

## P44-EXPRESSION PATTERN of APIN and AMELOTIN DURING FORMATION and REGENERATION of the JUNCTIONAL EPITHELIUM.

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### Key words

apin; amelotin; cytokeratin 14; development; gingiva; junctional epithelium; odam; regeneration.

### Introduction

The junctional epithelium (JE) is the component of the dentogingival junction that adheres to the tooth surface, and seals off periodontal tissues from the oral environment (1). This unique, incompletely differentiated epithelium is formed by the fusion of the reduced enamel organ (REO) with the oral epithelium (OE). Attachment of the gingiva to the enamel surface is provided by a structural complex called the epithelial attachment. This complex consists of an inner basal lamina (BL) formed and maintained by the superficial JE cells to which they are attached by hemidesmosomes. This BL is considered atypical because it contains laminin-5 but not other typical components, such as  $\gamma 1$  chain-containing laminins, and type IV and VII collagens. The exact mechanisms by which the JE maintains its adhesive relationship with the tooth surface through the BL and establishes its unique incompletely differentiated cellular status are still not known. Efforts to identify the secretome of the epithelial cells responsible for creating tooth enamel have led to the identification of genes encoding for two proteins called Apin (APIN, also known as odontogenic ameloblast-associated, ODAM) and amelotin (AMTN) (2;3). Unexpectedly, they are also strongly expressed in the JE. Our objective was to investigate the presence and distribution of APIN and AMTN at various stages of tooth eruption and in regenerating JE following gingivectomy.

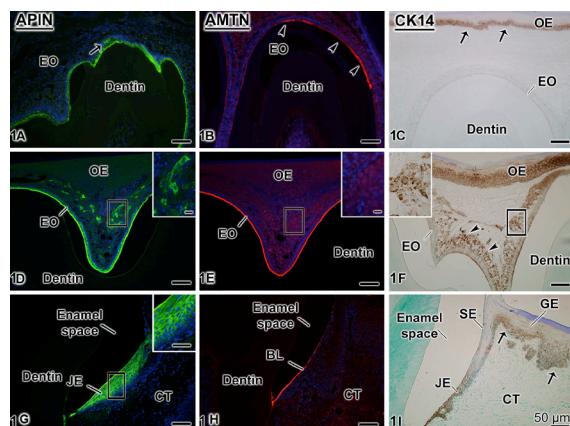
### Materials and Methods

The pattern of expression of APIN and AMTN during the formation of the primary JE was evaluated using immunofluorescence carried out on erupting maxillary molars of Wistar rats of 2, 3 and 8 weeks of age. Immunofluorescence was also done on samples of regenerating JE harvested following surgical ablation of the gingiva on the palatal side of maxillary molars from 250-300 g Wistar rats. Animals were sacrificed

immediately after gingivectomy (day 0) and at 3, 5, 7 and 14 days post-surgery. Immunoperoxidase labeling was used to detect cytokeratin 14 (CK14) and Ki67, a nuclear protein expressed during the active phases of the cell cycle.

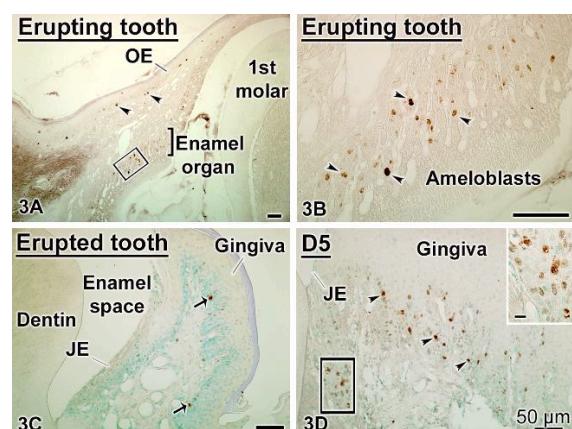
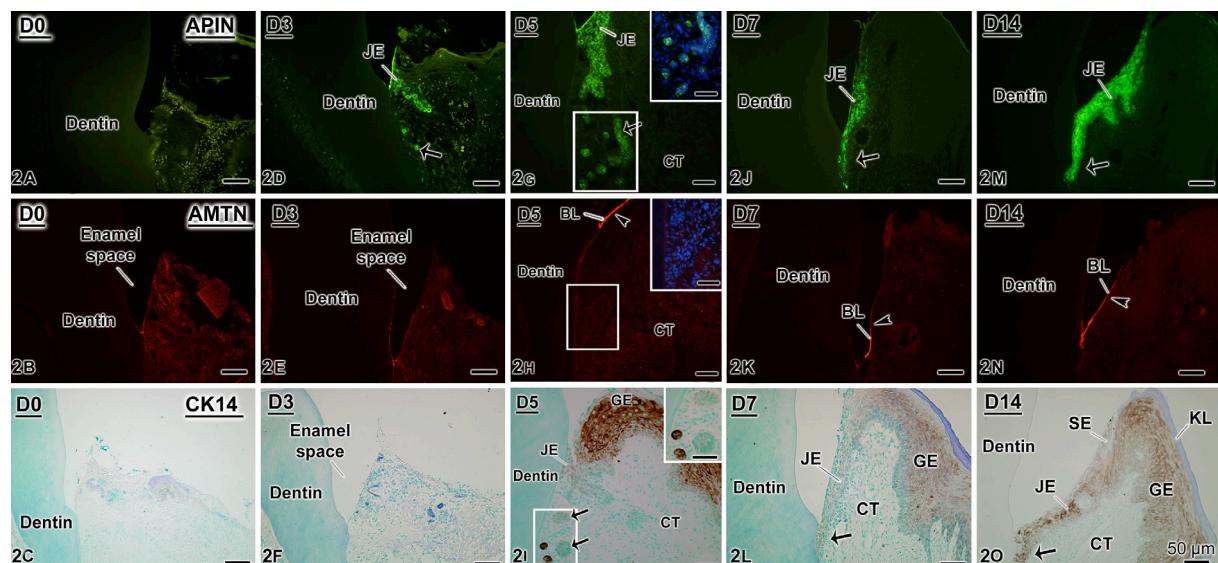
### Results

During tooth eruption, APIN (Figs 1A, 1D) and AMTN (Figs 1B, 1E, arrowheads) were immuno-detected at the REO-tooth interface. APIN was also distinctively expressed by cell clusters present between the REO and OE (Fig 1D). Cytokeratin 14 was detected in the basal cells of the OE (Fig 1C, arrows), but starting at 3 weeks, labelling was noted in clusters of cells present between the REO and OE, and in cells of the papillary layer (Fig 1F). In established JE, APIN (Fig 1G) and AMTN (Fig 1H) were likewise found at the cell-tooth interface, where the inner BL is found, but APIN was additionally observed among JE cells. Cytokeratin 14 was found in the basal cells of the gingival epithelium (GE) and throughout the JE, but a weaker signal was observed in the sulcular epithelium (SE) and suprabasal cells of the GE (Fig 1I, arrows).



During early JE regeneration, APIN (Fig 2D) was detected in association with cells at the leading wound edge of the OE and some immunolabeled cell clusters (arrow) were seen in the underlying connective tissue (CT). No AMTN (Fig 2E) and CK14 (Fig 2F) were detected at the wound site. At the later phase, both APIN (Fig 2G) and

AMTN (Fig 2H, arrowhead) were present at the interface with the enamel surface, but only APIN was observed among cell clusters in the CT (arrow). Cytokeratin 14 (Fig 2I) started to appear in the reforming GE and weak to intense labelling was noted for cell clusters in the CT (arrows). At day 14, despite the presence of a long JE, the immunolabeling for APIN (Fig 2M) and AMTN (Fig 2N) was comparable to that seen in control samples (see Fig. 1G and H). Different from normal gingiva (see Fig. 1I), CK14 was detected in the entire cell layer of the regenerating GE, JE and SE (Fig 2O).



Immunoperoxidase preparations for Ki67 was carried out in erupting molars of 3 weeks-old rat (Figs 3A and 3B, arrowheads) and several epithelial cells on the outer layer (boxed area) of the enamel organ and basal cells of the OE showed cell division activity. In erupted tooth, basal cells of the GE were less frequently immunolabeled (Fig 3C, arrows) and the fully-formed JE showed

scarce or no stained cells. At 5 days post-gingivectomy (Fig 3D, arrowheads), the basal cells of the reforming gingiva and cells of the clusters found in the CT, showed more proliferation activity than what is seen in the control sample.

## Discussion

The dual localization of APIN suggests that it may be implicated in both cellular activities and in the molecular mechanisms that allow the JE to adhere to tooth surface. It may influence the apical extension of the gingival wound edge to the reestablishment of a functional JE and may

play a role in modulating the cell differentiation status of the JE. Instead, the late appearance of AMTN when the reforming JE reestablishes contact with the tooth, and its conspicuous localization in the area of the inner BL suggest that the role of AMTN is restricted to events taking place at the epithelium-tooth interface. As components of the inner BL of the JE, both APIN and AMTN may contribute, directly or indirectly by interacting with other components of the BL, to the attachment mechanism of the JE.

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## References

- Schroeder, H. E. and Listgarten, M. A. (1997) Periodontology 2000, 13, 91-120.
- Moffatt, P. et al. (2006) Biochem J, 399, (1) 37-46.
- Moffatt, P. et al (2008) J Cell Biochem, 103, (3) 941-956.