

# THE ENAMELYSIN KNOCKOUT MOUSE

Laboratory of John D. Bartlett, Ph.D., e-mail: jbartlett@forsyth.org  
 Harvard-Forsyth Department of Oral Biology & Forsyth Department of Cytokine Biology

## The Enamelysin Knockout Mouse



Enamelysin is a matrix metalloproteinase (MMP) that is found exclusively in developing tooth tissues. An enamelysin knockout mouse was engineered that has a severe and profound tooth phenotype. We have demonstrated that the enamelysin knockout mouse does not process amelogenin properly, possesses an altered enamel prism pattern, has hypoplastic enamel, has enamel that delaminates from the dentin, and has a deteriorating tooth morphology as enamel development progresses. These are exciting results because despite the severe tooth phenotype, these animals are able to eat and breed. No other MMP knockout mouse possesses these qualities. Thus, for the first time, we can design experiments to answer mechanistic questions concerning the importance of the MMP domain structure. This is accomplished by introducing modified enamelysin transgenes into the knockout background. The other essential component of the transgenic experiments is the enamelysin promoter. We now have 7 Kb of enamelysin promoter that contains consensus regulatory elements that are present in other tooth-specific co-regulated genes. With the knockout mouse and promoter we can now address fundamental issues concerning enamelysin biology that will be of interest to both Oral Biologists and MMP Researchers.

## Domain Structure of Enamelysin (MMP-20)

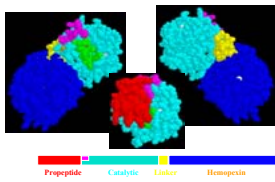


Figure 1. Molecular models of enamelysin. Bottom of figure: The linear domain structure of enamelysin is shown with the color of each domain matching those of the models. The color key for the enamelysin model is: propeptide domain (red), catalytic domain (cyan), linker domain (yellow), hemopexin domain, and active site region (green). The top-left and right-hand panels show the active protease. The right-hand panel shows the flip side of the model in the left-hand panel (rotated 180 degrees about the Y axis). The middle panel shows the catalytic domain of the inactive enamelysin zymogen. Note how the propeptide domain (red) covers the active site area (green).

## CASEIN ZYMOGRAPHY OF FOUR DAY-OLD MOUSE MOLARS

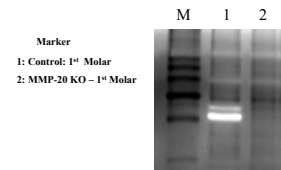


Figure 2. Tooth molar enzyme profile of a control versus enamelysin knockout mouse by casein zymography. First molars were dissected from mandibles of 4.0-4.5 day-old mice. The enzymes present in the molars were extracted and subjected to zymography. Clear bands or zones of substrate lysis indicate the presence of enzymes. Lane 1, normal control 1<sup>st</sup> molar; lane 2, enamelysin knockout 1<sup>st</sup> molar. Note that the doublet present at approximately 42-46 kDa is missing in the knockout molars. This doublet represents zones of enamelysin degradation.

## CONTROL OR ENAMELYSIN KNOCKOUT MOUSE MOLAR

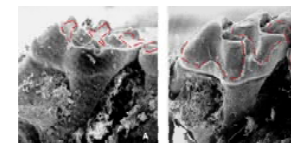


Figure 3. Scanning electron micrograph of a first maxillary molar from a control (A) and from an enamelysin knockout mouse (B). Dashed red lines encircle the enamel-free areas present within each molar. Note the pattern of enamel-free areas that are typical of rodent molars at the marsupial plateaus of the cusps (A). In contrast, the tooth from the enamelysin knockout mouse contains almost no enamel on the first cusp and most of the enamel is missing from the second cusp (B). Apparently, the enamel has delaminated from the underlying dentin as a result of normal mastication processes by the knockout mouse.

## PROTEINS EXTRACTED FROM CONTROL & KO 1<sup>ST</sup> MOLARS

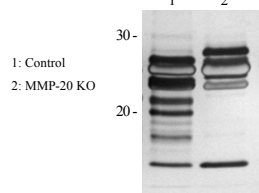


Figure 4. Extracted amelogenins from a control versus enamelysin knockout mouse. The first molar was dissected from the mandibles of 4.0-4.5 day-old mice. The amelogenins in the forming enamel were extracted and run on a 12% PAGE gel. Lane 1, first molar from the normal control; lane 2, first molar from the enamelysin knockout mouse. Note that the knockout mouse has a strong band of approximately 27 kDa (lane 2) whereas the control has a very weak band at this position (lane 1). Also note that several lower MW bands are missing in lane 2 when compared to bands present in lane 1.

## DENTAL ENAMEL FROM A CONTROL OR MMP-20 KNOCKOUT MOUSE

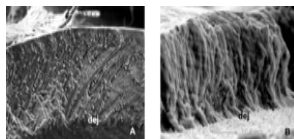


Figure 5. Scanning electron micrograph of fractured incisor enamel from a control (A) and from an enamelysin knockout mouse (B). Note that the micrograph of the control enamel is at a much lower resolution than that from the knockout animal (see 10 μm bars in each panel). This was necessary so that full-thickness of the enamel from the dentin/enamel junction to the tooth surface could be observed with clarity for each micrograph. The enamel thickness is approximately 115-120 μm in the control (A) and is only approximately 35-40 μm in the knockout animal (B). The typical decussating inner enamel rod pattern can be observed in the control (A), but is absent in the enamel from the knockout mouse (B). Also note that for the enamel from the knockout mouse, the enamel did not fracture in the same plane as the dentin, indicating a faulty dentin/enamel junction.

## Morphology of Control and Enamelysin Knockout Teeth

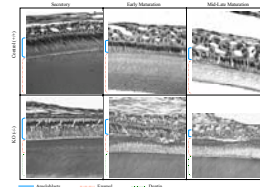


Figure 6. Examination of enamel thickness and ameloblast morphology at three defined stages of enamel development. Top panels, demineralized sections from a control mouse incisor and bottom panels, from an enamelysin -/- mouse. Solid blue brackets designate the ameloblast layer, red dashed-dot brackets designate the enamel layer, and for the lower panels, green dot brackets designate the dentin layer. Note the highly significant difference in enamel thickness between the knockout and control incisors (upper versus lower panels). Also note that as development progresses (from left panels to right) the ameloblast morphology becomes increasingly disorganized in the knockout incisor as compared to the control.

## CONCLUSIONS Enamelysin Knockout Mouse

Enamelysin Knockout Mouse has a Severe and Profound Tooth Phenotype Characterized By:

- ❖ Improper Amelogenin Processing.
- ❖ An Altered Enamel Prism Pattern.
- ❖ Hypoplastic Enamel.
- ❖ Enamel that Delaminates From the Dentin.
- ❖ A Deteriorating Tooth Morphology As Enamel Development Progresses.