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SCANNING ELECTRON MICROSCOPY OF INTRACELLULAR ORGANELLES IN THE YOUNG ODONTOBLASTS OF RATS

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Abstract

Intracellular structures of the odontoblasts were studied by scanning electron microscopy (SEM) using a modified AODO (aldehyde prefixed-osmium-DMSO-osmium) method. Well-developed flattened and layered rER (rough endoplasmic reticulum), paved with its associated ribosomes on its outer surface, were clearly observed in the odontoblast. Branched tubular mitochondria with nodules and swollen endings, interposing between and passing through the fenestrated layered rER, were demonstrated in the functional cells. Oblique and cross-sections of both the rER system and tubular mitochondria showed orthodox configurations similar to those usually described in transmission electron microscopy (TEM) studies. Many finger-like projections constructing the cristae directing towards the inner mitochondrial chamber were observed, and external chamber extending into the tubular cristae was also demonstrated.

Key Words: Rough endoplasmic reticulum (rER), mitochondria, odontoblast, rats, AODO (aldehyde prefixed-osmium-DMSO-osmium) method, scanning electron microscopy.

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Introduction

Transmission electron microscopic (TEM) studies on the continuously growing incisors and molar tooth germs of some rodents have shown developmental sequence of cell maturation in the odontoblastic layer; the morphologic changes associated with characteristic arrangement and distribution of organelles to occupy specific regions relating to differentiation stages were found. For example, an well-developed Golgi apparatus, surrounded with a large amount of granular endoplasmic reticulum (rER) and mitochondria, in particular, at their distal aspect, were distinguishing in the polarized preodontoblast. An enlarged Golgi area containing many secretory vesicles, cytoplasmic bodies, supranuclear region composed of rER showing parallel canalicular cisterns, elongated mitochondria containing many cristae and dense matrix, and many secretory vesicles were evident in the young odontoblasts; besides, transformation of the rER to smooth endoplasmic reticulum (sER) was noticed in the old odontoblasts (Gartner *et al.*, 1979; Provenza and Seibel, 1986; Takuma and Nagai, 1971; Ten Cate, 1980; Tsuboi, 1968). Moreover, morphology of the rER cisterns and electron density of its content have been found closely related to the cyclic activity of the odontoblasts (Reith, 1968).

The rER system is usually described to be cisternal interconnecting membrane-lined channels; which increases greatly with attached ribosomes during active synthesis of proteins. On the contrary, the sER is tubular or vesicular in form. Mitochondria are also membrane-bound intracellular organelles which increase in number in the cells with a high metabolic rate; they change in sizes and shapes, divide and distribute according to regional energy requirements (Leeson *et al.*, 1988, Williams and Warwick, 1980). Furthermore, reversible ultrastructural transformations between the orthodox and condensed configurations, of the energized and de-energized liver cell mitochondria, have been reported to be closely related with oxidative phosphorylation in the organelles (Hackenbrock, 1968, 1972; Hackenbrock *et al.*, 1971).

Hypotonic osmium tetroxide (OsO_4) solution maceration method, the ODO (O: OsO_4 solution, D: DMSO:

dimethyl-sulfoxide solution) method, for revealing membranous cell organelles has been conducted on scanning electron microscopic (SEM) study of soft tissues (Tanaka and Naguro, 1981). By the same method, relationships between functions and morphological changes in the Golgi complex of the secretory and resorptive cells have been reported (Kasuga and Harada, 1989; Miyamoto and Osatake, 1981; Fukudome and Kinose, 1981). On the other hand, OsO_4 containing Zenker's solution has been used in SEM studies, on the subplasmalemmal cytoskeletal elements in human odontoblasts, by removing both the membranous structures and soluble proteins (Sögaard-Pedérson and Boye, 1990). However, there is still no SEM study on the subcellular organelles in odontoblasts which are densely encased in hard tissues. In the present study, we modify the AODO (aldehyde prefixed-osmium-DMSO-osmium) method (Tanaka and Mitsushima, 1984) to study intracellular organelles in young odontoblasts, and use it as a model for further study on the odontoblasts in different stages.

Materials and Methods

The mandibular incisors were removed from 50 rats (Wistar strain; 7 weeks old male; body weight: 200-250 grams) after intracardiac perfusion with chilled (4 °C) fixatives containing both 0.5% glutaraldehyde and 0.5% formaldehyde (in 1/15 M sodium phosphate buffer). The apical third of tooth was trimmed to expose the dental pulp tissue and rinsed in the same buffer for 1 to 3 days. The samples were then postfixed in cold 1% osmium tetroxide (OsO_4) buffered solution for 1 hour, rinsed, and immersed in a graded series of DMSO (25-50%). They were freeze-fractured using metal contact method in an Eiko TF-1 equipment (Eiko Engineer., Tokyo, Japan). After thorough washing for one hour to remove the DMSO, the samples were fixed again in 1% OsO_4 solution for 30-60 minutes. Specimens were rinsed and macerated in several alternative changes of buffer solution and 0.1% OsO_4 (20 °C; 3-4 days). The tissues were washed and stained in 1% OsO_4 solution at 4 °C for 1 hour. They were rinsed again and treated with 2% tannic acid for 1-2 hours at 4 °C. They were thoroughly rinsed and stained again with 1% OsO_4 cold solution for 30-60 minutes. After rinsing, the specimens were dehydrated in an ascending series of ethyl alcohol. They were frozen and dried in an Eiko ID-2 drier after substitution with t-butyl alcohol. The specimens were coated with gold-palladium using a Hitachi E-1030 ion coater (Hitachi, Tokyo, Japan) and examined in a Hitachi S-4000 field mission SEM.

For comparative TEM studies, the specimens were fixed in a solution containing 2.5% glutaraldehyde and 2.0% paraformaldehyde (Gartner *et al.*, 1979; Karnovsky, 1965), rinsed overnight and postfixed in 1.0% OsO_4 solution. Some samples were processed using Epon freeze-fracture method (Tanaka, 1972; Takagi and Yamada, 1977) and some were embedded in Epon 812. The thin sections were examined in a Hitachi H-800 TEM operated at an accelerating voltage of 100 kV.

Figure Legends

Abbreviations used in Figures 1-13: A: Ameloblasts; G: Golgi apparatus; M: Mitochondrion; N: Nucleus; O: Odontoblasts; rER: rough Endoplasmic Reticulum.

Figure 1. Survey transmission electron micrograph showing odontoblasts (O) and ameloblasts (A) in early dentinogenesis. Note that the nucleus (N) and the well-developed rER (arrowheads) are distributed at the proximal- and distal-end of the secretory cells, respectively.

Figure 2. The young odontoblasts contain rER exhibiting parallel canalicular profiles having narrow cisternae, the well developed Golgi apparatus (G) and round and ovoid mitochondria (arrowheads) containing dense matrix.

Figure 3. Higher magnification showing the rER containing many parallel channels, and round mitochondria (arrowheads) containing cristae.

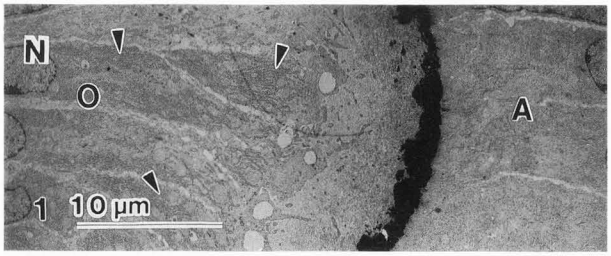
Figure 4. Scanning electron micrograph of the young odontoblasts (O) using conventional the epon freeze-fracture method. Micrograph shows the nucleus (N) at the proximal end and many cavities (arrowheads) in the cytoplasm of the functional cells.

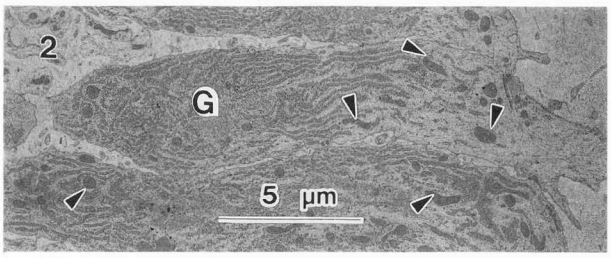
Figure 5. Scanning electron micrograph showing intracellular structures in the fractured odontoblasts prepared by the modified AODO method. The matrix in the nucleus (N) and cytoplasm of the cells are macerated and washed out showing that the nuclear membranous structures are continuous with the ER. Asterisks (*) indicate the ER system showing parallel canalicular structures. Arrowheads indicate many fractured mitochondria interposed between the ER.

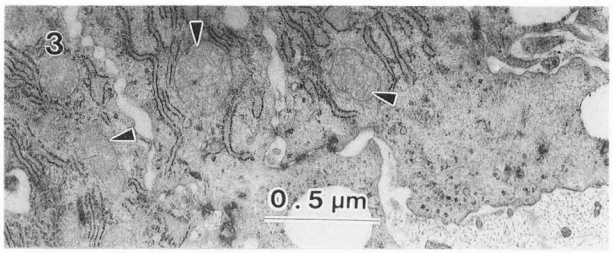
Figure 6. Scanning electron micrograph of the laminated rough endoplasmic reticulum (rER). The concentric rER lamellae were connected with many branches and tubular structures (arrowheads). Note many fine granules paving on the rER surface.

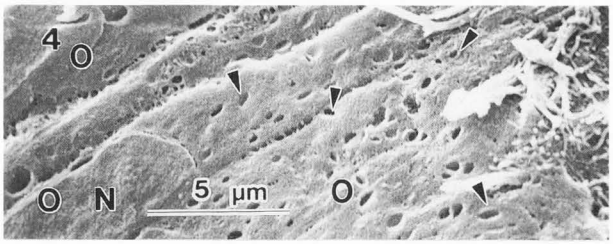
Results

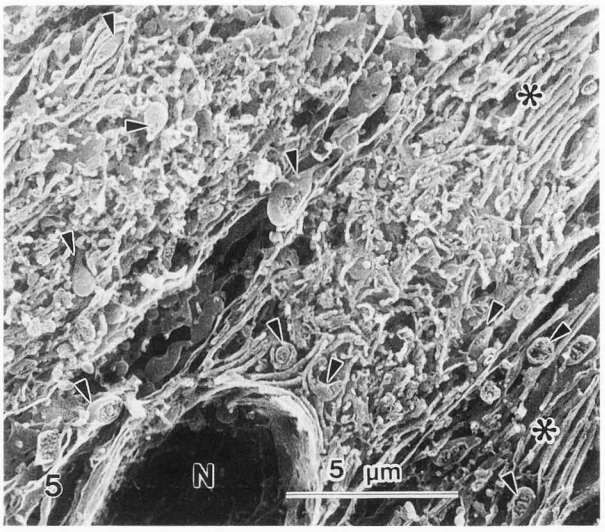
TEM observations showed initial dentinogenesis and amelogenesis at the labial aspect in apical third of the cross-sectioned incisor specimens. The odontoblasts, measuring 25-40 μm in length and 5-7 μm in width with nucleus at the proximal end, were arranged perpendicular to the dental hard tissues sending their distal process extending into the predentin (Fig. 1). The cytoplasm of the young odontoblasts contained a large rough endoplasmic reticulum (rER) system exhibiting parallel canalicular profiles, Golgi apparatus in the supranuclear area, many interposing cytoplasmic bodies, and elongated mitochondria (Figs. 2 and 3).

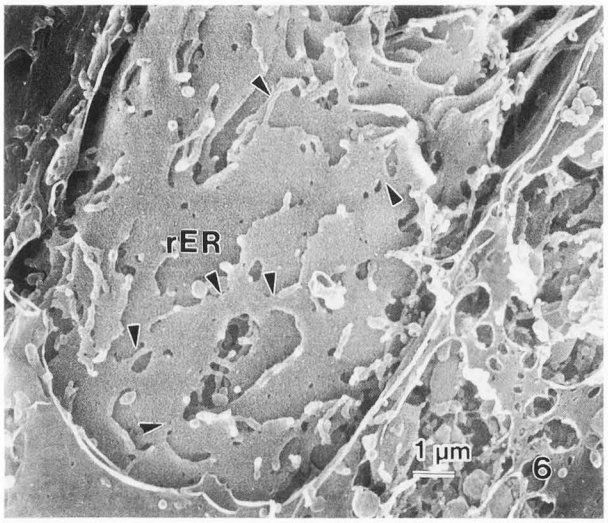












Comparative SEM study of the young odontoblasts using the Epon 812 freeze-fracture method show topological relationships of the organelles. The nucleus was located at the proximal end and many cavities of different sizes were distributed all over the cytoplasm (Fig. 4). We postulate that these cavities might be the sites representing the morphoplasm excavated during freeze-fracture.

In the present study, the modified AODO method (by changing the time for fixing and maceration), showed best results for studying the subcellular organelles in the odontoblasts. Young odontoblasts fractured through the mid-portion along the long axis demonstrated with ER showing complicated canalicular structures (Fig. 5). The cell membrane of some odontoblasts were removed showing that the ER system was mainly composed of concentric fenestrated lamellae shifting and blending with each other to form a well-developed onion-like structure. Some connecting branches and tubular structures were also seen. Moreover, the young odontoblasts contain mainly rER paved with fine ribosome granules on the outer surface of the ER. The sER was not prominent (Figs. 6 and 7).

Many tubular and branched mitochondria interposing between the layered rER and passing through the fenestrations were observed (Figs. 7 and 8). The mitochondria always had a pear-shaped free ending with a neck portion (Figs. 9 and 10). Higher magnification of the mitochondria in cross and oblique sections showed many finger-like projections from the membrane compartment running in different directions towards the inner chamber (Figs. 8 to 13). Some mitochondria containing plate-like cristae and condensed chambers were found (Fig. 13). In addition, some slits and lumens in

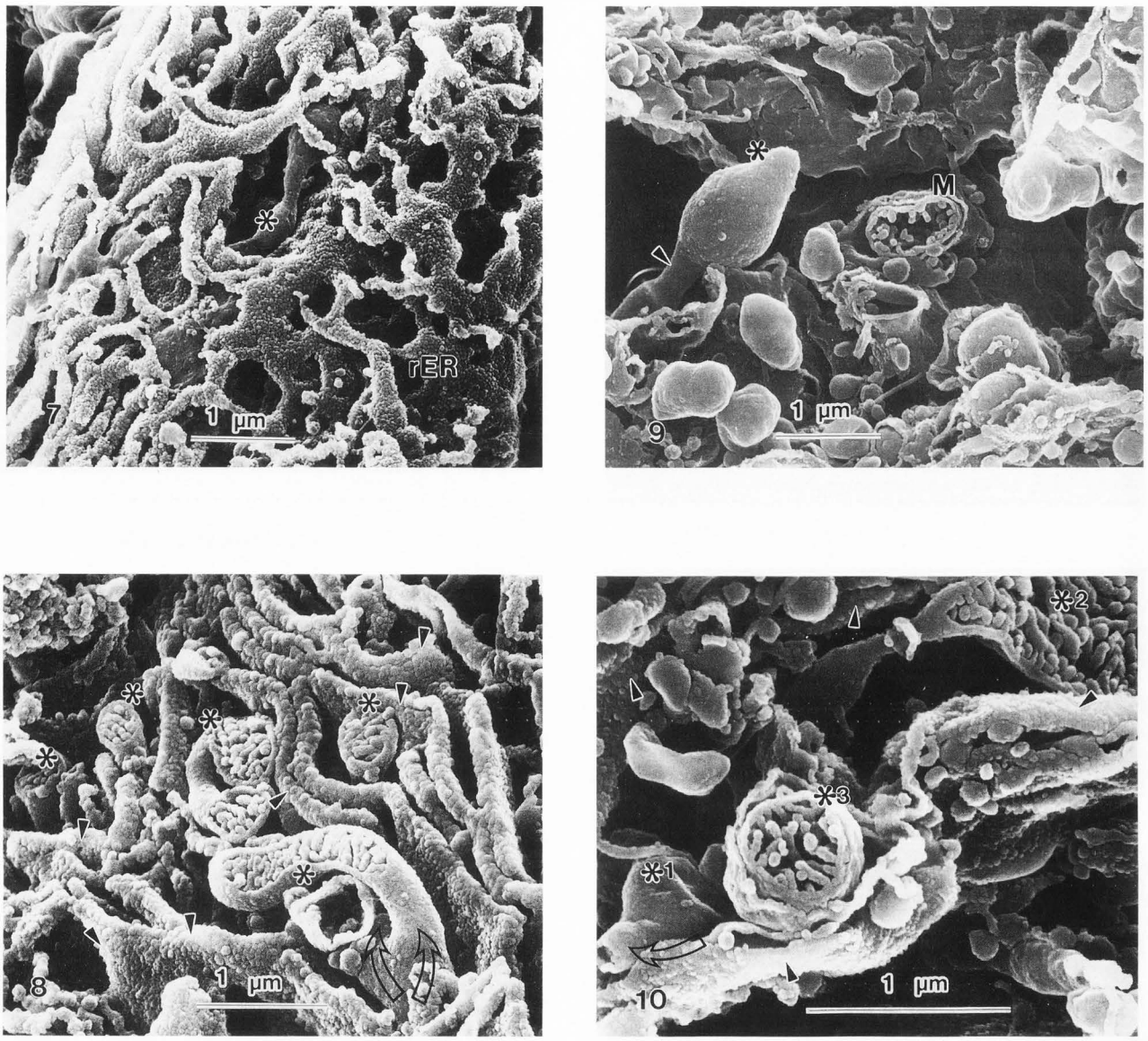


Figure 7. Scanning electron micrograph showing the complicated network of the rER and a nodular tubular mitochondria (*) running between the layered rER.

Figure 8. Tubular mitochondria (*) are fractured to expose many finger-like cristae in the inner chamber. They are branched (blank arrows) and interposed between the rER (arrowheads).

Figure 9. Micrograph showing a pear-shaped free end (*) and its neck portion (arrowhead) of a tubular mitochondrion. A fractured mitochondrion (M) in cross-section showing orthodox configurations is also observed.

Figure 10. Two swollen free endings (*1 and *2) of tubular mitochondria are found; one (*1) is just fractured at the neck portion (blank arrow) and the other (*2) is split at the bulbous ending to expose its inner contents. A cross-sectioned mitochondrion (*3) interposed between the rER (arrowheads) is also seen.

the finger-like cristae representing the outer chamber were noted (Fig. 12). Only a small amount of cristae were found in the thin tubular portion. Vacuolation and destruction of the cristae in the mitochondria were not observed (Figs. 10 and 11).

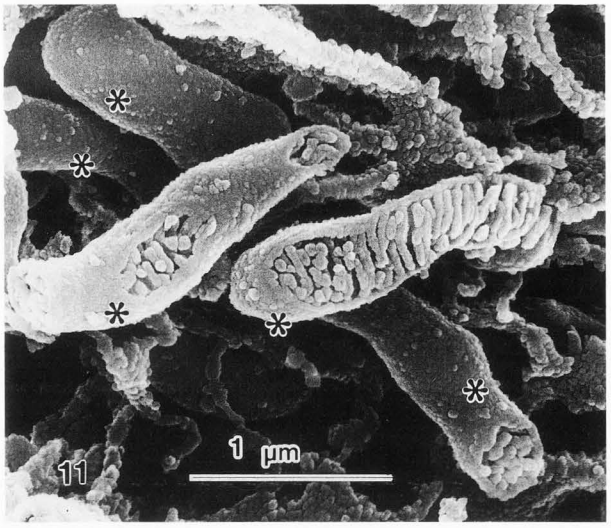
Discussion

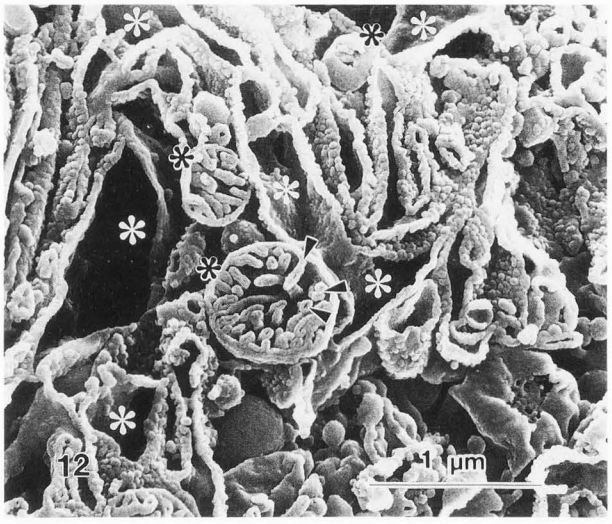
TEM studies of the young odontoblasts showed a large amount of rER and their associated organelles, particularly in the adjacent supranuclear region (Gartner

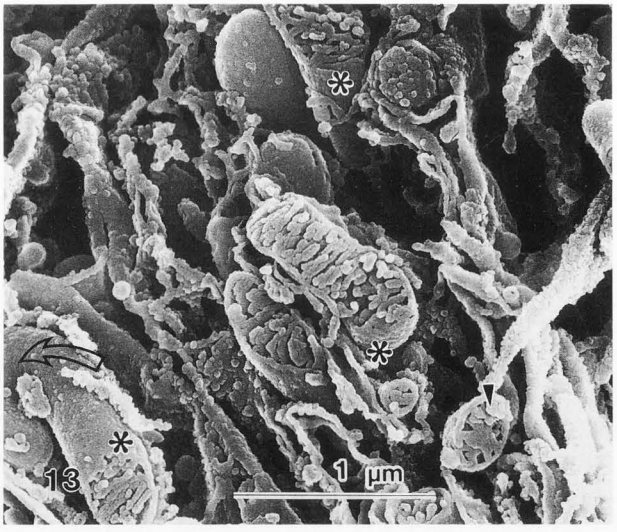
Figure 11. Scanning electron micrograph of many tubular mitochondria (*) cutting in different directions to show their interiors.

Figure 12. Micrograph showing some mitochondria (*) interposed between the rER. Arrowheads indicate the external chamber in the tubular cristae and wall of a cross-sectioned mitochondria. Note the inner surface (large white asterisks *) of the fractured cisternal rER is smooth.

Figure 13. Many fractured orthodox de-energized type mitochondria (*) containing tubular cristae and a condensed energized-type mitochondrion (arrowhead) containing broad cristae are demonstrated. A tortuous (blank arrow) mitochondrion at the left corner is also observed.







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et al., 1979; Provenza and Seibel, 1986; Takuma and Nagai, 1971; Ten Cate, 1980). We tried to view the intracellular structures using SEM following the Epon freeze-fracture method and identified the nucleus and some cavities, representing the excavated morphoplasm, in odontoblasts.

The ODO method and its modifications were useful in SEM study of cellular structures, because most of the intracellular organelles were fixed in the low concentration of osmium tetroxide solution and washed with buffer solution (Tanaka and Naguro, 1981; Fukudome and Tanaka, 1988). In the present study, we modified the AODO method (Tanaka and Mitsushima, 1984). The pulp tissues containing odontoblasts were perfusion-fixed with low-concentration aldehydes for preservation of the cellular configuration. The samples were then thorough washed, freeze-fractured and macerated for removal of the soluble matrix and most of the paraplasm, but still preserved the membranous structures in the odontoblasts.

The present SEM study indicates that the ER in young odontoblasts is mainly the concentric rER containing fenestrated lamellae and flat cisterns. The lamellae sent out many connecting flat branches and some tubular structures to form the complicated rER containing continuous cisterns. The present SEM study using the AODO method elucidates distinctive features in the rER corresponding to functions of odontoblasts (Gartner *et al.*, 1979; Provenza and Seibel, 1986; Takuma and Nagai, 1971; Ten Cate, 1980).

Mitochondria are usually described to be tubular or occasionally spherical structures measuring 0.5-2.0 μm wide and 3-4 μm long (William and Warwick, 1980; Rawn, 1991). Many SEM and TEM studies have pointed out that expansion of the outer chamber, condensation of the matrix in the inner chamber of the mitochondria, and change of the cristae from plate-like to tubular structures, were closely related to the enzymatic activity in cells (Fernadez-Moran *et al.*, 1964; Hackenbrock, 1968, 1972; Hackenbrock *et al.*, 1971; Hanaki *et al.*, 1984; Kirschner, 1981; Weiss, 1983; Vertel and Fischman,

1977). Furthermore, an ultrastructure study on the organelles in the muscle cells of hypertensive rats using the AODO method, has identified numerical and morphological changes in the mitochondria; decrease in number, vacuolation, and destruction of the cristae were characteristic in the pathologic cells (Goto *et al.*, 1987).

A SEM study on pharyngeal muscles prepared with HCl-collagenase has revealed that, other than the ovoid- or short-rod-shaped mitochondria, many large, long and sometimes branched L-shaped and U-shaped mitochondria were observed, in particular, at the medial portion of the vocalis muscle which contains mainly slow-twitch muscle fibers (Hirano and Ito, 1981). In addition, by the ODO method, round mitochondria arranged in chains and columns giving off branches have been demonstrated in the muscle (Ogata and Yamasaki, 1985, 1987; Shiozaki and Shimada, 1990). Furthermore, quite complicated external and internal morphologies of mitochondria showing tubular cristae also has been observed in the muscle, pancreas cells and axons using the same method (Fukodome and Kamitani, 1983).

In the present study, we demonstrated many tubular and branched mitochondria distributed between the rER sheets and network; some of them pierced the fenestrations. Further, the tubular mitochondrion always had a pear-shaped free ending with a neck portion. The tubular mitochondria, in particular, the bulging ends were fractured showing many finger-like and tubular cristae from the membrane compartment running in different directions but mainly directing towards the inner chamber. Cross and oblique sections of the mitochondria also revealed the round, oval and branched mitochondria similar to what has been usually described in TEM studies. Concerning the number of the cristae in different portions, only a small amount of cristae were found in the thin tubular portion, but vacuolation and destruction of the cristae representing pathologic and artificial changes in the mitochondria were not observed in our SEM study. However, some slits and lumens in the longitudinal and cross-sectioned tubular cristae representing the outer chamber were observed in our study.

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Discussion with Reviewers

Reviewer I: What is the advantage of the AODO method?

Authors: The AODO method is effective for SEM examination on the intracellular membranous structures. Using the method, both the intercellular and intracellular soluble contents were completely washed out while the subcellular organelles were selectively preserved after maceration and OsO_4 fixation. Different methods for tissue preparations were developed for suitable TEM and SEM studies for fundamentally different purposes. Thus, we compared the results of a SEM study conducted with the AODO method with conventional epon-embedded TEM preparations, which usually showed good results. The SEM results revealed that histology of the fractured intracellular organelles in young odontoblasts were similar to those described for the normal TEM findings.

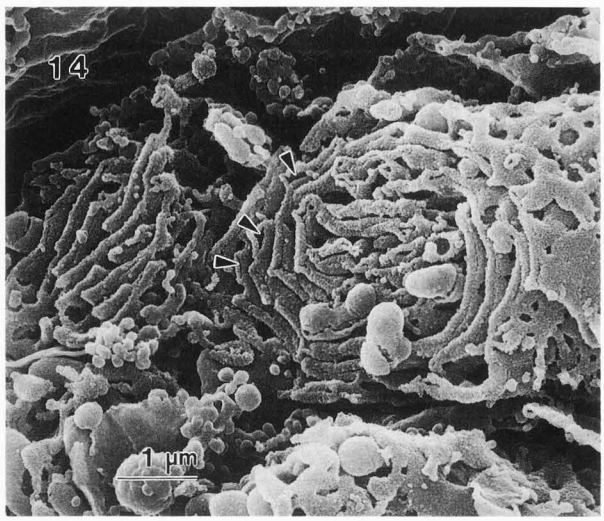

Figure 14. Scanning electron micrograph showing cross-sectioned ER in the odontoblasts. The ER contains many cisterns (arrowheads), and the outer surface is paved with many granules.

H. Mishima: Incisor dentine is divided into enamel-covered labial dentine and cementum-covered lingual dentine. Could you find any differences between odontoblasts in labial and lingual aspects?

Authors: The hard tissue producing cells in different developmental stages arranged along the long axis of the erupting incisor have been reported in many TEM studies; besides, cyclic morphologic changes in the intracellular organelles were also mentioned. The present study focuses on three-dimensional intracellular structures. Using conventional TEM, we found that the apical third region of the incisors contained many young odontoblasts, then using a modified AODO method in the study of the same region, SEM revealed that the young odontoblasts contained layered rER and many branched, tortuous tubular mitochondria. In addition, the mitochondria in the odontoblasts contained mainly tubular cristae. The differences in odontoblast structures between labial and lingual dentin are under study.

T. Ogata: The three-dimensional structure of the rER of the odontoblast seems to differ from that of other cells, for example, of the pancreas acinar cell, which was already reported by K. Tanaka. Please comment.

Authors: An additional micrograph (Fig. 14) reveals that the cross-sectioned odontoblasts contained an onion-like cisternal rER structure paved with many granules on the outer surface (Fig. 14). The micrographs in Figs. 6

and 7 also show odontoblasts having their cell membrane removed by the AODO method. They demonstrate the general view of the interior showing the ER composed of sheet-like and tubular structures. On the other hand, in Figs. 8, 10 and 12, we observe that channels and cisterns in the cross-sectioned rER resembled the histology of young odontoblasts. Moreover, in a personal discussion of these findings with Dr. Tanaka, he agreed to our observations.