

## P32-DISTRIBUTION AND STRUCTURE OF THE INITIAL DENTAL ENAMEL FORMED IN INCISORS OF YOUNG WILD-TYPE AND TABBY MICE

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### Introduction

The distribution and structure of mouse incisor enamel resemble that of the rat. The enamel, which covers only the labial aspect of the tooth, can be divided into four layers: a thin inner prism-free layer, inner enamel with prism decussation, i.e. transverse rows of prisms with prisms inclined medially and laterally in alternate rows, outer enamel with parallel prisms inclined incisally and a thin superficial prism-free layer (1,2). This is also the order of formation of the layers, since the enamel formation starts at the dentin surface and the new layers are apposed in external direction. However, it is not known how this elaborate organization of the enamel is established in the initial enamel formed on the unerupted and unworn incisal tip of the incisors. We wanted to study how this complex structural organization is established in the very first enamel formed, in wild-type mice and also in Tabby mice where enamel coverage varies considerably.

### Materials and Methods

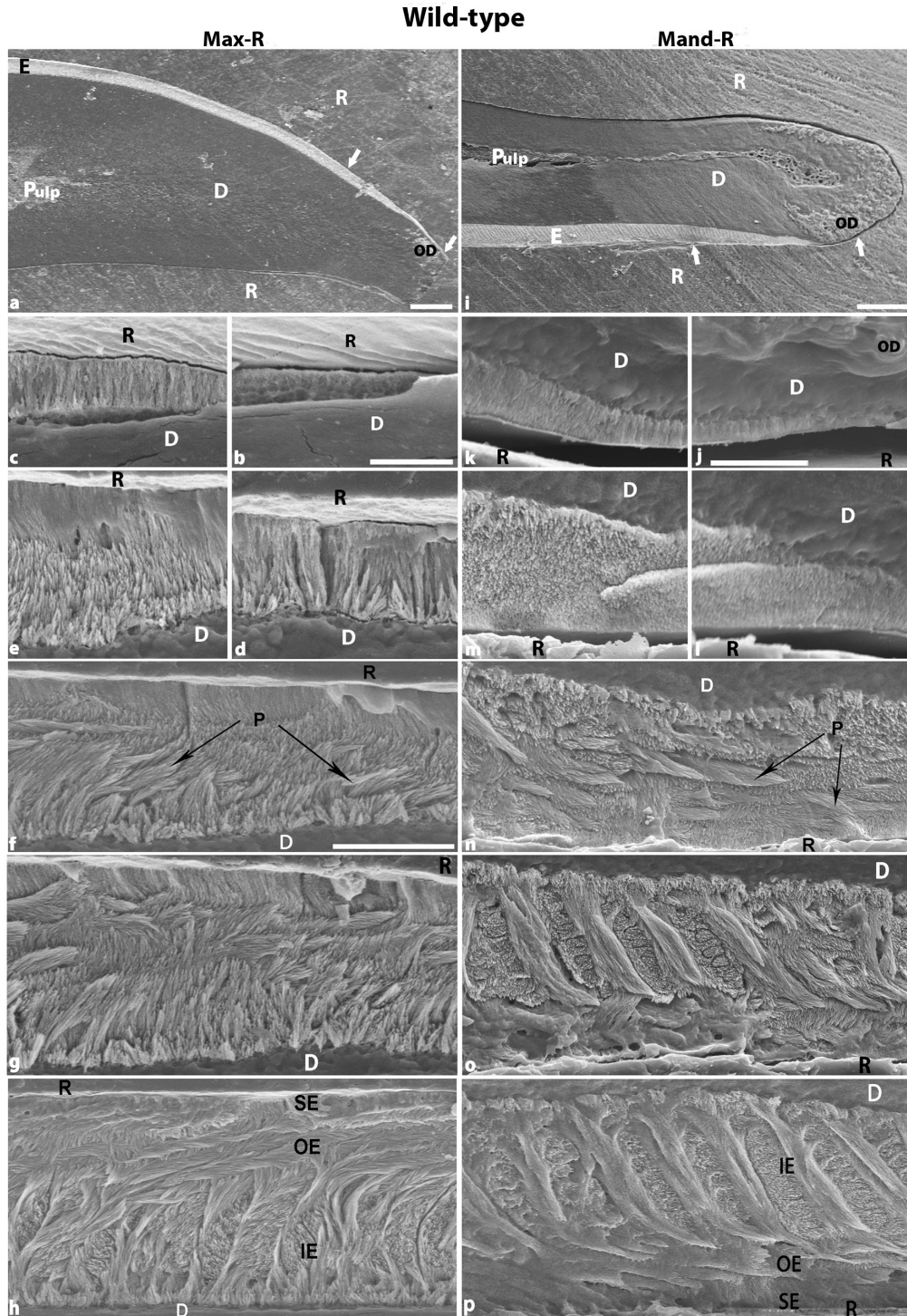
Unworn incisors from young female wild-type and Tabby mice were ground, etched, and observed in SEM. Some incisors were ground transversely, while the contralateral teeth were ground longitudinally through the median part of enamel. For the transversely ground incisors the whole procedure was repeated four times, creating transversely ground planes for observation. The first plane (T1) just touched the incisal tip. Subsequent planes were ground about 200, 400 and 800  $\mu\text{m}$  apical to the T1 plane, using identifiable markings in the investing resin as reference points.

### Results

The incisal tip was intact and unworn, and tooth diameter increased gradually in apical direction. In both wild-type and Tabby mice, the enamel tended to extend somewhat further lingually on the lateral than on the medial aspect. Distribution of enamel in Tabby mice exhibited a considerable variability. In two mandibular incisors the enamel covered the whole circumference in the incisal part of the tooth to at least 800  $\mu\text{m}$  from the T1 plane. In both wild-type and Tabby mice, the establishment of the enamel structural characteristics in the initially formed enamel proceeded as follows, going from the incisal tip in apical direction (Fig. 1): 1) zone with prism-free enamel, 2) zone with occasional prisms most often inclined incisally, and 3) zone where prism decussation was gradually established in the inner enamel.

### Discussion

In zone 1, the prism-free enamel increases in thickness in apical direction and becomes thicker than the prism-free superficial layer of fully established enamel in zone 4. Since the enamel increases in thickness in an apical direction as the different layers are established, the ameloblasts producing the enamel may obey a gradient of increasing ameloblast life span in an apical direction. The most incisally positioned ameloblasts may only have time and/or capacity to produce and mature a very thin layer of prism-free enamel. Since the enamel is prism-free, it means that these ameloblasts did not develop Tomes' processes. As the ameloblasts further apically probably are allowed longer life spans, indicated by the thicker enamel that they produce, they, in increasing numbers, develop Tomes' processes and produce prismatic enamel. Since development of Tomes' processes takes



Legend to Fig.

Figure 1 SEM of longitudinally ground planes of young wild-type mouse incisors demonstrating the sequence in establishment of the characteristic structural pattern of incisor enamel. a-h) Longitudinally ground planes through centrolabial part of enamel of right maxillary incisor (Max-R). i-p) Longitudinally ground plane through centrolabial part of enamel of right mandibular incisor (Mand-R). b-h) Higher magnification of initial enamel, between arrows in a. j-p) Higher magnification of initial enamel, between arrows in i. b-e, j-m) The most incisally situated enamel is prism-free. f-g, n) Appearance of more or less well-defined, isolated, and mostly incisally inclined prisms, corresponding to the prisms in the outer enamel (OE) in h and o, p. Possibly attempted prism decussation in enamel close to dentin. o) Transition from tentative (right) to fully established (left) decussation. h, p) Fully established enamel with three distinct layers. Bar is 100  $\mu\text{m}$  for a and i, 10  $\mu\text{m}$  for b-e, and for j-m, 20  $\mu\text{m}$  for f-h and n-p. E = enamel, D = dentin, OD = osteodentin, R = resin, SE = superficial enamel, OE = outer enamel, IE = inner enamel, P = prism.

some time, the innermost enamel is largely prism-free. The movement of the ameloblasts, as deduced from the orientation of the prisms they produced, is at first somewhat variable. However, the main tendency for the first ameloblasts with Tomes' processes is to move in an incisal direction. These ameloblasts eventually lose their Tomes' process and produce a thin superficial prism-free enamel. Further apically ameloblasts with Tomes' process are probably being organized in transverse rows (3) facilitating a transverse movement of ameloblasts resulting in prism decussation in the inner enamel layer. Then they move in incisal direction creating the outer enamel layer and finally lose their Tomes' process to form the thin superficial prism-free layer.

In accordance with previous findings in adult mice (4), incisors from young Tabby mice exhibited a variable distribution of enamel, while the enamel structure was largely normal. In young Tabby mice the crown-analogue compartment was both absolutely and relatively increased compared to the root-analogue compartment. The variable extent and coverage of enamel medially and laterally may perhaps be related to the expression pattern of genes that encode for ectodysplasin and its receptor Edar; while ectodysplasin expression is restricted to the outer dental epithelium, the gene that encodes for its receptor Edar is expressed only in inner dental epithelium (5), from which the ameloblasts originate. The gene that is not being expressed in the inner dental epithelial cells due to the missing ectodysplasin signal from the outer dental epithelium cells, is not known. Ectodysplasin is evidently not a signal necessary for enamel formation. However, it seems to be important for defining the extent of the enamel-producing domain of the inner enamel epithelium. The unequal distribution of enamel in rodent incisors seems to be linked to a complex gene regulatory network operating in the stem cell compartments of the incisor's cervical loop

and involving such factors as activin, bone morphogenetic protein (BMP), fibroblast growth factor (FGF), follistatin and sprout (6,7). Interestingly, ectopic ameloblasts differentiate on the lingual aspect of lower incisors in mice that are null for follistatin (6) or sprout (7,8), which may be due to an upregulation of FGF signalling in the follistatin and sprout mutants (6,7). Specific roles of EDA and FGF in determining enamel producing domains should be focused on in the future.

### Conclusions

The sequence of initial enamel formation in mouse incisors mimics a development from a primitive (prism-free) to an evolved structure. It is suggested that genes controlling enamel distribution are not associated with genes controlling enamel structure. The control of ameloblast configuration, life span, organization in transverse rows, and movement is important for establishing the characteristic mature pattern of mouse incisor enamel.

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