSION

The most abundant wild-type amelogenin (M180) increases Amelx null enamel thickness from a thin 10-μm layer to up to 85% of wild-type enamel in molars. Since 100% rescue was not observed, it could be proposed that insufficient transgenic protein was secreted or that the other 14 or so amelogenins produced by alternative splicing of the primary transcript are required for normal enamel to be produced. The abundant alternative splicing seen in many species (54) may be a mechanism to fine-tune the process or to produce species-specific variations in enamel structure. It will be interesting to measure nanosphere and crystal dimensions in the various animal models described here to determine the effect of amelogenin amount and to compare the effect of the 180-amino-acid amelogenin and the P70T mutation on crystal dimensions and growth.

Acknowledgments—We thank Drs. Colin Robinson, Rochelle Lindemeyer, and Eric Everett for informative discussions and Steven Labadessa and Sylvia Decker for technical contributions. We acknowledge the School of Dental Medicine vivarium staff for attentive husbandry of the mice.

REFERENCES

1. Thesleff, I., Vahtokari, A., and Partanen, A. M. (1995) Int. J. Dev. Biol. 39, 35–50
2. Tucker, A. S., and Sharpe, P. T. (1999) J. Dent. Res. 78, 826–834
3. Robinson, C., Briggs, H. D., Atkinson, P. J., and Weatherall, J. A. (1979) J. Dent. Res. 58 (Spec. Issue B) 871–882
4. Smith, C. (1998) Crit. Rev. Oral Biol. Med. 9, 128–161
5. Simmer, J. P., and Hu, J. C. C. (2002) Connect. Tissue Res. 43, 441–449
6. Robinson, C., Shore, R. C., Brooks, S. J., Strafford, S., Wood, S. R., and Kirkham, J. (2000) Crit. Rev. Oral Biol. Med. 11, 481–495
7. Termine, J. D., Belcourt, A. B., Christner, P. J., Conn, K. M., and Nylen, M. U. (1980) J. Biol. Chem. 255, 9760–9768
8. Delgado, S., Ishiyama, M., and Sire, J.-Y. (2007) J. Dent. Res. 86, 326–330
9. Fincham, A. G., Belcourt, A. B., Termini, J. D., Butler, W. T., and Cothran, W. C. (1983) Biochem. J. 211, 149–154
10. Fincham, A. G., Moradian-Oldak, J., and Simmer, J. P. (1999) J. Struct. Biol. 126, 270–299
11. Brooks, S. J., Robinson, C., Kirkham, J., and Bonass, W. A. (1995) Arch. Oral Biol. 40, 1–14
12. Moradian-Oldak, J. (2001) Matrix Biol. 20, 293–305
13. Margolis, H. C., Beniash, E., and Fowler, C. E. (2006) J. Dent. Res. 85, 775–793
14. Hu, C. C., Ryu, O. H., Qian, Q., Zhang, C. H., and Simmer, J. P. (1997) J. Dent. Res. 76, 641–647
15. Li, Y., Yuan, Z. A., Aragon, M. A., Kulkarni, A. B., and Gibson, C. W. (2006) Eur. J. Oral Sci. 114, Suppl. 1, 190–193
16. Bartlett, J. D., Ball, R. L., Kawai, T., Tye, C. E., Tsuchiya, M., and Simmer, J. P. (2006) J. Dent. Res. 85, 894–899
17. Li, W., Mathews, C., Gao, C., and DenBesten, P. K. (1998) Arch. Oral Biol. 43, 497–504
18. Papagerakis, P., Ibarra, J. M., Inozentseva, N., DenBesten, P., and MacDougall, M. (2005) J. Dent. Res. 84, 613–617
19. Nakahori, Y., Takenaka, O., and Nakagome, Y. (1991) Genomics 9, 264–269
20. Salido, E. C., Yen, P. H., Koprivnikar, K., Yu, L. C., and Shapiro, L. I. (1992) Am. J. Hum. Genet. 50, 303–316
21. Chapman, V. M., Keitz, B. T., Distech, C. M., Lau, E. C., and Sneh, M. L. (1991) Genomics 10, 23–28
22. Wright, J. T. (2006) Am. J. Med. Gen. 140A, 2547–2555
23. Ozdemir, D., Hart, P. S., Ryu, O. H., Choi, S. J., Ozdemir-Karatas, M., Firatli, E., Piesco, N., and Hart, T. C. (2005) J. Dent. Res. 84, 1031–1035
24. Hart, P. S., Hart, T. C., Simmer, J. P., and Wright, J. T. (2002) Arch. Oral Biol. 47, 255–260
25. Wright, J. T., Hart, P. S., Aldred, M. J., Seow, K., Crawford, P. J. M., Hong, S., Gibson, C., and Hart, T. C. (2003) Connect. Tissue Res. 44, Suppl. 1, 72–78
26. Sneh, M. L. (2003) Connect. Tissue Res. 44, (Suppl 1), 47–51
27. Gibson, C. W., Yuan, Z. A., Hall, B., Longenecker, G., Chen, E., Thygaramjan, T., Sreenath, T., Wright, J. T., Decker, S., Piddington, R., Harrison, G., and Kulkarni, A. B. (2001) J. Biol. Chem. 276, 31871–31875
28. Gibson, C. W., Yuan, Z. A., Li, Y., Daly, B., Suggs, C., Aragon, M. A., Alawi, F., Kulkarni, A. B., and Wright, J. T. (2007) J. Dent. Res. 86, 331–335
29. Sauk, J. J., Jr., Lyon, H. W., and Witkop, C. J., I. (1972) Am. J. Hum. Genet. 24, 267–276
30. Collier, P. M., Sauk, J. J., Rosenblom, J., Yuan, Z. A., and Gibson, C. W. (1997) Arch. Oral Biol. 42, 235–242
31. Chen, E., Yuan, Z. A., Wright, J. T., Hong, S. P., Li, Y., Collier, P. M., Hall, B., D’Angelo, M., Decker, S., Piddington, R., Abrams, W. R., Kulkarni, A. B., and Gibson, C. W. (2003) Calcif. Tissue Int. 73, 467–495
32. Oliver, W., and Pharr, G. (1992) J. Mater. Res. 4, 564–1586
33. Gibson, C. W., Kucic, U., Callier, P., Shen, G., Decker, S., Bashir, M., and Rosenblom, J. (1995) Connect. Tissue Res. 32, 109–114
34. Moeremans, M., Daneels, G., and De Mey, J. (1985) Anal. Biochem. 145, 315–321
35. Paine, M. L., Zhu, D. H., Luo, W., and Sneh, M. L. (2004) Cells Tissues Organs 176, 7–16
36. Ravassipour, D. B., Hart, P. S., Hart, T. X., Ritter, A. V., Yamauchi, M., and Kulkarni, A. B., D’Angelo, M., Sire, J.-Y. (2007) J. Mater. Res. 22, 1476–1481
37. Fearnhead, R. W. (1960) Nature 188, 509–510
38. Robinson, C., Fuchs, P., and Weatherall, J. A. (1981) J. Crystal Growth 53, 160–165
39. Fincham, A. G., Moradian-Oldak, J., Diekwisch, T. G., Lyaruu, D. M.,
Amelogenin Null Rescue

Wright, J. T., Bringas, P., Jr., and Slavkin, H. C. (1995) J. Struct. Biol. 115, 50–59
41. Robinson, C., Shore, R. C., Wood, S. R., Brookes, S. J., Smith, D. A. M., Wright, J. T., Connell, S., and Kirkham, J. (2003) Connect. Tissue Res. 44, Suppl. 1, 65–71
42. Moradian-Oldak, J., and Goldberg, M. (2005) Cells Tissues Organs 181, 202–218
43. Moradian-Oldak, J., Paine, M. L., Lei, Y. P., Fincham, A. G., and Snead, M. L. (2000) J. Struct. Biol. 131, 27–37
44. Li, W., Gibson, C. W., Abrams, W. A., Andrews, D. W., and DenBesten, P. (2001) Matrix Biol. 19, 755–760
45. Li, W., Gao, C., Yan, Y., and DenBesten, P. (2003) Arch. Oral Biol. 48, 177–183
46. Ravindranath, R. M. H., Moradian-Oldak, J., and Fincham, A. G. (1999) J. Biol. Chem. 274, 2464–2471
47. Ravindranath, R. M. H., Tam, W. Y., Nguyen, P., and Fincham, A. G. (2000) J. Biol. Chem. 275, 39654–39661
48. Hoang, A. M., Klebe, R. J., Steffensen, B., Ryu, O. H., Simmer, J. P., and Cochran, D. L. (2002) J. Dent. Res. 81, 497–500
49. Oida, S., Nagano, T., Yamakoshi, Y., Ando, H., Yamada, M., and Fukae, M. (2002) J. Dent. Res. 81, 103–108
50. Papagerakis, P., MacDougall, M., Hotton, D., Bailleul-Forestier, I., Oboeuf, M., and Berdal, A. (2003) Bone 32, 228–240
51. Nagano, T., Oida, S., Ando, H., Gomi, K., Arai, T., and Fukae, M. (2003) J. Dent. Res. 82, 982–986
52. White, S. N., Paine, M. L., Ngan, A. Y. W., Miklus, V. G., Luo, W., Wang, H. J., and Snead, M. L. (2007) J. Biol. Chem. 282, 5340–5345
53. Wen, H. B., Moradian-Oldak, J., and Fincham, A. G. (2000) J. Dent. Res. 79, 1902–1906
54. Gibson, C. W. (1999) Crit. Rev. Eukaryotic Gene Expression. 9, 45–57