New Perspectives on Amelotin and Amelogenesis

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This issue reports the first enamel malformations in amelotin (*Amtn*)-ablated mice (Nakayama et al. 2015). Among the proteins necessary for enamel formation, amelotin is one of the more recently discovered. It was discovered independently by 2 different laboratories (Iwasaki et al. 2005; Moffatt et al. 2006). The amelotin protein is enriched in proline, leucine, glutamine, and threonine (52% of total) and contains a perfectly conserved protein kinase CK2 phosphorylation site. Amelotin is a member of the secretory calcium-binding phosphoprotein (SCPP) family of proteins that evolved in vertebrates by gene duplication from a single ancestral gene (Kawasaki and Weiss 2003). Subsequent reiterative tandem gene duplication created 2 classes of genes, acidic SCPPs and proline/glutamine-rich SCPPs (Kawasaki et al. 2009). The SCPP genes encode secreted proteins with an SXE motif for phosphorylation by Golgi Casein Kinase (encoded by *FAM20C*) (Tagliabracci et al. 2012). In humans, there are 5 acidic and 18 P/Q-rich SCPP genes, which encode many of the secreted proteins that play prominent roles in enamel or dentin formation, such as amelogenin (AMELX), enamelin (ENAM), ameloblastin (AMBN), amelotin, odontogenicameloblast-associated (ODAM), secretory calcium-binding phosphoprotein-proline-glutamine-rich 1 (SCPPPQ1), dentin matrix protein 1 (DMP1), and dentin sialophosphoprotein (DSPP). AMTN, ODA, and SCPPPQ1 are expressed during the maturation stage of enamel development, when protein is removed from the fully thick enamel as it becomes the hardest substance in the body. The proteins encoded by these genes localize together within the basement membrane of maturation-stage ameloblasts and mediate the attachment of these epithelial cells to the mineralized tooth surface (Moffatt et al. 2014). Specifically, it was demonstrated that amelotin expression begins at the transition stage of enamel development and continues to be expressed throughout the maturation stage and into the reduced stage prior to when the incisor erupts (Somogyi-Ganss et al. 2012). Interestingly, Northern blots showed that amelotin was also expressed at low levels in the rat lung, and RT-PCR analysis showed it to be expressed at low levels in the mouse thymus, but not the lung (Moffatt et al. 2006). The detected low-level *Amtn* expression in the rat lung and mouse thymus are not likely biologically significant, since the EST databases show no amelotin transcripts from the lung in mice or humans and just 2 amelotin transcripts out of over 100,000 characterized from the mouse thymus, while human amelotin thymus transcripts are nonexistent.

Amelotin, like many of its SCPP relatives, functions specifically in teeth. No phenotype outside of the dentition has been observed in *Amtn* null mice. Amelotin expression is restricted to the inner enamel epithelium and the epithelia derived therefrom. Strikingly, an enamel phenotype was observed only in the mandibular incisors, which showed delayed mineralization and a tendency for the enamel to chip off the incisal tip. It is not clear why, in these mice, the molars and maxillary incisors appear to have normal enamel, but other protein(s) may functionally overlap with AMTN. Such candidates would include ODA and SCPPPQ1. Unfortunately, it will be difficult to determine if these genes can substitute for AMTN, because all 3 genes reside on the same chromosome, which excludes breeding of the various knockouts to obtain double (*Amtn*, *Oda*) or triple (*Amtn*, *Oda*, *Scppq1*) knockout mice.

Although the phenotype is mild and selective, demonstration of enamel defects in *Amtn* null mice is the first experimental evidence to support the functional importance of a specialized basement membrane in enamel development. This basement membrane replaces the secretory-stage mineralization front apparatus that functioned to expand the enamel layer to its final dimensions, and it provides a novel attachment apparatus that secures the epithelia to the enamel surface (Simmer et al. 2012). The amelotin present within the basement membrane appears to allow a more consistent hardening of the enamel, because without amelotin, the incisor enamel tends to chip. Perhaps more importantly, this specialized basement membrane persists following tooth eruption in the junctional epithelium, where firm attachment of the soft tissue to the enamel helps seal the gingival collar and protect the underlying periodontal attachment.

Kindler syndrome is an autosomal-recessive condition caused by mutations in *FERMT1* (Jobard et al. 2003). One of the features of this disease is severe early-onset periodontal

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disease (Larjava et al. 2014). Histologic findings suggest that the junctional epithelium in Kindler syndrome may be abnormal and could explain why these patients have periodontal disease (Wiebe et al. 2008). The protein product of *FERMT1* is KIND1, a key intracellular component of focal adhesions, which tightly connects cells to the extracellular matrix. Focal adhesions contain clusters of transmembrane integrin adhesion receptors and intracellular proteins that link integrins to the actin cytoskeleton and to signaling pathways (Brahme et al. 2013). Therefore, it is possible that molecular defects in the basement membrane could weaken the attachment of junctional epithelium to tooth surfaces, which could increase the susceptibility of a patient to periodontal disease (Wiebe and Larjava 1998). It will be of interest to identify the molecular functions of AMTN, ODAM, and SCPPPQ1 in normal amelogenesis and determine the potential for defects in the genes encoding these proteins in the etiologies of amelogenesis imperfecta and periodontal disease susceptibility.

Identification of the genes involved in amelogenesis has provided evolutionary biologists with the ability to use genomics to discern how and when enamel, like that found in mice and humans, first formed and how it evolved over time. The enamel-related genes *Odam*, *Amtn*, *Amel*, *Enam*, and *Ambn* are found in the coelacanth genome (Kawasaki and Amemiya 2013). The coelacanth is a “fossil” fish related to lungfish that evolved about 400 million years ago. Enamel in lungfish forms like enamel in mammals, by the elongation of thin mineral ribbons at a mineralization front along the distal membranes of ameloblasts (Satchell et al. 2000). Therefore, all tetrapods with enamel-covered teeth, including reptiles, amphibians, and mammals, express AMTN. In fish with gills, such as the teleosts, the tooth surfaces are covered with enameloid, which is a hypermineralized tissue different from enamel formed by the deposition of long, organized mineral ribbons (Kawasaki and Suzuki 2011). Teleosts that have been characterized genomically (such as fugu and zebrafish) contain *Odam*, but lack *Amtn*, *Amel*, *Enam*, and *Ambn*. It appears that early fish were evolving various means to harden the surface-covering of their teeth and that *Odam* was already in place to facilitate cell attachment to this mineralized surface. The introduction of *Enam*, *Ambn*, and *Amel* is associated with the development of a novel form of appositional growth that centered upon the deposition of oriented mineral ribbons. It is clear that amelotin is not active during this period of appositional growth, because it is not normally expressed during the secretory stage (Fig.) and, in the *Amtn*-ablated mice, the enamel layer is full-thickness and shows normal rod decussation. The enamel mineral ribbons deposited during the secretory stage harden by growing in width and thickness during the subsequent maturation stage.

Figure. Amelotin expression during various stages of amelogenesis in the 10-day-old mouse incisor from the incisal end to the cervical loop. Magnified views (a–i) show details of the restricted expression in maturation-stage ameloblasts. Bar indicates 10 μ m. [Adapted with permission from Fig. 3 in Iwasaki et al. J Dent Res. 2005;84(12):1127–1132.]

This process benefits from the expression of *Amtn*, without which there is a delay in enamel maturation and a tendency for the incisor enamel to chip at its working edge.

These findings strongly support the perspective that mammalian-like enamel is an evolutionary innovation that arose during the evolution of fish through gene duplication(s) that generated multiple new SPP genes and that these genes became specialized for the formation of dental enamel. Amelotin was one of those genes. *AMTN* has survived as a gene for 400 million years through natural selection for its necessary participation in tooth development, presumably through contributing to the hardening of dental enamel and potentially through improving the adherence of the junctional epithelium to the tooth surface.

AMTN has not yet been proven to be part of the etiology of amelogenesis imperfecta (AI). This is true despite the fact that *AMTN* has been a logical candidate gene for AI ever since its discovery as an ameloblast-specific protein (Iwasaki et al. 2005; Kawasaki and Weiss 2006). It appears, however, that the current list of AI candidate genes accounts for only about half of all non-syndromic AI cases (Chan et al. 2011). *Amtn* null mice show only a mild enamel phenotype. So, it may be some time before we fully understand the critical function served by AMTN upon which natural selection has acted to maintain the integrity of this gene for 400 million years in so many vertebrates.

Author Contributions

J.D. Bartlett, J.P. Simmer, contributed to data interpretation, drafted the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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