An Electron Microscopic Study of Ectomesenchymal Contacts in Rat Incisors

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Abstract

In order to clarify connections between epithelium and mesenchymal tissue in the early stage of odontogenesis, the formation of ectomesenchymal contacts and the presence of coarse-textured material at the interface between ameloblasts (AMs) and odontoblasts (ODs) on growing rat incisors were studied by electron microscopy.

The presecretory zone was classified into five regions according to the ultra-structure of each preameloblast (PA). The region where collagen fibrils could be observed was designated PZ-2, the region where predentin existed PZ-3, the region where the basal lamina began to disappear PZ-4, and the region where the basal lamina had disappeared PZ-5. In PZ-2, the cytoplasmic processes of PAs penetrated the basal lamina and reached the dental papilla cells. In some locations, ectomesenchymal contacts were also observed, in which the cytoplasmic processes of the PAs were in contact with those of the preodontoblasts (POs).

In PZ-3, the distal cytoplasm contained large amounts of type-I vesicles, and secretory granules. In the distal membrane of the PA, membrane invaginations containing fibrillar structures were visible. Also in some areas, the cytoplasmic processes at the distal ends of the PAs invaded the predentin. In the predentin, a large amount of coarse-textured material, considered to be the precursor of the enamel matrix, was observed. In PZ-3, a large number of cytoplasmic processes extended into the predentin, while in PZ-4, microvillus-like processes extended from the distal ends of the PAs, showing a high frequency of ectomesenchymal contact.

It was suggested that secretory activity of the PAs was induced after ectomesenchymal contact had been accomplished, but that the PAs did not undergo morphological change into secretory ameloblasts.

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Introduction

Enamel and dentin are formed at the interface between the ectodermal cells and mesenchymal cells, which elaborate the hard tissue constituting a tooth. When these hard tissues are formed, odontoblasts (ODs) and ameloblasts (AMs), by exchanging various kinds of information, secrete a matrix of enamel and dentin in a certain order, and are also capable of mineralizing this matrix\(^{1-4}\). It is considered that these two types of cell, AM and OD, transmit information by some morphological method of conduction.

This morphological transmission has not been clarified by light microscopy to the problem of resolution. However, as a result of electron microscopic studies\(^{2,4-12}\), it has become clear that "ectomesenchymal contact" exists between AMs and ODs, by which the cytoplasmic process of each comes into contact at an early stage of tooth development.

This contact may affect AMs and ODs at the beginning of their differentiation and matrix-formation\(^{13-15}\). However, it has not been clarified at which stage of tooth formation this contact forms.

Therefore, using the growing end of rat incisors, the authors carried out an electron microscopic study of the interface of ectodermal and mesenchymal tissues.

Materials and Methods

Six male Wistar rats, each weighing about 40 g, were used for this experiment. Each rat was perfused from the left ventricle with 2.5% glutaraldehyde solution containing 2% paraformaldehyde diluted in 0.05 M phosphate buffer (pH 7.4). After perfusion, the upper incisors were dissected out and decalcified in 0.1 M EDTA (pH 7.4). Their growing ends were cut transversely to the long axis of the tooth, then cut again mid-sagittally with a razor blade. These tissues, after washing with 0.08 M cacodylate buffer (pH 7.4), were post-fixed with 1% OsO\(_4\) diluted with 0.08 M cacodylate buffer (pH 7.4), then dehydrated in ethanol and embedded in Araldite. Ultrathin sections of the AMs and ODs were cut in a longitudinal direction and double stained with uranyl acetate and lead citrate. The ectomesenchymal interfaces were then observed sequentially with a Hitachi H-500 electron microscope.

Results

As to the criteria used to indicate the stages and regions of the AMs and ODs of rat incisors, the classification by KASAMO\(^{12}\) was followed, and the PAs in the presecretory zone were observed in each of the regions from PZ-1 to PZ-5, according to the morphological features of the mesenchymal layer under the PA. The region in which microfibrils were attached was designated PZ-1, the region in which collagen fibrils could be observed PZ-2, the region in which predentin existed PZ-3, the region in which the basal lamina began to disappear PZ-4, and the region in which the basal lamina had disappeared PZ-5.

1. PZ-1

PAs and the dental papilla cells (DPCs) are divided by a basal lamina with
microfibrils. The PAs show a cuboidal form.

The DPCs have a stellate shape and are located distally from the basal lamina. The distal cytoplasmic processes of cells extend to the basal lamina (Fig. 1). Ectomesenchymal contact cannot be seen in this region.

2. PZ-2

PAs show a low columnar shape, and the Golgi apparatus is mainly located lateral to the nucleus. Preodontoblasts (POs) which have differentiated from the DPCs have a low columnar shape with more abundant distal cytoplasm. The POs are situated closer to the basal lamina than those in PZ-1 (Fig. 2). In this region, microfibrils are attached to the basal lamina, and collagen fibrils can be seen under the microfibrils as well as between the POs. The cytoplasmic processes extending from the distal ends of the PAs penetrate the basal lamina and reach the cytoplasm of the POs (Figs. 3, 4). Occasionally, the cytoplasmic processes of the PAs make contact with those of the POs (Fig. 5).

3. PZ-3

Since PAs and POs have more abundant distal cytoplasm, the cells become columnar in shape. Predentin with a thickness of about 2-4 μm is formed (Fig. 6). The Golgi apparatus of the PAs mainly occupies the supranuclear region, and a distal terminal bar appears at the distal end (Figs. 6-9). The distal cytoplasm contains large amounts of type-1 vesicles\(^8\), tubular structures and secretory granules. In the distal cell membrane of PAs in this region, narrow membrane invaginations containing fibrillar structures are recognized. Furthermore, in some locations, the cytoplasmic processes at the distal end of PAs penetrate the basal lamina and invade the predentin (Figs. 7, 8). In the predentin of this region, large amounts of coarse-textured material can also be found (Figs. 7, 9).

4. PZ-4

Numerous cytoplasmic processes extend from the distal end of the PAs and invade the predentin. However, the cytoplasmic processes of PAs and POs rarely make contact. In PZ-4, more coarse-textured material is present in the predentin than in PZ-3 (Fig. 10).

5. PZ-5

The basal lamina is absent in PZ-5. Numerous microvillus-like processes extend from the distal end of the PAs toward the dentin. Large amounts of coarse-textured material can be seen near the distal cell membrane of the PAs and within the dentin (Fig. 11).

**Discussion**

Although a large number of electron microscopic studies have been done on ectomesenchymal contact, there is still no consensus as to the stage when contact is actually formed. SLAVKIN et al.\(^2\) and PANNES\(^5\) considered ectomesenchymal contact to form before dentin formation. REITH\(^6\), KALLENBACH\(^8\), SILVA et al.\(^9\) and BURGESS et al.\(^4,10\) reported that it occurred when the thick layer of the predentin had been formed, and LESTER\(^7\) and HURMERINTA et al.\(^11\) emphasized that contact was seen simultaneously with mineralization of the predentin. On the other hand, KASAMO\(^12\) observed the existence of ectomesenchymal contact in the
latter region of PZ-1. However, since the POs are closer to the basal lamina in PZ-2 than in PZ-1, the frequency of ectomesenchymal contact in PZ-2 could be considered to be higher than in PZ-1.

KOLLAR et al.[1], WARTIOVAARA et al.[13,14], SLAVKIN et al.[15] and THESLEFF et al.[3] showed on the basis of tissue culture and electron microscopy that, as a result of the “instruction” transmitted to ectodermal cells from mesenchymal cells, the PAs differentiated abruptly into secretory ameloblasts (SAs) and started the secretion of enamel. However, COHN et al.[16], BANERJEE[17], THESLEFF[18] and SLAVKIN et al.[19] indicated that, with regard to the morphogenesis of AMs, the existence of the basal lamina was extremely important. Meanwhile, many studies[20–24] have shown that types I and IV collagen, which comprise the extracellular matrix secreted by ODs or AMs are extremely important for the morphogenesis of AMs or for cell differentiation. In the present observations of rat incisors, no evidence was obtained for major morphological differentiation into SAs in the PZ-3 to PZ-5 regions after the formation of ectomesenchymal contact. This process of differentiation is not triggered directly by ectomesenchymal contact but occurs gradually in the region between PAs and AMs. The differentiation of AMs is further stimulated by phenomena such as dentin mineralization and disappearance of the basal lamina.

A large amount of coarse-textured material was observed in the intercellular spaces between PAs and in the predentin from the PZ-3 region, where ectomesenchymal contacts were formed. This material is considered to correspond to the stippled material reported by FEARNSHEAD[25] and REITH[6]. Based on the results of an immunocytochemical study, INAGE et al.[26] suggested that this material might contain amelogenins and enamelins. Therefore, it is suggested that the ectomesenchymal contact observed in PZ-2 is closely associated with the secretion of enamel proteins synthesized by PAs.

From the above, it is considered that the “instructions” transmitted from mesenchymal cells through the ectomesenchymal contacts play a role in stimulating the secretory activity of PAs. Furthermore, these “instructions” may not be involved in the morphogenesis of PAs to SAs.

References


Fig. 1  PZ-1: Preameloblasts (PAs) and dental papilla cells (DPCs) are divided from the basal lamina (BL). The DPCs extend cytoplasmic processes toward the basal lamina (arrow). x2,400

Fig. 2  PZ-2: The preodontoblasts (POs) have a low columnar shape and are located closer to the basal lamina. PA: preameloblasts. BL: basal lamina. x11,500

Figs. 3-4  PZ-2: The distal cytoplasmic processes of PAs (arrows) penetrate the basal lamina (BL) and reach the dental papilla. MF: microfibril. CF: collagen fibril. x11,500

Fig. 5  PZ-2: The cytoplasmic processes of PAs penetrate the basal lamina (BL) and make contact with the cytoplasmic processes of POs. x17,000

Fig. 6  PZ-3: The distal cytoplasm of PAs and POs have developed, and the thickness of the predentin (PD) reaches 2-4 μm. Arrow: distal terminal bar of PA. x2,000

Figs. 7-9  PZ-3: Numerous matrix vesicles (MV) and coarse-textured material (CTM) can be seen within the predentin. In the distal portion of PAs, the distal terminal bar (DTB) is observed. The distal cytoplasm includes type-1 vesicles (T1), tubular structures (TS) and secretory granules (SG). In Figs. 7 and 8, the cytoplasmic processes of PAs invade the predentin (double arrows). Arrow: membrane invagination. BL: basal lamina. PO: preodontoblasts. x11,500

Fig. 10  PZ-4: From the distal end of PAs, numerous cytoplasmic processes extend (arrows). CTM: coarse-textured material. D: dentin. x16,000

Fig. 11  PZ-5: No basal lamina can be recognized. From the distal end of PAs, numerous microvillus-like processes (arrows) extend. CTM: coarse-textured material. D: dentin. x11,500