Three-dimensional Architecture of the Keratin Filaments in Epithelial Cells Surrounding Taste Buds in the Rat Circumvallate Papilla

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Summary. The three-dimensional architectures of the perigemmal cells and their keratin bundles in the rat circumvallate papillae were studied by transmission and scanning electron microscopy. The perigemmal cells were classified into three layers: basal, middle and upper. The basal layer consisted of polygonal cells located close to the basal lamina, the middle layer comprised longitudinally elongated cells fitting the lateral convexity of the taste bud, and the upper layer was imbricating flat cells along the upper portion of the taste bud. When fresh specimens were jointly treated with Triton X-100 and sonication, the taste buds were often detached and the cytoplasmic matrices of the perigemmal cells were effectively removed. Consequently, we were able to demonstrate an extensive network of the subplasmalemmal keratin bundles of the perigemmal cells. The framework appeared either as a thin lacework, a thick fence-like structure, or a lattice work in the basal, middle, and upper layers, respectively. The thin lacework in the basal layer was considered to be a developing process of the framework. The thick fence-like structure in the middle layer probably plays a primary role in supporting the taste bud. The lattice work in the upper layer is believed to reflect a remodeling in reducing the keratin framework.

Keratin filaments, which represent an epithelial type of intermediate filament, are major cytoskeletal components in epithelial cells (FRANKE et al., 1978; SUN et al., 1979; SCHLEGEL et al., 1980; DENK et al., 1982). They play important roles both in stabilizing the cell shape and in attaching to adjacent epithelial cells and the basal lamina (STAEHELIN and HULL, 1978; FUCHS and CLEVELAND, 1998).

The trench wall epithelium of the circumvallate papilla contains a number of oval taste buds. The taste buds are surrounded by a layer of epithelial cells called perigemmal cells in which keratin filaments are aggregated into bundles to provide a structural framework for maintenance of the oval outline of the taste buds (MURRAY and MURRAY, 1970; TAKEDA et al., 1990). Although the ultrastructures of these bundles have been studied by transmission electron microscopy (TEM), it has been difficult to understand their three-dimensional organization using only two-dimensional images. The present study therefore aims to examine the three-dimensional organization of the keratin bundles by scanning electron microscopy (SEM) after extraction of the cytoplasmic matrices which have prohibited the visualization of the bundles. In our specimen preparation, detaching the taste buds from perigemmal cells allowed us to demonstrate the subplasmalemmal keratin bundles of the perigemmal cells. We also examined the outer configuration of the perigemmal cells under SEM by exfoliating the taste bud after sodium hydroxide digestion.

This paper describes the arrangement of perigemmal cells and the three-dimensional organization of their keratin bundles, paying particular attention to how these bundles support the taste bud.

MATERIALS AND METHODS

Animals

Twelve adult female Wistar rats, weighing 200-350 g, were used in this study. The animals were deeply anesthetized with intraperitoneal injections of sodium pentobarbital (50 mg/kg body weight).

SEM after detergent treatment

The circumvallate papillae and surrounding tissues were excised from the tongue and cut into halves
with a razor blade. They were treated at 45 kHz for 2 h by a sonicator (Bransonic B-1200, Yamato Scientific Co., Ltd., Japan) in a 5 mM Tris buffer (pH 7.2) containing 5% Triton X-100, 1 mM ethylene glycol tetraacetic acid (EGTA), and 0.5 mM MgCl2. During the sonication, the solution was changed every fifteen minutes to keep the solution temperature below 30°C. After rinsing in 1/15 M phosphate buffer (PB; pH 7.4), all tissues were successively immersed in 25% and 50% dimethyl sulfoxide (DMSO) solutions for 30 min each. They were then frozen on a metal plate chilled with liquid nitrogen and cracked with a razor blade along a frontal plane (TOKUNAGA et al., 1974). To remove cytoplasmic remnants, they were further sonicated for 1 h in the same manner as mentioned above. After being rinsed in PB, the specimens were fixed with 2.5% glutaraldehyde buffered with PB for 30 min. They were then postfixed in 1% osmium tetroxide for 2 h and conductive-stained with 1% tannic acid and 2% osmium tetroxide (MURAKAMI, 1974). Following dehydization in ethanol of increasing concentrations, they were freeze-dried with t-butyl alcohol (INOUE and OSATAKE, 1988). The dried specimens were sputter-coated with platinum of an approximately 2 nm thickness and observed with a Hitachi S-4500 scanning electron microscope.

**SEM after alkali maceration**

Circumvallate papillae and surrounding tissues were excised and fixed in a mixture of 2% formaldehyde and 1% glutaraldehyde in PB (1/10 M, pH 7.4) for 12 h at room temperature. They were then fractured by the DMSO freeze-cracking method as mentioned above. The specimens were placed in 6N sodium hydroxide at 60°C for 12 min (TAKAHASHI-IWANAGA and FUJITA, 1986), and postfixed in 1% osmium tetroxide for 1 h. After conductive-staining, they were processed in the same fashion as described above. The lateral surface of the perigemmal cells facing the taste buds was photographed at a magnification of 6,000 ×, printed at the magnification of 10,000 ×, and finally assembled in a montage. The contour of each epithelial cell was traced on a transparent film.

**Light microscopy and TEM**

The animals were perfused through the left ventricle with a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Circumvallate papillae and surrounding tissues were excised and immersed in the same fixative for an additional 12 h. After postfixation in 1% osmium tetroxide for 2 h, the specimens were placed in 2% uranyl acetate for 6 h. They were dehydrated in ethanol of increasing concentrations and finally embedded in Epon 812 resin. Perpendicular sections of the taste buds and perigemmal cells were obtained by an ultramicrotome (Reichert Ultracut S, Leica, Austria). Semithin sections were stained with toluidine blue and observed with a light microscope. Ultrathin sections were stained with lead citrate and uranyl acetate and observed with a JEOL 100CX-II transmission electron microscope.

**RESULTS**

**Configuration of perigemmal cells**

In a perpendicular section of the circumvallate papilla, stratified squamous epithelium of the trench wall exhibited a number of oval taste buds (Fig. 1). The perigemmal cells, lined along the contour of the taste bud, were classified into three layers by their characteristic profiles: basal, middle and upper layers (Fig. 2). The basal layer consisted of two or three polygonal cells, the middle layer comprised two or three longitudinally elongated cells fitting the lateral convexity of the taste bud, and the upper layer was several flat cells imbricated along the upper portion of

![Fig. 1. A perpendicular section of a rat circumvallate papilla, stained with toluidine blue. A squamous epithelium of the trench walls contains many oval taste buds (arrows). ×120](image-url)
of the taste bud. When the perpendicularly fractured specimens containing the taste buds were prepared for SEM, the taste buds were often detached from the surrounding perigemmal cells (Figs. 3, 4a). Although plasma membranes were completely dissolved by the treatment with Triton X-100, three layers of the perigemmal cells could be inferred from the oval contour where the taste buds had been housed (Fig. 3).

The alkali maceration for SEM demonstrated the lateral surface of the perigemmal cells facing the taste buds (Fig. 4a). At high magnification, the boundaries of the perigemmal cells were clearly visible (Fig. 4b). They were traced as shown in Figure 4c. The contour of the perigemmal cells varied from the basal to the upper layers: they were polygonal in the basal and upper layers, whereas longitudinally elongated in the middle layer.

**Keratin bundles by TEM**

Perigemmal cells contained densely aggregated bundles of cytofilaments in all three layers. Since individual filaments in these bundles measured about 10 nm in diameter, they were interpreted to be keratin filaments.

The basal perigemmal cells contained short keratin bundles of 200-400 nm thickness. In the lateral cytoplasm facing the taste bud, perigemmal keratin bundles appeared as short fragments lined up about 100-350 nm below the plasma membrane (Fig. 5a). In the deeper cytoplasmic region, however, keratin bundles were distributed rather randomly.

In the middle layer, elongated perigemmal cells possessed thick keratin bundles measuring 350-600 nm in thickness beneath the plasma membrane facing the taste bud. These bundles, arranged longitudinally, partially anastomosed with each other (Fig. 5b). In the deeper cytoplasmic region of the cells, keratin bundles were thinner than beneath the plasma membrane.

In the upper layer, the perigemmal cells contained keratin bundles measuring 150-300 nm in thickness. These bundles were distributed beneath the plasma membrane and sometimes fused or crossed with each other (Fig. 5c).

Desmosomes were well developed between the perigemmal cells and other epithelial cells, and were receiving the termination of the keratin bundles (Fig. 5). In contrast, desmosomes were poorly developed between the perigemmal cells and taste bud cells.

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**Fig. 2.** Taste buds and adjacent perigemmal cells, stained with toluidine blue. The perigemmal cells are classified into basal, middle and upper layers. The basal layer (BL) consists of two or three polygonal cells. The middle layer (ML) has two or three longitudinally elongated flat cells fitting the convexity of the taste bud. The upper layer (UL) possesses flat epithelial cells arranged in an oblique direction along the tapering of the upper portion of the taste bud. ×700

**Fig. 3.** SEM image of a fractured surface through the taste bud following detergent treatment with Triton X-100 and sonication. The taste bud has been removed during specimen preparation, and the lateral surfaces of the perigemmal cells are exposed. Higher magnifications of these cells are shown in Figures 6a-c. BL basal layer, ML middle layer, UL upper layer. ×900
Perigemmal keratin bundles by SEM

At higher magnifications of the perigemmal cells where the taste buds had been removed during specimen preparation, keratin bundles beneath the perigemmal plasma membrane could be observed in three dimensions (Fig. 6a–c). The perigemmal keratin bundles formed an extensive framework surrounding each taste bud as a whole. The architecture of the framework of keratin bundles varied in the basal, middle and upper layers.

In the basal layer, perigemmal keratin bundles of about 200–400 nm in thickness were randomly oriented, and frequently anastomosed so that they looked like a lacework surrounding the basal part of the taste bud (Fig. 6a).

In the middle layer, perigemmal keratin bundles, measuring about 350–600 nm in thickness, were longitudinally oriented and frequently anastomosed with each other. They formed a fence-like structure.

Fig. 4a. SEM image of the perigemmal epithelium after sodium hydroxide treatment. The lateral surface of the perigemmal epithelial cells facing the taste bud are seen; the hollow space was formed after the removal of the taste buds. b. Higher magnification of the lateral surface of the perigemmal cells facing the taste bud. Cell boundaries are clearly visible. c. Tracing of boundaries of perigemmal cells. The lateral profiles of perigemmal cells are polygonal in the basal (BL) and upper layers (UL) while longitudinally extended in the middle layer (ML). Dotted lines show supposed outlines of the taste bud. a: ×1,400, b: ×12,000, c: ×1,400

Fig. 5. Transmission electron micrographs of vertical sections, showing the taste bud cells (TB) and perigemmal cells (PG) in the basal (a), middle (b) and upper (c) layers. a. The perigemmal keratin bundles appear as short fragments (arrows). In the interior of the cell, keratin bundles are distributed randomly and sparsely (arrowheads). b. Longitudinally arranged thick perigemmal keratin bundles are partly anastomosed with each other (large arrows). In the deeper cytoplasm, keratin bundles are thinner (arrowheads) than near the taste bud. Desmosomes are well developed between the epithelial cells (small arrows), but poorly so between the perigemmal cells and taste bud cells. Taste bud cells contain loosely aggregated keratin bundles (double arrowheads). c. Short perigemmal keratin bundles are seen beneath the cell membrane (arrows). An arrowhead shows a desmosome that connects to adjacent epithelial cells. Inset: Higher magnification of perigemmal keratin bundles crossing each other (arrows). a: ×13,000, b: ×11,000, c: ×10,000, Inset: 40,000

Fig. 6. SEM view of the perigemmal keratin bundles in the basal (a), middle (b) and upper (c) layers. a. A lacework of keratin bundles which are randomly oriented and anastomosed with each other. b. Longitudinally oriented thick keratin bundles forming a fence-like structure. c. A thin latticework of keratin bundles. a: ×22,500, b: ×20,000, c: ×22,000
Figs. 5 and 6. Legends on the opposite page.
as a whole, surrounding the middle convex portion of the taste bud (Fig. 6b).

In the upper layer, perigemmal keratin bundles, about 150-300 nm in thickness, were obliquely oriented along the upper portion of taste bud. They formed a general latticework by crossing with each other (Fig. 6c).

DISCUSSION

The present SEM study demonstrated the three-dimensional architecture of keratin bundles in the perigemmal cells surrounding the taste bud in the rat circumvallate papilla. After the removal of cytoplasmic components and plasma membranes by sonication in a Triton X-100 solution, the keratin bundles exhibited a well-developed specific framework.

Since taste buds are located in the oral epithelium, they frequently receive mechanical forces generated by mastication, swallowing, and tongue movement. Such mechanical forces could compress and/or deform taste buds if there were no proper supporting structures around the taste buds. It has been suggested that the keratin framework imparts mechanical integrity to epidermal cells (Chan et al., 1994; Rugg et al., 1994; Lloyd et al., 1995; Jonkman et al., 1996). Consequently, the framework of the densely aggregated perigemmal keratin bundles is believed to provide a firm supporting structure for the taste buds (Takeda et al., 1990). Besides this framework, the taste bud cells themselves also contain loosely aggregated keratin bundles (Takeda et al., 1988, 1990; Kanazawa, 1993). The taste buds thus seem to be supported externally by dense keratin bundles in the stratified squamous epithelial cells—particularly by the strong perigemmal keratin bundles, and internally by weaker keratin bundles within the taste bud cells.

The three-dimensional architecture of keratin filaments has been suggested to be influenced by externally applied mechanical forces (Görmar et al., 1990; Galou et al., 1997). The present SEM studies demonstrate that the frameworks of the perigemmal keratin bundles differ among the three cell layers (Fig. 6a-c). The keratin bundles form a thin latticework, thick fence-like structure, or latticework in the basal, middle and upper layers, respectively. The fence-like structures in the longitudinally elongated perigemmal cells of the middle layer probably play a chief role in supporting the taste bud. The thin latticework in the basal perigemmal cells is thought to represent a process of development of the perigemmal keratin bundles. The latticework in the upper perigemmal cells may reflect a reductive remodeling of the keratin bundles without losing their supportive ability. The perigemmal keratin bundles thus exert their supportive function while transforming their keratin bundles as perigemmal cells move upward.

It has been reported that keratin composition changes during the transition of epidermal cells from the basal to the outer cornified layer (Fuchs and Green, 1980; Woodcock-Mitchell et al., 1982). It is likely that such a change may also occur in the perigemmal cells. Previous immunohistochemical studies showed differences in keratin subtypes between basal cells and spinous and granular cells in the mouse circumvallate papilla (Takeda et al., 1988, 1990). They suggest that the aggregation and distribution pattern of keratin filaments may reflect differences among keratin subtypes. It would be of scientific value to perform further immunohistochemical studies on the perigemmal keratin bundles during their transformation in order to determine the differentiation of keratin subtypes.

Several SEM studies have visualized the three-dimensional architecture of cytoplasmic filaments after treatment with detergents. Some investigators used Triton X-100 as the detergent for the liver (Ökanoue et al., 1985) and pituitary cells (Senda et al., 1988), whereas others used saponin for the small intestine and kidney cells (Fukudome and Tanaka, 1992). In our preliminary studies, however, these detergents were ineffective in demonstrating keratin bundles in the stratified squamous epithelial cells. This study was able to obtain favorable results by the use of Triton X-100 together with sonication. The sonication increased the effect of the Triton X-100 treatment without conspicuous artifacts. Unfortunately, this method was inapplicable for observing keratin filaments in the taste buds because these cells were removed by sonication. However, it is expected that this method will be useful in SEM examination of keratin filaments in other thick squamous epithelia, e.g., the oral epithelium, esophageal mucosa, the epidermis and those epithelia with pathological conditions.

REFERENCES


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