supranuclear region, usually representing a "U" shape as a whole (Fig.3). Most of the Golgi vacuoles and vesicles having been unconnected to membranous structures were generally removed during the specimen preparation procedure. However, Golgi vesicles firmly attached to the Golgi cisternae were preserved. The mitochondria, surrounded by the meshwork of the ER, were rod-shaped, branching and anastomosed to each other (Figs. 2 and 4).

The tubular smooth ER appeared to be continuous all over the interior of the ciliated cells. The rough ER with ribosomes was sometimes observed around the Golgi apparatus and nucleus.

Conventional rats

Ciliated cells were a major component of the epithelium. Mucous cells were often seen between the ciliated cells (Fig. 1a), and non-ciliated cells were scarcely visible.

Many cilia, together with a few microvilli, were distributed on the apical surface of the ciliated cells (Fig. 2a). The Golgi apparatus was highly developed and many Golgi vesicles were attached to the lateral margin of the Golgi cisternae (Fig. 3a). The Golgi stack was composed of five to eight flattened cisternae which were piled up in close parallel array.

The plate-like rough ER was observed around the nucleus (Fig. 4a). Many ribosomes were attached to the surface, some of them forming polysomes. The rough ER had a few fenestrations about 0.02 to 0.2 μm in diameter. The tubular smooth ER extended from the margin of the rough ER, linking to the adjacent rough ER.

SPF rats

The surface of the trachea was composed of ciliated cells and non-ciliated cells. The ciliated cells were less frequently observed in SPF rats than in conventional rats (Fig. 1b). Mucous cells were also less frequently visible than in conventional rats. The apical surface of the non-ciliated cells was covered with short micro-

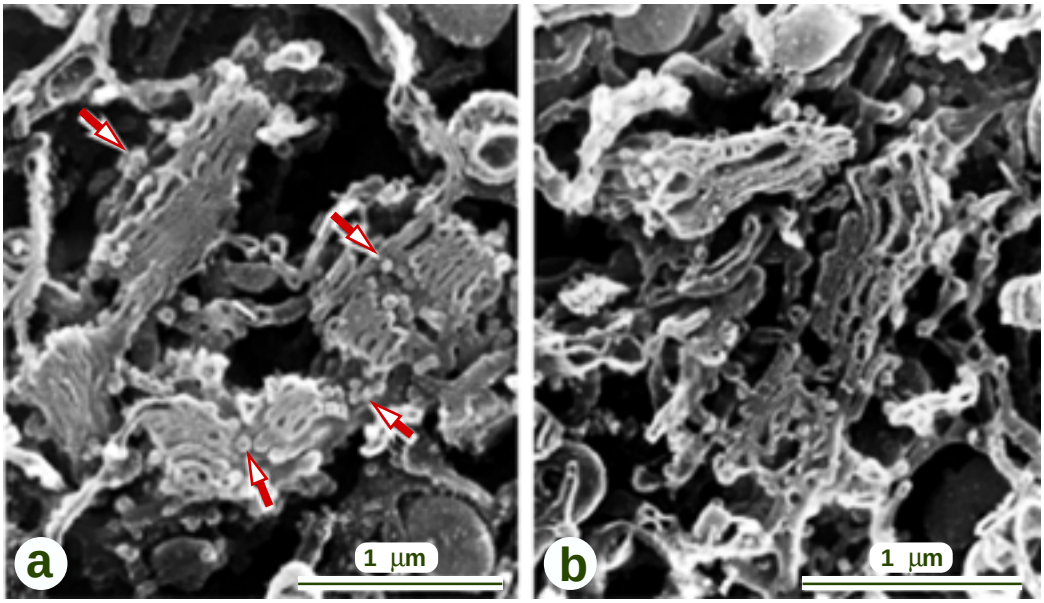

Fig. 3. The Golgi apparatus of tracheal ciliated cells in a conventional rat (**a**) and a SPF rat (**b**). **a:** The Golgi stack is composed of five to eight flattened cisternae which are piled up in a close parallel array. Note numerous Golgi vesicles (arrows). × 23,000. **b:** The Golgi stack is composed of four to seven fenestrated cisternae. × 28,000.

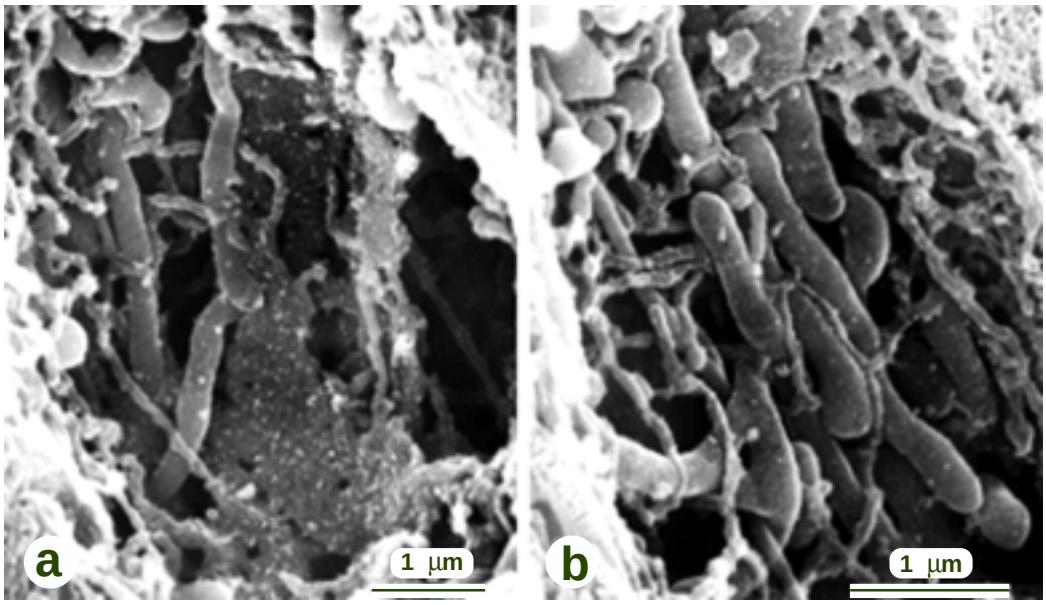

Fig. 4. The perinuclear intracellular structures exposed by the removal of the nucleus. **a**: A conventional rat. The tubular smooth endoplasmic reticulum extends from the margin of a plate-like rough endoplasmic reticulum. The rod-shaped mitochondria run parallel to the long axis of the cell. × 14,000. **b**: A SPF rat. A network of the tubular smooth endoplasmic reticulum is observed in the perinuclear region; the rough endoplasmic reticulum is not as highly developed as in conventional rats. × 17,000.

villi, bulging into the tracheal lumen. The cilia were not so highly developed as in conventional rats. Instead, numerous microvilli were visible (Fig. 2b). The Golgi apparatus was not so highly developed either. The Golgi stack was composed of four to seven cisternae. Golgi vesicles attached to the Golgi cisternae were fewer than those in conventional rats.

The ER in the perinuclear space was mostly smooth and tubular. The rough ER was scarcely visible (Fig. 4b).

Discussion

The intracellular structures of tracheal ciliated cells have been precisely examined by transmission electron microscopy (TEM) (Rhodin and Dalhamn, 1956; Jefferry and Reid, 1975; Marin et al., 1979). However, it has been difficult to understand the three-dimensional configuration of intracellular structures by two-dimensional TEM images. Although the development of specimen preparation for SEM made it possible to demonstrate intracellular structures three-dimensionally (Tanaka, 1980; Tanaka and Naguro, 1981; Inoué, 1982; Tanaka and Mitsushima, 1984; Inoué, 1985; Inoué and Osatake, 1989), intracellular structures of the tracheal ciliated epithelium have not been sufficiently studied by SEM. The osmic maceration procedure of the A-O-D-O method (Tanaka and Mitsusima, 1984) was effective to remove excess cytoplasmic matrices, thus intracellular structures such as the mitochondria, ER and Golgi apparatus were three-dimensionally observed as shown in Figs. 1 to 4.

The tracheal surface of conventional rats was densely covered with cilia as reported by Alexander et al. (1975). In contrast, there were fewer ciliated cells in SPF rats than in conventional rats. Non-ciliated cells of SPF rats were more often visible than those of conventional rats. The mucous cells were more frequently observed in conventional rats than in SPF rats. This apparently indicates that ciliated cells and

mucous cells are closely related to microorganisms in the air.

The most interesting finding obtained in this study was a three-dimensional network of the smooth ER. This network appeared to be continuous all over the interior of the ciliated cells. Such continuity of the ER has been shown three-dimensionally in rat spermatids by SEM (Inoué, 1982). An intensive network was demonstrated beneath the basal bodies, partly enclosing the underlying mitochondria (Fig.2). An amorphous layer, referred to as the hypobasal hyaline zone, was described between the basal bodies and underlying mitochondria by light microscopy (Hioki, 1942). Later, electron microscopy proved that the hypobasal hyaline zone contains the smooth ER (Graf and Stockinger, 1966), which is identical to the intensive ER network demonstrated in this study.

Kanamura (1975) considered that tracheal ciliated cells probably require a large amount of glucose-6-phosphate for ciliary movement. In addition, acetylcholine has been proven to have a close relationship with this movement (Kordik et al., 1952; Burn, 1954; Salathe and Bookman, 1995). Graf and Stockinger (1966) proved acetylcholinesterase activity in the ER of rat respiratory ciliated cells using histochemical techniques. Rhodin (1960) speculated that mitochondria under the basal bodies furnish the energy required for the ciliary beat. The close relationship between the smooth ER, basal bodies and mitochondria indicates that these intracellular organelles under the basal bodies are engaged in the supplementation of energy for ciliary movement.

This study showed that the Golgi apparatus and rough ER were highly developed in conventional rats (Fig. 3), but less so in SPF rats. The development of the Golgi apparatus can be estimated by the increased number of the Golgi cisternae and Golgi vesicles. Although no significant morphological differences were noted in the smooth ER under the basal bodies (Fig. 2), the plate-like rough ER was seen in the perinuclear region only in conventional rats (Fig. 4a). Since proteins, including enzymes, are synthesized and processed in the Golgi apparatus and rough ER, it is reasonable that such intracellular structures are highly developed for ciliary formation and movement. Probably, protein synthesis can be activated in conventional rats, thus the tubular smooth ER transformed into a plate-like rough ER.

Aoki et al. (1986) demonstrated that peroxidase activity in the rat tracheal epithelium was higher in conventional rats than in SPF rats. Kinbara et al. (1992) also showed similar findings in peroxidase activity, suggesting that peroxidase plays a role in mucosal antimicrobial defense mechanisms. However, the relationship between the peroxidase activity and intracellular morphology has not been clarified. The present morphological study on intracellular structures has been proven useful as an another approach when considering the defense mechanism of tracheal ciliated cells.

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