Dentin Sialoprotein and Dentin Phosphoprotein Overexpression during Amelogenesis*

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The gene for dentin sialophosphoprotein produces a single protein that is post-translationally modified to generate two distinct extracellular proteins: dentin sialoprotein and dentin phosphoprotein. In teeth, dentin sialophosphoprotein is expressed primarily by odontoblast cells, but is also transiently expressed by presecretory ameloblasts. Because of this expression profile it appears that dentin sialophosphoprotein contributes to the early events of amelogenesis, and in particular to those events that result in the formation of the dentino-enamel junction and the adjacent “aprismatic” enamel. Using a transgenic animal approach we have extended the dentin sialoprotein or dentin phosphoprotein expression throughout the developmental stages of amelogenesis. Overexpression of dentin sialoprotein results in an increased rate of enamel mineralization, however, the enamel morphology is not significantly altered. In wild-type animals, the inclusion of dentin sialoprotein in the forming aprismatic enamel may account for its increased hardness properties, when compared with bulk enamel. In contrast, the overexpression of dentin phosphoprotein creates “pitted” and “chalky” enamel of non-uniform thickness that is more prone to wear. Disruptions to the prismatic enamel structure are also a characteristic of the dentin phosphoprotein overexpressing animals. These data support the previous suggestion that dentin sialoprotein and dentin phosphoprotein have distinct functions related to tooth formation, and that the dentino-enamel junction should be viewed as a unique transition zone between enamel and the underlying dentin. These results support the notion that the dentin proteins expressed by presecretory ameloblasts contribute to the unique properties of the dentino-enamel junction.

Dentin is an ectomesenchymal derived, collagen containing, calcified tissue that forms the major part of the tooth. Genetic diseases that result in abnormal dentin are classified in a subgrouping of dentinogenesis imperfecta. Dentinogenesis imperfecta type I (DI-I)1 is always associated with one of the osteogenesis imperfectas, where mutations have been characterized in the following collagens: COL1A1, COL1A2, COL5A1, and COL2A1 (1), whereas dentinogenesis imperfecta type II (DI-II) is a disorder in dentin mineralization. Dentinogenesis imperfecta type III (DI-III) has a more extreme abnormal dental phenotype that impacts beyond dentin to the enamel and the pulps of affected teeth (2, 3).

In dentin, two of the noncollagenous dentin matrix proteins are dentin sialoprotein (DSP) and dentin phosphoprotein (DPP). Both DSP and DPP are products of a single gene called dentin sialophosphoprotein (DSPP). Following transcription and translation of DSPP, the resulting product is post-translationally cleaved to produce DSP and DPP that are primarily expressed in the dentin matrix (4). DPP (also known as phosphophoryn) is a highly acidic protein and is the major noncollagenous matrix component of dentin (~90% of the dentin matrix is collagen) (5, 6). DSP is a glycoprotein also present in dentin in low but measurable quantities (5–7). As early as 1988 it was suggested that mutations to the “dentin” phosphophoryn (sic) gene locus were a causative factor in DI-II (8, 9). DI-II has been mapped to 4q21-q23 by linkage studies (10), which are consistent with the mapping of the human DSPP gene locus to chromosome 4q21.3 (4). Recently, mutations to the DSPP gene have been characterized in DI-II and DI-III family pedigrees (11–13). A Dspp-null mouse model has dentin defects that most closely resemble DI-III (3). Previous studies (14) suggest that DSPP also has a role in enamel mineralization, being localized to the initial enamel matrix proximal to the dentino-enamel junction (DEJ).

Because of the severity of the phenotype in patients with DI-III, and the recently presented animal model representative of the same disease, we sought to create animals with Dspp up-regulation at the DEJ, and also within the enamel matrix. The reasoning was to observe the impact that DSP or DPP overexpression would have on the dimensions and structural and mechanical properties of the DEJ. Enamel mineralization proceeds through an organic matrix phase, and this organic matrix is competent to direct its own replacement by the mineral phase (15). It is likely that the unique materials properties of enamel and the DEJ are owed to the proteins that regulate the mineral phase. During development, Dspp is expressed by presecretory ameloblasts at a time when the interface between dentin and enamel is forming (14, 16–18). We hypothesized that this transient sharing of proteins between the dentin and enamel might account for some of the unique physical properties of the DEJ, for example, resistance to fracture (19–21). In this paper we report our findings from these animals.

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§ The abbreviations used are: DI, dentinogenesis imperfecta; DEJ, the dentino-enamel junction; DPP, dentin phosphoprotein; DSP, dentin sialoprotein; DSPP, dentin sialophosphoprotein; SEM, scanning electron microscopy; TEM, transmission electron microscopy; VSV, vesicular stomatitis virus.

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Enamel Matrix Protein Interactions

Experimental Procedures

Transgenic DNA Cassettes—Two plasmid cassettes were prepared using the pGEM-7zf(+) vector (Promega Corp., Madison, WI) as the backbone: one to express the Dsp and the other to express the Dpp regions of mouse dentin sialophosphoprotein (Dpp; clone 5B2; GenBank™ accession numbers U67916 and NM_010080) (4). The reasons for the use of the amelogenin promoter (22), the inclusion of an intron, the use of the Dpp signal peptide (4), the selection of the epitope tags (either c-myc or VSV-G at the amino-terminal of the gene products) (23–25), and the use of the Dpp 3’ untranslated region (4) have been discussed previously (26). However, each of these features is included to ensure the efficient transcription, translation, and secretion of the transgene product. The Dsp and Dpp protein regions (either c-myc or VSV-G at the amino-terminal of the gene products) (27) by direct comparison using ClustalW alignment (28). The complete details of the construction of the two cassettes are not included in this paper but will be provided upon request. Briefly, the Dsp region was PCR amplified from the mouse Dspp cDNA using oligonucleotide primers, including SN260 (forward) and PA85 (reverse) (Fig. 1, panels A and B). The Dpp region was removed from the Dspp cDNA using PflMI and EcoRV restriction enzymes. The carboxyl-terminal 49 amino acids of Dsp, and a 9-amino acid region separating Dsp from Dpp are included in this Dpp transgene construct (Fig. 1, panel B). Epitope tags were included in the transgenes using single-stranded synthetic DNA and PCR methodologies. A schematic of each final construct (pVSV-Dsp and pmyc-Dpp) and their translated transgene product (to be referred to as VSV-Dsp and myc-Dpp) are presented (Fig. 1, panels A and B). For each transgenic construct, the entire coding region was sequenced to ensure no PCR or cloning errors had occurred during their synthesis, and that the correct open reading frame was established. The pGEM-7zf(+) vector backbone was removed from the DNA prior to generating transgenic animal lines using PvuI and BamHI restriction enzymes (Fig. 1), followed by gel purification of the transgene DNA.

Transgenic Animals—All vertebrate animal manipulation complied with Institutional and Federal guidelines. Transgenic mice lines were prepared as described elsewhere (29, 30). Briefly, fertilized eggs for microinjection were harvested from superovulated 6-week-old female F1(C57BL/6J × DBA/2J) mice impregnated by inbred adult male CBA/J mice. For embryo transfer, pseudo-pregnant females were produced by mating CD1 adult females with a vasectomized CD1 adult male. Microinjection of DNA and oviduct transfer of injected zygotes was performed as described (29). In the course of this study, any animal deemed to show any discomfort was immediately sacrificed and their teeth examined macroscopically.

Animals were analyzed for transgene status by Southern blot hybridization of genomic DNA (31, 32). Hybridization was to random primed 32P-labeled PCR-generated DNA to the VSV-G or c-myc region of the transgenic construct (22) (data not shown). In addition, animal transgene status was confirmed by PCR using primer pairs PA32 and PA57 (VSV-Dsp transgenic animals) to give a 896-bp product, and PA93 and PA20182 (myc-Dpp transgenic animals) to give a 527-bp product, and PA93 and PA20182 (myc-Dpp transgenic animals) to give a 527-bp product (data not shown; primers are listed in Fig. 1). Primer PA32 is located in the amelogenin promoter region, whereas PA20182 is located between the epitope region and the Dpp untranslated region (Fig. 1). For each transgenic construct, the entire coding region was sequenced to ensure no PCR or cloning errors had occurred during their synthesis, and that the correct open reading frame was established. The pGEM-7zf(+) vector backbone was removed from the DNA prior to generating transgenic animal lines using PvuI and BamHI restriction enzymes (Fig. 1), followed by gel purification of the transgene DNA.

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region of the myc-DPP construct (region identified in blue in Fig. 1B). For either transgene, three (VSV-Dsp lines 214, 244 and 245) or five (myc-Dpp lines 1, 10, 12, 13, 46, 47, and 53) transgenic animal lines were selected and bred.

Southern analysis was done to ascertain approximate transgene copy numbers in each of the animal lines studied (Fig. 2). Genomic DNA was isolated from early generation and presumably heterozygous animals, digested with NcoI, and subjected to Southern analysis (31) using a 32P-labeled 1,311-bp NcoI-NcoI DNA fragment from the original mouse Dspp clone (5B2) (4). This 1,311-bp NcoI-NcoI DNA fragment is contained entirely within exon 5 of Dspp, and is also located entirely within its 3′ untranslated region. The endogenous Dspp gene, and both transgenes carry this 1,311-bp region. Transgene copy numbers in each of the animal lines studied was assessed by quantitation of the images generated for Southern blot analysis (isotope hybridization [panel B]) and normalized to the ethidium bromide staining image) using ImageQuant™ TL software (Amersham Biosciences). Southern analysis was performed on genomic DNA from two wild-type animals (lanes 1 and 2), myc-Dpp transgenic animals (lanes 3-12), and from VSV-Dsp transgenic animals (lanes 13-18). Lane 19 contains the 1,311-bp Dspp NcoI-NcoI insert as a positive control, and lane 20 contains the DNA size marker. Two animals were assessed in each line: for myc-Dpp line 10 (lanes 3 and 4), line 13 (lanes 5 and 6), line 16 (lanes 7 and 8), line 47 (lanes 9 and 10), and line 53 (lanes 11 and 12); for VSV-Dsp line 214 (lanes 13 and 14), line 244 (lanes 15 and 16), and line 245 (lanes 17 and 18).

Transmission Electron Microscopy—Thin sections from 4-day postnatal lower incisor teeth from three independent lines of VSV-Dsp transgenic mice, and three independent lines of myc-Dpp transgenic mice, were prepared for TEM analysis. In addition, three age-matched non-transgenic control animals taken from the same breeding stock were prepared for TEM analysis. Samples were taken from the growing end of the incisor and included secretory ameloblasts, ameloblast Tomes processes, and immature enamel. Methodology for sample preparation and imaging by TEM were previously reported and followed without modification (32, 34, 35). For each sample, approximately 10 representative fields were viewed and photographed for analysis.

Scanning Electron Microscopy—Methodology for sample preparation and imaging by SEM were used as previously reported (36–38). Six-week-old animals were sacrificed for SEM imaging. All SEM images were collected from the transitional zone of enamel from the mandibular incisor (26). The teeth were fractured coronally through the enamel transition zone and there was no acid etching of the samples done prior to SEM analysis. Teeth from a single animal from each transgenic line (VSV-Dsp or myc-Dpp) were subjected to SEM analysis. In addition, three unique lines of compound transgenic animals, bearing both the VSV-Dsp and myc-Dpp transgenes, were subjected to SEM analysis. All data described or illustrated are representative of the defects noted in these transgenic animals.
is the dentino-enamel junction (red arrow Sial cells (Od) are identified, as Odameloblasts, and the enamel matrix. Ameloblasts (4). Transgene expression is limited to the cytoplasm of the secretory patterns have been seen in all transgenic animal lines that Dpp transgenic animal is presented (Fig. 3). Similar expression epitope. An example of this gene expression profile for a myc-VSV-G epitope (data not included), and for myc-Dpp transgenic individual transgene expression was observed to be restricted solely to the cytoplasm of the secretory ameloblasts, and the enamel matrix. Ameloblasts (Amp), odontoblast cells (Od), and cells of the stratum intermedium (Si) are identified, as is the dentino-enamel junction (red arrow and red line). Black arrowheads define the pre-dentin region (panel F), and the black arrow points to Tomes processes. Asterisks indicate a separation artifact within the enamel. Scale bars are: 200 μm (panel A), 100 μm (panels B–E), and 50 μm (panel F).

RESULTS

Transgene Copy Numbers—Southern blot analysis was performed on heterozygous animal lines to assess transgene copy numbers in individual animal lines (Fig. 2). Data for transgenic animals were compared with wild-type animals that carry two copies of Dcpp. For all transgenic animal lines studied it was assessed that a single copy of the transgene was integrated into the animals genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; except myc-DPP line 47, which had incorporated an order of magnitude greater transgene copies (per animal genome; 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At 6 weeks of age excessive attrition was noted in some of myc-DPP line 47 transgenic animals. Fitted enamel is clearly evident in myc-Dpp line 47, and enamel that was sheared or fractured from the DEJ was a frequent observation (Fig. 4). This severe phenotype was less apparent in myc-DPP lines 10, 13, 46, and 53. When myc-Dpp line 47 transgenic animals were crossed (bred) with any of VSV-Dsp transgenic animals, a similarly severe enamel phenotype resulted in animals carrying both transgenes.

TEM Data—An extensive and comparative TEM analysis was carried out on incisor teeth for multiple transgenic animals and age-matched non-transgenic animals from the same breeding stocks. Of particular interest and focus of our attention was the size and orientation of the forming crystalites relative to Tomes processes, and the size and spatial arrangement of radiolucent spherical structures previously discussed as amelogenin nanospheres (34). Specifically, we were interested in the very early stages of enamel formation, hence our intention was to observe enamel, the DEJ, and Tomes processes in the same field (Fig. 5). A typical image for the DSP overexpressing animals is presented for VSV-Dsp line 214 (Fig. 5, panels C and D). A typical image from a Dpp overexpressing animal from myc-Dpp line 47 is shown (Fig. 5, panels E and F). One notable difference was seen in the involve the amelogenin promoter, which is regulated in a tissue-specific manner (22, 26, 32). Using an antibody to amelogenin protein (26), no gross quantitative or qualitative changes to the expression pattern for the endogenous amelogenin gene were observed (data not shown). The absence of gross changes in the expression pattern for the endogenous amelogenin gene also suggests that targeted recombination disrupting the endogenous amelogenin gene has not occurred (38). Gross Enamel Phenotype of VSV-Dsp and myc-Dpp Transgenic Animals—The breeding of the transgenic lines was not affected by the enamel phenotype. The animals diet was constant for both non-transgenic and transgenic animals. Transgenic animals developed normally and there was no evidence of malnutrition.

No gross abnormalities were detected in the dentition of any of the VSV-Dsp transgenic mouse lines at the time of the eruption of the incisor or molar teeth. No gross abnormalities were observed for the molar or the incisor teeth of VSV-Dsp transgenic animals at 6 weeks of age. At 3 weeks of age, a delay in molar teeth eruption was evident in myc-DPP line 47 transgenic animals when compared with non-transgenic control animals (data not included). At 6 weeks of age excessive attrition was noted in some of myc-Dpp line 47 transgenic animals. Pitted enamel is clearly evident in myc-Dpp line 47, and enamel that was sheared or fractured from the DEJ was a frequent observation (Fig. 4). This severe phenotype was less apparent in myc-DPP lines 10, 13, 46, and 53. When myc-Dpp line 47 transgenic animals were crossed (bred) with any of VSV-Dsp transgenic animals, a similarly severe enamel phenotype resulted in animals carrying both transgenes.

Tissues beyond the mandible were not included for immunohistochemistry using a monoclonal antibody to the c-myc epitope. Mature end (M) and growing end (GE) of the incisor is identified in Panel A. Regions identified in Panel A are enlarged in panels B (section 1), C (section 2), D (section 3), and E (section 4). Transgene expression is limited to the cytoplasm of the secretory ameloblasts, and the enamel matrix. Ameloblasts (Am), odontoblast cells (Od), and cells of the stratum intermedium (Si) are identified, as is the dentino-enamel junction (red arrow and red line). Black arrowheads define the pre-dentin region (panel F), and the black arrow points to Tomes processes. Asterisks indicate a separation artifact within the enamel. Scale bars are: 200 μm (panel A), 100 μm (panels B–E), and 50 μm (panel F).
VSV-Dsp and myc-Dpp transgenic animals when compared with non-transgenic animals. This difference equated to an apparent increase in enamel crystallites proximal to the DEJ (Fig. 5, panels C and E, when compared with panel A), which is suggestive of both Dsp and Dpp acting as accelerators of dental hard-tissue mineralization.

Negative images from TEM analysis were scanned into the computer, and using Image®Pro Plus version 4.0 software (Media Cybernetics, Newburyport, MA), nanosphere diameters were calculated. Because the sections were prepared at random angles, the maximum diameter for each nanosphere within a field was measured. Fifty nanospheres in each field were measured, and six different microscopic fields were analyzed for the non-transgenic and VSV-Dsp transgenic animals. All transgenic animal lines were represented in data collection. The amelogenin nanosphere size distributions for the non-transgenic control animals are 11.7 nm (standard deviation of 1.67 nm) and for the VSV-Dsp transgenic animals 9.3 nm (S.D. of 1.50 nm). No significant differences were noted in nanosphere dimensions between the non-transgenic and VSV-Dsp transgenic animals. No apparent differences were noted in crystallite size or orientation between non-transgenic and transgenic VSV-Dsp animals. In contrast, a similar analysis of the enamel of the myc-Dpp transgenic animals was not possible. In the myc-Dpp animals, forming enamel rods were not evident, and individual crystallites were rarely seen running parallel to neighboring crystallites. Amelogenin nanosphere dimensions have generally been visualized and measured between immature crystallites running in parallel, thus it is difficult to assess nanospheres in the myc-Dpp animals.

Dsp Overexpressing Homozygotes—A detailed SEM analysis was carried out on the incisor teeth of animals from each of the VSV-Dsp transgenic animal lines (214, 244, and 245) bred to homozygosity, and these images were compared with incisor teeth of age-match non-transgenic control animals. No significant differences in morphology were noted between the wild-type animals and the VSV-Dsp transgenic animals.

Dpp Overexpressing Homozygotes—A detailed SEM analysis was carried out on the incisor teeth of animals from each of the myc-Dpp transgenic animals (lines 10, 13, 46, 47, and 53) bred to homozygosity, and these images were compared with incisor teeth of age-match non-transgenic control animals. No significant differences in morphology were noted between the wild-type animals and the VSV-Dsp transgenic animals.

**Fig. 5. TEM analysis of wild-type, VSV-Dsp, and myc-Dpp transgenic animals.** Thin sections from the lower incisor of a 4-day postnatal wild-type animal (panels A and B), a VSV-Dsp (line 214) transgenic animal (panels C and D), and a myc-Dpp (line 47) transgenic animal (panels E and F). Panels B, D, and F are magnifications of the region identified by an asterisk in panels A, C, and E, respectively. The asterisks (panels A, C, and E) and arrows (panels B, D, and F) identify the same regions in the respective sections. Enamel rods (R) are evident in wild-type and VSV-Dsp transgenic animals only. Tomes processes (TP) are seen in panel C. Dentin (De) and DEJ are labeled in panels A, C, and E. Scale bars: 500 nm (panels A, C, and E) and 100 nm (panels B, D, and F).
compared with wild-type controls in panels A and B). This
dysmorphology covered large and defined regions where no
prismatic structure was apparent (Fig. 6, panel F). The pitting
of the enamel surface was apparent (Fig. 2, panel B, and Fig. 6,
panel G). This phenotype was most pronounced in line 47, and
the likely explanation for this is that line 47 carried many more
copies of the myc-Dpp transgene than any of the other
lines studied.

**Phenotype of Animals Homozygotic for Both the VSV-Dsp and myc-Dpp Transgenes—**No additional information was ob-
tained by SEM studies of crossed animals carrying both the
VSV-Dsp and myc-Dpp transgenes. The general finding was
that the severity of enamel defects in these double transgene-
bearing animals mirrored that of the more severe phenotype
seen the respective parental lines, which in all cases was a
phenotype seen in the myc-Dpp homozygotic transgenic
animals.

**DISCUSSION**

The DEJ can be viewed as a mechanical transition zone
separating enamel and dentin. Microindentation and microradiographic profiling of human incisor teeth suggests that this
transition zone could be in the order of 100 μm, a figure that
relates to a hardness profile as one moves from the enamel
proper to the dentin proper (20, 39). Another feature of the DEJ
is its unique gene expression profile during development. Den-
tin is a collagen-based hard tissue, whereas enamel contains no
collagen (40). Collagen bridging the DEJ has been identified and postulated to serve in the toughening of this transition
from bulk dentin to bulk enamel (41, 42). The mineral compo-
nent of both dentin and enamel is a substituted hydroxyapat-
tite. Enamel, under normal circumstances, does not shear away
from the underlying dentin, suggesting a biologically derived
union of these dissimilar materials. The organic material ex-
pressed and secreted by the ameloblasts and odontoblasts, and
the subsequent protein-protein interactions that dictate bi-
ominalization events can likely explain this transition zone of
the DEJ (Fig. 7).

From a gene expression perspective, many gene products of
the dentin, and enamel, are “relatively” unique to those tooth
matrices. For example, amelogenin, ameloblastin, and enam-
elin are generally considered enamel-specific, whereas dentin-
matrix protein-1 and DSPP have generally been considered
dentin-specific. As molecular probing techniques have become
more sensitive, these gene expression pattern profiles are being
challenged and/or corrected. In mice, the mRNAs of ameloge-
nin, ameloblastin, and enamelin, in addition to being a product
of ameloblast cells, are also apparent in presecretory and se-
crety odontoblasts (18, 43–46). The mRNA and protein ex-
pression of dentin-matrix protein 1 and Dspp, in addition to
odontoblasts, are also apparent in presecretory ameloblasts
(14, 16, 17, 47, 48). This transient duplication of tooth-specific
genesis products during the early stages of dentin and enamel
formation is suggestive of a molecular mechanism responsible
for the creation of a transition zone at the DEJ (Fig. 7).

Recent data suggest that the enamel proteins amelogenin,
ameloblastin, and enamelin have the biochemical ability to
interact with a number of the collagens, including the type II
(Col2a1) and the type V collagens (Col5a1 and Col5a3) (49).
These potential enamel protein-collagen interactions were
identified using the yeast two-hybrid assay. If any of these
enamel protein-collagen relationships prove to be physiologi-
cally valid, this would help to explain the DEJ as a transition
zone, rather than an interface between the two dissimilar bio-
logical materials. It appears that no comprehensive cataloging
of the collagens in dentin has been done recently, however,
many collagens are expressed by odontoblasts including types
I, III, IV, V (50), and VII (bite-it.helsinki.fi).

We suggest that the overexpression of Dsp during tooth
formation in transgenic mice causes ameloblasts to produce
bulk enamel more like that of more highly mineralized “aprismatic” enamel found proximal to the DEJ (40, 51, 52). This
could be partly or entirely explained by the accelerated miner-
alization of the enamel matrix, as evident from our TEM anal-
ysis of forming enamel proximal to the DEJ (the aprismatic
enamel). Alternatively, although we do not have data to sup-
port this, the increased mineral content seen in the VSV-Dsp
transgenic animals could be as a result of increased nucleation
events. If this were the case then Dsp (and thus Dspp) could be
acting as a nucleator of enamel crystallites. These explanations
are not inconsistent with the temporal pattern of Dspp expres-
sion by pre-ameloblasts in wild-type animals. Microscopists
have known about the specialized, highly mineralized apri-
matic enamel proximal to dentin for the past century (51, 52),
but its significance to the function of the DEJ was not recog-
nized. Although the DEJ appears to be an abrupt discrete
interface upon imaging and nanoprofiling (19, 21, 53), the
interface is only one component of a broad structural and functional DEJ zone containing specialized aprismatic enamel and "mantle" dentin. This specialized zone of dentin-adjacent aprismatic enamel is now known to be harder than bulk enamel, more radiodense than bulk enamel, and to be related to the mechanical function of the broad DEJ zone (20, 39, 54). However, relatively little is currently known of DEJ mechanics, toughening mechanisms, or failure modes (19, 21, 53). In contrast to Dsp overexpressing animals, Dpp overexpression has resulted in softer enamel. These remarkable, and opposing functional results, or biomechanical effects, of two proteins encoded by a single gene is suggestive of both protein products having very different structural or instructive roles in the formation of dentin and aprismatic enamel.

Amelogenin nanosphere dimensions were calculated to establish if amelogenin self-assembly properties were disrupted in either the VSV-Dsp or myc-Dpp transgenic animals. No significant differences in nanosphere dimensions were noted between the VSV-Dsp transgenic animals and their non-transgenic kindred, suggesting that Dsp overexpression in the enamel matrix does not disrupt amelogenin self-assembly. In contrast, it was not possible to determine nanosphere dimensions for the myc-Dpp transgenic animals as too few nanospheres could be visualized in any field studied. We interpreted this as suggesting that overexpression of Dpp in the enamel matrix directly or indirectly influences amelogenin self-assembly. Here we report the diameter of amelogenin nanospheres in non-transgenic mice to be ~11.7 nm. Previously we had reported this dimension to be ~19.4 nm (32), and another in vivo report, using mice as their model, calculated this figure at ~20 nm (34). These differences probably relates to the methodologies used to calculate dimensions, as well as investigator bias when establishing nanosphere boundaries. In the past we have produced black-and-white photographs and used a calibrated lenticular to determine the diameter of each nanosphere (32), however, in this study we have used computer images and software to measure nanosphere dimensions directly. In this study all animal samples were prepared, visualized, and measured in an identical manner. We believe that our conclusion that non-transgenic and VSV-Dsp transgenic animals have similar nanosphere dimensions to be accurate.

Other than the human and animal data that suggest that disruptions to the DSPP/Dspp genetic code can result in abnormal dentin, very little data is available describing a functional role for the Dssp protein. Physiologically Dssp is cleaved after it is translated and secreted to form two distinct and unique protein products, DSP and DPP. In this study, animal models overexpressing Dsp or Dpp in enamel each have a very different resulting enamel phenotype. The overexpression of Dpp results in pitted and chalky enamel, and the severity of this defect is dose-dependent. Similar findings of deleterious effects on enamel by the overexpression of other mutated and non-mutated enamel proteins have been noted before (22, 26, 32, 35, 55, 56). Overexpression of Dsp in the enamel resulted in an acceleration of mineralization events, as evident from TEM images taken at the early stages of enamel formation. Superior hardness properties of the final bulk enamel may be expected in VSV-Dsp transgenic animals based on these observations. Based on our observations, we would liken any enhanced mechanical properties of the enamel in the VSV-Dsp transgenic animals to the more highly mineralized aprismatic inner enamel layer.

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