Dentin Matrix Protein 1 Regulates Dentin Sialophosphoprotein Gene Transcription during Early Odontoblast Differentiation*

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Dentin mineralization requires transcriptional mechanisms to induce a cascade of gene expression for progressive development of the odontoblast phenotype. During cytodifferentiation of odontoblasts there is a constant change of actively transcribed genes. Thus, tissue-specific matrix genes that are silenced in early differentiation are expressed during the terminal differentiation process. Dentin sialophosphoprotein (DSP) is an extracellular matrix, prototypical dentin, and a bone-specific gene, however, the molecular mechanisms by which it is temporally and spatially regulated are not clear. In this report, we demonstrate that dentin matrix protein 1 (DMP1), which is localized in the nucleus during early differentiation of odontoblasts, is able to bind specifically with the DSPP promoter and activate its transcription. We have identified the specific promoter sequence that binds specifically to the carboxyl end of DMP1. The DNA binding domain in DMP1 resides between amino acids 420 and 489. A chromatin immunoprecipitation assay confirmed the in vivo association of DMP1 with the DSPP promoter. Interactions between DMP1 and DSPP promoter thus provide the foundation to understand how DMP1 regulates the expression of the DSPP gene.

Odontoblast differentiation is a well designed process that couples cell-cycle withdrawal with the synthesis of dentin matrix proteins. A number of macromolecules are involved in dentin mineralization. One such protein, dentin sialophosphoprotein (DSP) is produced by terminally differentiated odontoblasts and is known to modulate dentin mineralization. DSP is a compound gene encoding for two proteins namely DSP (dentin sialoprotein) and PP (phosphophoryn) (1). Earlier reports have indicated DSPP as the sole odontoblast-specific gene (2); however, a recent report demonstrates low levels of expression in osteoblasts and calvarial tissue (3).

Tissue-specific gene expression is complex and, among many events, requires the orderly binding of transcription factors to specific gene regulatory sequences, alteration of chromatin structure by acetyltransferases and demethylases, and recruitment of the RNA polymerase II complex (4). The temporal and spatial expression of DSPP is tightly regulated by the transcriptional elements in the promoter (5). In an attempt to identify the regulatory elements controlling the temporal and spatial expression of DSPP, a DSPP promoter of ~1.7 kb has been cloned and characterized to a certain extent (6). Deletion mapping experiments indicated the presence of various regulatory sequences (7). The activity of this promoter has been demonstrated by transgenic technology with a reporter construct expressing LacZ under the control of the DSPP promoter (5). It was reported that DSPP transcription is regulated by several positive and negative regulators of gene transcription in combination with the various signaling pathways/networks. Some of the negative factors known to down-regulate DSPP promoter activity have been reported earlier, including transforming growth factor β1 (8), CAAT/enhancer-binding protein β (6), and Nrf1 (6). Odontoblast-specific overexpression of transforming growth factor β1 in the transgenic mouse model showed a reduced expression of DSPP (8) indicating that transforming growth factor β1-mediated signaling pathway down-regulates DSPP expression. Similarly we have shown that CAAT/enhancer-binding protein β and Nrf1 interact and down-regulate the expression of DSPP in odontoblasts (6). RUNX2 has been shown to differentially regulate the DSPP promoter activity during differentiation of odontoblasts (9). At the cellular level, RUNX2 down-regulates DSPP promoter activity in preodontoblasts while increasing its expression during terminal differentiation (9).

At the molecular level, DSPP gene also undergoes several post-transcriptional modifications, a common one being alternative splicing. Several transcripts of various lengths have been identified for the DSPP gene (10). However, a predominant message of ~5 kb has been reported (11). Currently, the significance of alternative splicing and DSPP function is not known. There is speculation that, after post-translational modification, DSPP synthesized as a “pro” form may be proteolytically cleaved to DSP and PP moieties. One major observation that supports this hypothesis is the absence of the holoprotein (DSPP) in mineralized dentin or in odontoblasts (12). Pathologically, the DSPP gene has been indicated as the candidate gene for dentinogenesis imperfecta type II and III genetic disorder

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*This work was supported by National Institutes of Health Grants DE13836 and DE11657. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2.

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2 The abbreviations used are: DSP, dentin sialophosphoprotein; DMP1, dentin matrix protein 1; EMSA, electrophoretic mobility shift assay; ChIP, chromosomal immunoprecipitation; DMS, dimethyl sulfate; aa, amino acid(s); Ab, antibody.
(13–18). However, the DSPP knock-out mice possessed a dentinogenesis imperfecta type III phenotype. This further complicates the significance of DSPP in these genetic disorders but also suggests that there may be one or more potential candidate genes for the dentinogenesis imperfecta type II genetic disorder.

Dentin matrix protein 1 (DMP1) is a non-collagenous protein expressed in mineralized tissues (19). DMP1 is an acidic protein rich in aspartic acid, glutamic acid, and serine residues. Most of the serine residues are phosphorylated by casein kinase II (20–22). Various lines of evidence indicate that DMP1 functions as an important regulatory molecule in the bio-mineralization process. The role of DMP1 in the initiation of hydroxyapatite nucleation was demonstrated recently (23), which directly supports its role at the mineralization front. Furthermore, overexpression of DMP1 in a cell culture system augments the mineralization process both qualitatively and quantitatively (24). Conversely, blocking translation of DMP1 by antisense techniques inhibited the mineralization process (24). In summary, these results indicated that DMP1 has an important regulatory function in mineralization. However, Dmp1−/− mice had no major phenotypic abnormality in dentin and bone during early development, on the other hand, there were defects in chondrocyte differentiation and cartilage formation (25, 26). With progression of age, defects in bone and dentin were noted (25). Overall, the results from the DMP1 knock-out suggest that other compensatory mechanisms might be responsible for bone and dentin formation during early development.

Apart from its role in mineralization, DMP1 has a putative function in modulating gene expression. Overexpression studies in mesenchymal stem cells have given insights into the role of DMP1 in regulating the expression of specific molecular markers of the osteoblast and odontoblast lineage, such as osteocalcin, alkaline phosphatase, and DSPP (24). Furthermore, blocking DMP1 expression by antisense techniques inhibited the expression of these genes (24). More recently, DMP1 has been implicated in more complex biological processes. We have shown by immunostaining that the primary localization of DMP1 is in the nucleus (27). Further, Ca^{2+} was responsible for the export of DMP1 into the extracellular matrix during osteoblast differentiation. This bimodal pattern of localization provided direct evidence that DMP1 promotes the development of the odontoblast and osteoblast phenotype and performs other functions associated with their differentiated state.

In this report, we have provided evidence to support the function of DMP1 as a transcriptional regulator of the DSPP gene. We have demonstrated the binding of DMP1 to the DSPP promoter using several lines of evidence, namely by DNA footprinting and electrophoretic mobility shift assay (EMSA). Transient transfection assays with DSPP promoter indicate that DMP1 positively regulates the DSPP promoter activity. Furthermore, when mutations to this binding site were introduced, these constructs failed to respond to the overexpression of DMP1. The in vivo binding of DMP1 to the DSPP promoter was further confirmed by ChIP analysis. We have also identified a potential DNA binding region within DMP1. With these data, we propose that DMP1 acts as a potential transcriptional regulator with respect to the regulation of DSPP during early odontoblast differentiation.

**MATERIALS AND METHODS**

**Plasmids and Adenoviral Vectors—**A 1.7-kb genomic fragment was amplified from rat genomic DNA to obtain the DSPP promoter (6). The promoter was sequence-verified and subcloned into pGL3 basic (Promega) vector at NheI and SmaI restriction sites. DSPP promoter deletions were made using specific primers.

The adenoviral construct and viral infection of odontoblast cells were made as described earlier (24). DMP1 deletions were made by PCR reactions using HF-Taq polymerase (Clontech). The amplified fragments were sequence-verified for open reading frame and cloned into gluthathione S-transferase fusion protein expression (pGEX4T-3) vector.

**Cell Culture and Transfections—**An odontoblast cell line T4-4, developed by human telomerase reverse transcript (hTERT)-mediated immortalization as reported (28), was used in this study. Odontoblast cells were grown in Dulbecco’s modified Eagle’s medium/F-12 medium containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% Amphotericin B. Transient transfections with reporter plasmids were performed with SuperFect (Qiagen) as per the manufacturer’s protocol. Reporter transfections were carried out in triplicates and repeated thrice to obtain a mean value. All transfections contained an internal control vector pRL-SV40, which contains a Renilla luciferase gene driven by the SV40 promoter. Statistical significance was calculated using Student t test.

**Luciferase Assay—**A dual luciferase assay system was purchased from Promega and used in the experiments. The luciferase activity was measured using an automated luminometer from Dynex as reported earlier (29).

**Dimethyl Sulfate Footprinting—**DMS footprinting was followed as published (30 and 31). Briefly, a ^32P-end-labeled DNA fragment was incubated with DMP1 protein in footprinting buffer (20 mM HEPES, pH 8.0, containing 1 mM EDTA and 5 μg of bovine serum albumin), and the binding was carried out for 30 min at 4 °C. 27 mM DMS (Aldrich Chemical Co.) in 0.6 mM sodium cacodylate (pH 8.0) was added, and the mixture was incubated further for 1 min. The DNA-protein complex was precipitated in the presence of 0.3 mM sodium acetate and 200 μg/ml yeast tRNA with 3 volumes of cold 100% ethanol. The DNA pellet was resuspended with 20 μl of 20 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and incubated for 10 min at 90 °C. 2 μl of 1 M NaOH was added, and the mixture was incubated at 90 °C for 5 min. DNA was then precipitated by ethanol. The DNA pellet was resuspended in sample buffer containing 80% (v/v) formamide and subjected to electrophoresis on a 8% acrylamide gel in the presence of 8.3 μl urea. The gel was dried and subjected to autoradiography.

**Mobility Shift Assay—**EMSA was carried out to monitor the protein binding sites on the DSPP promoter. Oligonucleotides were synthesized as listed in Table 1. The oligonucleotides were annealed and labeled using T4 polynucleotide kinase in the presence of [γ-^32P]ATP. Protein-DNA interactions were per-
formed for 45 min in 4 mM Tris (pH 8.0) containing 60 mM KCl, 5 mM MgCl₂, 4% glycerol, and 100 ng of poly(dG-dC) along with 100 μg/ml bovine serum albumin. Typically, 10 ng of purified DMP1 was incubated with 5 fmol of labeled oligonucleotides. Supershift experiments were carried out as described above except that the samples were preincubated for 30 min at 4 °C with DMP1 antibody. For competition binding assay, unlabeled double-stranded oligonucleotides were used at different concentrations.

**Site-directed Mutagenesis**—Site-directed mutagenesis was carried out to mutate the DMP1 binding site on DSPP promoter (same as EMSA-MUT shown in Table 1) using a site-directed mutagenesis kit (Promega).

**Northern Blotting**—Messenger RNA was isolated from cultured cells at log phase using an Invitrogen mRNA isolation kit. RNA gel electrophoresis and Northern blotting were done as described by Sambrook et al. [32]. 5 μg of mRNA was resolved on an 8% agarose gel containing formaldehyde. The RNA was transferred to a Hybond nylon membrane (Amersham Biosciences). The membrane was prehybridized using PerfectHyb (Sigma) and probed with a randomly labeled appropriate probe. Random labeling was carried out using the Decaprime kit (Ambion Inc.).

**ChIP Assay**—A ChIP assay kit obtained from Upstate Inc. was used in this experiment. Briefly MC3T3-E1 cells were transfected with plasmid containing DSPP promoter. The DNA-protein complex was cross-linked with 1% formaldehyde for 10 min at 37 °C. The cells were washed with phosphate-buffered saline containing protease inhibitors mixture and scraped into a conical tube. The cells were lysed with SDS-lysis buffer (provided in the kit) for 10 min. The DNA was recovered from the DMS footprinting assay are presented in Fig. 4, which shows that in the presence of DMP1 there is a protection of DNA between 417 and 353 nucleotides. The sequence that corresponds to this region is indicated in Fig. 4.

**RESULTS**

**DMP1 Influences DSPP Expression**—Overexpression studies conducted earlier indicated that expression of DMP1 in mesenchymal cells influence the expression of DSPP gene, indicating the role of DMP1 in the transcriptional regulation of DSPP [24]. Blocking DMP1 expression by antisense DMP1 constructs further confirmed that DSPP transcription is under the control of DMP1 [24].

In this study, transient transfections performed in odontoblasts with increasing concentration of DMP1 expression plasmid, resulted in a steady increase in the DSPP promoter activity. Overall, a 70% increase in the promoter activity in the presence of 0.3 μg of DMP1 expression plasmid (Fig. 1) was observed.

The role of DMP1 in the regulation of DSPP gene was confirmed by overexpression studies using the adenoviral construct. Odontoblasts were infected with the cytomegalovirus-driven DMP1 adenoviral constructs in both sense and antisense orientation. mRNA was isolated from these cells and analyzed for the expression levels of DSPP mRNA. Results in Fig. 2A indicate an increase in 40% of the message level of DSPP with the overexpression of DMP1. On the other hand, the overexpression of antisense DMP1 decreased the expression level of DSPP by 30% (Fig. 2B). Thus overexpression of DMP1 under cytomegalovirus promoter influences DSPP promoter activity.

**DMP1 Binding to the DSPP Promoter**—The direct binding of DMP1 to the DSPP promoter was analyzed by EMSA experiments. The 1.7-kb DSPP promoter was PCR-amplified into smaller fragments of ~500 bp using gene-specific primers. These fragments were end-labeled and used in EMSA studies. Results from these studies indicate that DMP1 binds to DSPP promoter between −450 bp to +80 bp relative to the transcription start site (Fig. 3). This fragment was further analyzed for specific DMP1 binding sequence by footprinting.

**Identification of the DMP1 Binding Sequence on the DSPP Promoter**—DNA footprinting was carried out to identify the DMP1 binding sequence. A 450-bp DSPP promoter was PCR-amplified between −450 bp and +1 bp and was used in the DNA footprinting assay. DNA footprinting was carried out using DMS. One of the reasons for selecting chemical footprinting is due to the problem encountered with the Dnase I methodology. The presence of calcium in the Dnase I reaction buffer affected the DNA binding property of DMP1. Results from the DMS footprinting assay are presented in Fig. 4, which shows that in the presence of DMP1 there is a protection of DNA between −417 and −353 nucleotides. The sequence that corresponds to this region is indicated in Fig. 4.
Identification of the Core Binding Sequence—To identify the core sequence of the binding region, we performed EMSA with various oligonucleotides designed from the footprinting studies (Fig. 5). The 64-bp region identified by footprinting was used as positive control (Fig. 5, lanes 1–3), oligonucleotides synthesized from 22 to 64 bp also contain the binding region as shown in lanes 4–6. However, oligonucleotides made in the region 1–30 bp failed to interact with DMP1 protein (lanes 7–9), whereas oligonucleotides containing the region from 22 to 50 bp was shown to interact with the DMP1 protein (Fig. 5, lanes 10–12). These results indicate that the region spanning 22–50 bp of the sequence identified by the DNA footprinting assay contained the DMP1 binding region. The specificity of this binding region was further confirmed by competition assay in the presence of varying concentrations of cold oligonucleotides. Results from the EMSA study demonstrated that, with increasing concentration of cold oligonucleotides, the binding of DMP1 to the labeled oligonucleotides reduced gradually, indicating the specificity of the binding interaction (Fig. 6A). The specificity of DMP1 binding to the DNA was further confirmed by the supershift EMSA in the presence of DMP1-specific antibody (Fig. 6B). A supershift was observed in the presence of DMP1 antibody as presented in lane 3, indicating the specific binding of DMP1 to its responsive element. Mutations were introduced in the oligonucleotides (in the conserved region shown in the supplementary data) and DMP1 binding was analyzed by EMSA. Results suggested that DMP1 failed to interact with its binding site upon mutation (Fig. 6B, lane 2).

Binding of DMP1 to the DSPP promoter region was further substantiated by introducing mutations (EMSA-MUT) on the DSPP promoter using primers listed in Table 1. Mutations were specifically incorporated at the DMP1 binding site. Promoter activity assay further revealed that the DSPP promoter failed to respond to the overexpression of DMP1 in odontoblasts when compared with the wild-type promoter activity (Fig. 7).

Identification of the DNA Binding Domain within DMP1—Deletion analyses were performed to identify the DNA binding domain within the DMP1 protein. Initially DMP1 deletions were made in such a way to express two truncated proteins; one polypeptide containing the N-terminal domain (1–321 aa) and the other C-terminal domain (321–489 aa). These proteins were purified and used in EMSA studies. Results from EMSA indicated that the C-terminal region of DMP1 has the DNA binding potential (Fig. 8A). To narrow down the binding site further, additional deletions (as shown in Table 1) were made within the C-terminal region of DMP1, and these polypeptides were used in EMSA (Fig. 8B). Results in Fig. 8B indicate that the DNA binding region resides between 420 and 489 aa (QDSRSEENRDSQDSRSEENRSGQKSLQEGGRIDQDD-NDCQDG).
**ChIP Assay Demonstrating DMP1 Binding to the DSPP Promoter**—To demonstrate the in vivo interaction between DMP1 and DSPP promoter, a ChIP assay was performed. This analysis was carried out using affinity-purified DMP1-specific antibody. The region of interest of DSPP promoter was amplified upon immunoprecipitation with DMP1 antibody. Results from the ChIP assay demonstrate that DMP1 binds to the DSPP promoter, on the other hand, DMP1 failed to bind to DSPP promoter, which had mutated DMP1 binding sites (Fig. 9). These results clearly indicate that DMP1 has the potential to bind to the DSPP promoter in vivo.

**DISCUSSION**

Molecular mechanisms controlling tissue-specific gene expression is a challenging topic. DSPP is specifically expressed by the odontoblasts (2) and recently reported to be also synthesized at very low levels by osteoblasts (3). It is now known that DSPP is a complex gene coding for two protein products namely DSP and PP (7).

DMP1 is a secreted, extracellular matrix-associated, acidic protein that regulates many cellular functions, including differentiation and matrix production by both osteoblasts and odon-
DMP1 Regulates DSPP Gene Transcription

Studies have shown that, early on during the cytodifferentiation of osteoblasts and odontoblasts, DMP1 can be localized in the nucleus (27). DMP1 localized in the nucleus is unphosphorylated, and we speculate that this non-post-translationally modified DMP1 can act as a transcriptional regulator. In this report, we provide evidence for a functional role for DMP1 in the nucleus; namely, it acts as a transcriptional enhancer for the tooth/bone-specific gene DSPP. Several lines of evidence presented here indicate that DMP1 binds directly to the DSPP promoter. The DMP1 binding site on the DSPP promoter was identified by DNA footprinting. The core binding region was identified by EMSA, and mutation of the DMP1 binding region abolished the DMP1-mediated activation of DSPP promoter activity. Furthermore, the DNA binding region on DMP1 was mapped by deletion analysis. Initial N-terminal and C-terminal deletions indicated that the DNA binding region resides within the C-terminal region of the protein. Further deletions narrowed down the DNA binding region from 420 to 489 aa.

**TABLE 1**

Primers used in the study

| Primers for EMISA | Primers for DMP1 deletions (underlined bases were the added bases for the ease of cloning) |
|------------------|-----------------------------------------------------------------------------------|
| FL-S: G GAT CCC ATG AAG ACT GTC CTC GTT ACC | FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | C-Term-S: G GAT CCC AGC AGC GAG TCT CAG GAA |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | C-Term-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | Del 1-S: G GAT CCC AGC AGC GAG TCT CAG GAA |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | Del 1-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | Del 2-S: G GAT CCC AGC AGC GAG TCT CAG GAA |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | Del 2-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | Del 3-S: G GAT CCC AGC AGC GAG TCT CAG GAA |
| FL-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT | Del 3-AS: GC GCC CTC GTA GCC ATC TGT GCA ATC ATT |

**FIGURE 7.** DMP1 regulates the DSPP promoter activity by binding to its responsive element. Mutations were introduced on the DMP1 binding sites (EMSA-MUT, Table 1) of the DSPP promoter. These constructs were analyzed for their activity in an odontoblast cell line. The promoter activities of the mutant constructs were compared with that of the wild-type constructs. Transient transfections were carried out in triplicates, and the dual luciferase assay was performed. The value (ratio between firefly and Renilla luciferase) for the full-length DSPP promoter was taken as 1-fold. Average values obtained from three independent experiments after normalizing for transfection efficiency were shown with appropriate standard errors. Filled bars indicate wild-type DSPP promoter; empty bars indicate DMP1-responsive element-mutated construct. An increase of 1.5-fold activity in the wild-type type and mutated DSPP promoters (p < 0.05).

**FIGURE 6.** Competition and supershift assay. Oligonucleotides corresponding to 22–50 bp (EMSA-Del3) of the sequence identified by DMS footprinting were end-labeled. The competition assay (A) was carried out in the presence of -fold excess of cold (unlabeled) oligonucleotides. Lane 1 represents 5 fmol of labeled oligonucleotides incubated with 5 ng of purified DMP1. Lane 2 represents 5-fold excess of cold oligonucleotides, and lane 3 represents 10-fold excess of cold oligonucleotides. The supershift assay (B) was carried out in the presence of affinity-purified DMP1 antibody. 5 ng of DMP1 protein was incubated with 1 μg of anti-DMP1 IgG in the binding buffer prior to incubation with the labeled oligonucleotides. The antibody-protein complex was incubated with end-labeled oligonucleotides and analyzed on SDS-PAGE. In B: lane 1, with DMP1; lane 2, EMSA-mut; and lane 3, with DMP1 and DMP1 antibody.
Gene knock-out studies provide a powerful tool to investigate complex biological phenomena. DMP1 knock-out mice have been developed, and they demonstrate defects in chondrocyte differentiation and cartilage mineralization resembling chondrodysplasia-like defects (25). An interesting observation was that increased proliferation of chondrocytes indicates a time lapse in the differentiation of these cells into a mature mineral-forming tissue. With respect to DSPP expression, DMP1 mice showed a reduced level of expression compared with the wild-type mice (26). This resulted in a hypo-mineralization phenotype and reduced thickness of dentin leading to a wider pre-dentin in DMP1 null mice (26). Similarly, results obtained from our laboratory by overexpressing DMP1 in undifferentiated mesenchymal stem cells induced their differentiation toward an odontoblast-like cell phenotype, with the transcription of the DSPP gene (24). This response was found to be cell type-specific that is, only mesenchymal-derived cells underwent this phenotypic change (24). An extension of this study further demonstrated that DMP1 is localized in the nucleus (27). These studies suggest that DMP1 regulates the expression of DSPP gene. However, during bio-mineralization, we have demonstrated that DMP1 is phosphorylated and transported to the extracellular matrix where it functions in hydroxyapatite crystal nucleation and assembly of the dentin matrix (23).

DMP1 is one of the early genes expressed during the commitment of neural crest-derived cells into odontoblasts. Expression of DSPP follows DMP1 expression, indicating a probable control over regulation of the DSPP gene during early odontoblast differentiation events (31). However, with their progression into the maturation stage of the odontoblasts, expression of DMP1 decreases while DSPP expression continues to increase (2, 7, 21, 35, 36). The question that still needs to be addressed is the molecular control that DMP1 exerts over DSPP expression during the mineralization process. One possible explanation would be that DMP1 initiates the basal expression of DSPP during the early stages of odontoblast cytodifferentiation, whereas during the terminal differentiation process the expression of DSPP is independent of DMP1. Immunohistochemical analyses with DMP1-specific antibody on developing tooth sections in mouse have supported this hypothesis. Results demonstrate a high level of DMP1 expression at the earlier stages of odontoblast
maturation (36, 37). However, we do not have any substantial data to support the mechanism that operates to control the expression of DSPP during the secretory stage of the odontoblasts. A possible explanation could be the remodeling of chromatin structure during the odontoblast differentiation process. There are several reports indicating chromatin remodeling as a common mechanism that occurs during various stages of cellular differentiation (33, 38–40).

DSPP null mice showed a similar phenotype as the DMP1 null mice at later stages of development (12). DSPP−/− mice had an extended pre-dentin area and possessed a reduced mineralized dentin region (12). These results are in concurrence with our observations presented here in this report. DSPP promoter has been characterized to a certain extent. Several transcriptional binding sites and their effects on the transcriptional activity of DSPP promoter were studied in a cell–culture system and a transgenic mouse model (4, 5). The tight programmed regulation is believed to be achieved by the orchestrated manipulation performed by various transcription factors and signaling pathways. Runx2, an osteoblast-specific transcription factor, has been shown to differentially regulate the DSPP expression during the odontoblast differentiation and maturation process (8,34). Runx2 down-regulates the DSPP promoter activity in pre-odontoblasts, whereas it activates the DSPP promoter activity in differentiated odontoblasts. It is presumed that partners for Runx2 might play a role in this differential regulation of the DSPP promoter.

Another target gene we have identified downstream of DMP1 is osteocalcin (data not shown). DMP1 regulates the osteocalcin promoter through a different mechanism. Overexpression of DMP1 in osteoblasts had no effect on the expression of the osteocalcin gene; however, overexpression of antisense DMP1 significantly decreased the expression level of the osteocalcin gene (24). This result further suggests that other mechanisms might be operative through which DMP1 regulates the expression of bio-mineralization-specific genes. Future studies will focus on the detailed mechanism by which DMP1 mediates the regulation of such genes.

Acknowledgments—We thank Dr. Gerard Karsenty (Baylor College of Medicine, Houston, TX) for the kind gift of osteocalcin promoter in luciferase reporter plasmid, and Dr. Gen He for the AFM studies.

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