

DMP1 is essential for osteocyte formation and function

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DMP1 is an acidic phosphorylated extracellular matrix protein that was originally identified from a rat incisor cDNA library and thought to have a primary function in regulation of dentinogenesis¹. DMP1 shares similar sequence, biochemical, and genomic DNA organizational properties with a family of proteins, called SIBLINGs for Small; Integrin-Binding Ligand, N-linked Glycoprotein. The members of this family include bone sialoprotein (BSP), osteopontin (OPN), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE). All of these family members contain an RGD sequence for integrin binding and can bind to hydroxyapatite^{2,3}. BSP, OPN and DMP1 can also bridge complement Factor H to cell surface receptors, an alternative complement pathway in order to prevent cell lysis^{2,3}. OPN and DMP1⁴ have been demonstrated to bind to CD44, a membrane bound protein thought to interact with the ERM (ezrin, radixin, moesin) family of adapter proteins that link to actin in the cytoskeleton.

Our recent observations on the expression pattern of DMP1 and of the phenotype in mice lacking the DMP1 gene underscore the critical importance of DMP1 in osteocyte function. These findings include: 1) although DMP1 is expressed in all tissues that undergo mineralization, its expression in osteocytes is much higher than in any other cell type as determined by *in situ* hybridization, lac Z knock-in expression, and immunostaining; 2) by immunostaining, DMP1 appears to be highly abundant in the dendritic processes of osteocytes and by immuno-EM appears to be localized on the canalicular walls along the lamina limitans; 3) a dramatic increase in DMP1 expression is observed in osteocytes in response to mechanical loading both *in vitro*

and *in vivo*; 4) DMP1 null mice show major abnormalities in osteocyte morphology, with a 2-fold increase in lacunar size, abnormal "buckling" of the membrane surface of the dendrites, loss of integrity of the lamina limitans and obliteration of the canalicular space; 5) DMP1 null mice show a severe impairment in mineralization, with patchy and poorly organized mineral and an apparent delay in the transition from osteoblasts to osteocytes; 6) mechanical loading of the ulna from DMP1 null mice at 60 Hz produces strains 1.7 times higher than the strains in wild type and heterozygous littermates, indicating a significant change in the material (elastic moduli) and/or structural (stiffness) properties of the bones; and 7) a further striking observation in these mice is the progressive change in the skeletal properties with age with bony protrusions forming over time, appearing primarily at sites of muscle insertion, suggesting an abnormal response to mechanical loading in postnatal animals. We propose that osteocytes in the DMP1 null mice exist in a hyperstimulated state, due to the mechanically compromised skeleton in these mice and that the formation of abnormal bony protrusions over time reflects an abnormal adaptive response that is attempting to restore skeletal homeostasis.

Our laboratory has generated transgenic mice that lack the gene for DMP1⁵. Although these animals appear normal at birth, a profound defect is observed in dentinogenesis⁶, chondrogenesis⁷, and craniofacial development (Ye et al., *in press*, appendix). However, the most striking phenotype is mineralization and osteocyte dysfunction. DMP1 is almost exclusively expressed in osteocytes and shows a restricted localization along the dendrites and/or canalicular walls. We have developed several techniques to analyze morphological changes in osteocytes from DMP1 KO mice. First, a combination of injection of calcein/Alizarin Red in conjunction with DAPI nuclear counterstaining allows visualization of the mineralization front and its relationship with osteocytes. In the WT animals, there are three discrete lines of labeling, which are clearly separated from the osteocytes. In contrast, the labeling in the KO is absent in some areas and is highly diffuse in other areas, making it difficult to distinguish discrete lines of labeling. Osteocytes are surrounded by patchy fluorochrome label. These data suggest defective and disor-

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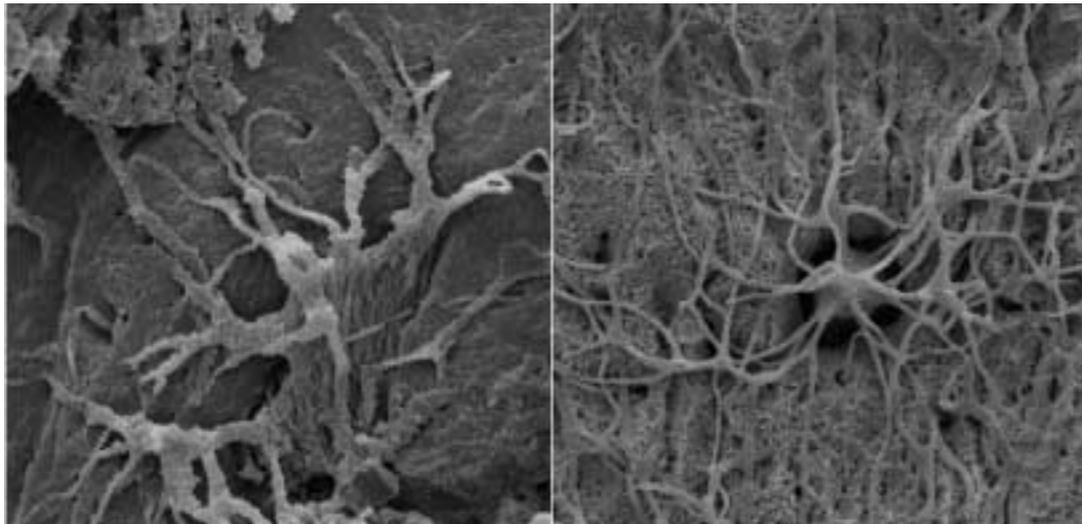


Figure 1. Scanning electron microscopy image of acid etched resin embedded cortical bone from wild-type (left) and DMP1-null mice (right). Note the smooth inner surfaces of canaliculi and extensive branching with consistent, continuous canaliculi diameter in the wild-type osteocyte lacuno-canalicular system. Note the rough and buckled appearance of the lacuna and canaliculi of osteocytes from DMP1-null mice. Fewer canaliculi are also present.

ganized mineralization in the DMP1 KO mice.

The second technique we have used to examine the lacuno-canalicular system in DMP1-KO mice is injection of procian red. This small molecular dye, when injected into the tail vein (0.8 %, 0.01ml/g), fills in lacunae and canaliculi but does not enter the cell. Thus, the dye can be used to give a visual representation of the organization of the lacuno-canalicular system within the skeleton. WT osteocyte lacunae are highly organized and spaced apart regularly, generally in linear arrays. The canaliculi are generally straight and run perpendicular to the long axis of the osteocyte. In contrast, the osteocyte lacunae in DMP1 KO mice appear much larger, the distribution of the osteocytes appears less organized and the canaliculi are less straight and more randomly oriented.

To quantify the lacunar area we have used atomic force microscopy (AFM) together with measurement by image analysis. This confirmed an approximately two-fold increase in osteocyte lacunar size in DMP1 KO mice. By TEM, striking abnormalities were observed in the canaliculi of DMP1 KO mice compared to WT controls. In WT mice the membrane surface of the dendritic processes was smooth, there was a clear space between the membrane and the canalicular wall, and the wall was defined by a clearly visible lamina limitans (arrowhead). In contrast, in KO mice, the membrane surface of the dendritic processes appeared irregular and buckled, the canalicular spaces surrounding the dendritic processes were poorly delineated, the lamina limitans was absent and the canalicular space appeared to be obliterated by collagen fibrils. These observations suggest that DMP1 may play an essential role in formation and/or maintenance of the canaliculi and canalicular space.

Another technique we have established for analyzing the morphology of the lacuno-canalicular system is acid-etched resin casting of the bones. In this technique, a polished surface from a resin embedded bone is etched with acid to remove mineral, leaving a relief cast of the non-mineralized areas that have been penetrated by resin. Using this technique, striking differences were observed in the appearance of the lacunae and canaliculi of DMP1 KO mice compared to WT controls, consistent with the abnormalities seen by TEM (Figure 1). Thus, the lacunae were larger and the surface of the lacunae and canaliculi appeared highly irregular and rough compared to the smooth surfaces of the lacunae and canaliculi in WT mice. There also appeared to be reduced numbers of canaliculi in the KO mice.

Taken together the above observations suggest that DMP1 is a major regulator of mineralization and that it may play a role in the transition of osteoblasts/preosteocytes to osteocytes perhaps through maintenance of structure of the lacunae and canaliculi. DMP1 may also be an important regulator of osteocyte-mediated responses to mechanical loading perhaps through its role as a regulator of mineralization. We hypothesize that these abnormalities in the osteocyte canaliculi/dendritic processes result in impaired fluid flow through the canaliculi, thus affecting the ability of the osteocytes to respond to mechanical signals.

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