Dynamic Behavior of Ciliated Centrioles in Rat Incisor Ameloblasts during Cell Differentiation

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Summary. Centriolar pairs of ameloblasts were examined in relation to cell differentiation at the growing end of rat incisors. In their undifferentiated phase, including many mitotic cells, most of the centrioles in the ameloblasts were located lateral to the nuclei; the pairs were closely associated to each other. During the differentiating phase, the centriolar pairs moved more distally, always accompanied by the Golgi apparatus; they often became separated from each other. In the early secretory phase, and also in the following secretory phase, the pairs were usually observed distally, often away from the nuclei, and were separated from each other more frequently than in the previous differentiating phase. Throughout these phases of the ameloblast differentiation, one centriole of the pair was invariably ciliated and the Golgi apparatus was commonly associated with the centriolar pair. Such movement and separation of the ciliated centrioles is considered to be involved in the morphological and functional differentiation of the cells.

The centriole is a unique cell organelle whose position has been thought to determine cell polarity. It has been determined that one of the centriolar pair (diplosome) is ciliated in various types of cells forming a primary cilium (WHEATLEY, 1982).

Studies on leukocytes and other cells in vitro have revealed the behavior and morphology of the centrioles, their movement, separation, and ciliation or deciliation, suggesting their importance in the organization of microtubules and direction of migrating cells (OSBORN and WEBER, 1976; ALBRECHT-BUEHLER, 1977; MALECH et al., 1977; ALBRECHT-BUEHLER and BUSHNELL, 1979; ANDERSON et al., 1982; SCHLIWA et al., 1982; SHERLINE and MASCARDO, 1982; KUPFER et al., 1982, 1983; BROOKS and RICHMOND, 1983; GOTLIEB et al., 1983; HERMAN and ALBERTINI, 1983; TUCKER, 1983; NEMERE et al., 1985). There is, however, little information about such behavior of the centrioles in vivo.

An ameloblast is one of the cell systems in which movement of the centrioles has been described by light microscopic observation during the course of its differentiation, albeit insufficiently (RENYI, 1933; KALLENBACH, 1971). The present study aims at a more precise observation of the behavior and morphology of the centrioles in the differentiating ameloblasts of the rat. Three-dimensional analysis will be performed using serial ultrathin sections, with the significance of the centrioles in vivo to be discussed.
MATERIALS AND METHODS

Wister male rats weighing about 200 g were used. Under anesthesia with sodium pentobarbital, the rats were perfused through the right or left common carotid artery (Akita and Kagayama, 1985) with either fixative (a) or (b), being:

a) a mixture of 2.5% glutaraldehyde, 1.5% acrolein, and 1.0% OsO₄ buffered with 0.1M cacodylate buffer, pH 7.4; or

b) a mixture of 2.0% glutaraldehyde and 2.0% paraformaldehyde buffered with 0.1M cacodylate buffer or 0.1M phosphate buffer, pH 7.4.

The growing ends of the rat lower incisors were excised, and after demineralization in 5.0% EDTA containing 7.0% sucrose at 4°C for about a month as required, only those specimens with (b) were postfixed in buffered 1.0% OsO₄ for about 1 hr at 4°C. The tissues were rinsed in distilled water, dehydrated in ascending concentrations of ethanol, and embedded in Epon 812. Serial sections approximately 0.1–0.2 μm thick were cut with diamond knives in an ultramicrotome and stained with uranyl acetate and lead citrate.

The centrioles in ameloblasts were examined in ten to twenty serial sections with a Hitachi H-700H electron microscope operated at 100 or 150 kV. As necessary, the entire area covering the ameloblastic layer in each serial section was photographed (about ten electron micrographs per section, printed at an equal enlargement) and reconstructions were made from the micrographs to analyze the behavior of the centrioles.

RESULTS

Forty-four ameloblasts were examined in serial sections with special reference to their centrioles. It was seen that the centrioles frequently appeared paired in the ameloblasts and that one centriole of each pair was ciliated, i.e., it issued a primary cilium, whereas the other was a non-ciliated one. Some of such primary cilia appeared at the cell surface; others, however, were proved by observations of several serial ultrathin sections to be entirely within cytoplasm, and not connected with the plasma membrane (Fig. 1). Although most cilia were relatively developed in form (Fig. 1), some were only rudimentary (Fig. 10). Most cells had a single set of centriolar pairs. Only two cells in the undifferentiated phase were found to have two pairs, or duplicated centriolar pairs per cell.

In the present study, the stage of ameloblast differentiation was divided into four phases to correlate the behavior of the centrioles in cell differentiation. As to the direction of the ameloblast, the term "distal" denotes that portion of the cell closer to the pulp or basal lamina, while the term "proximal" means just the opposite.

Hoffstein (1981) indicated that the above fixative (a) in Materials and Methods in this study acted so rapidly that it could preserve cytoskeletal architecture better than other standard fixation methods such as (b). In the present observation of the ameloblasts, however, there was little difference between results with (a) and (b) regarding morphological conditions of primary cilia and microtubules.

1. Undifferentiated phase

This phase shows many mitotic figures and has been also called the proliferation zone...
(Kallenbach, 1971), where ameloblasts are organized into a stratified low-columnar epithelium as in Figure 3.

In this phase, 11 ameloblasts were examined with serial sections with regard to their centrioles.

The centrioles were invariably accompanied by the Golgi apparatus (Fig. 2, 5a, b) and frequently located lateral to the nucleus (Table 1). The distance between the two centrioles of the pair measured less than 0.5 μm. Pericentriolar dense-bodies were often observed near the centrioles (Fig. 5a, b). There were few microtubules radiating from the centrioles, or from the pericentriolar dense-bodies. Two of the 11 cells showed a centriole under duplication, located lateral to the nucleus (Fig. 5a, b).

2. Differentiating phase

This phase was composed of cells which had finished terminal mitoses and begun differentiation into highly polarized ameloblasts (Fig. 4). Cells were developing in height and organizing a single layer from the stratified epithelium. A nucleus was located in the proximal part of the cell and mitochondria were gathered in more proximal part. Proximal and distal terminal bars were developed. The basal lamina was still clear and secretion of enamel matrix had not yet begun.

In this phase, 17 ameloblasts with their centrioles were examined with serial sections.

Most centrioles were located lateral or distal to the nucleus (Table 1) and observation in several serial sections proved each to be always associated with the Golgi apparatus (Fig. 8). The distance between the two centrioles of the pair was variable, ranging from less than 0.5 μm to 5.0 μm. When this distance surpassed 1.0 μm, the two centrioles were considered to be separated. As shown in Table 2, 8 out of the 17 pairs were separated in this phase.

Figures 6–8 demonstrate two cases of cells carrying separated centrioles. The one has separated pairs of centrioles located distal to the nucleus (Fig. 6, 7). In the other
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In the undifferentiated phase, the centriole pairs are separated to the opposite sides of the nucleus (Fig. 8). Closely associated centrioles, as shown in Figure 2 in the undifferentiated phase, could also be found in this phase, either distal or lateral to the nuclei. Rootlet-like structures which probably linked the two centrioles and pericentriolar dense-bodies around them were often seen (Fig. 6). Unlike the undifferentiated phase, no cell containing the duplication of a centriole was found.

Microtubules around the centrioles were rather obscure in this phase.

3. Early secretory phase

This phase was represented by highly polarized cells which had increased their height more than in the previous differentiating phase and had a remarkably developed Golgi apparatus and rough endoplasmic reticulum in their parts distal to the nuclei. The basal lamina began to disappear. The ameloblasts had just initiate secretion of enamel matrix but had not yet completed the formation of Tomes' processes at the cell tips.

In this phase, 9 ameloblasts were examined in serial sections with regard to their centrioles.

**Fig. 3.** The undifferentiated phase, which includes mitotic cells (*) and consists of stratified low-columnar epithelial cells. ×2,900

**Fig. 4.** The differentiating phase, which includes cells developing in height and organizing a single layer. The indicated centriole (●) can be seen more clearly in the adjacent section in Figure 6. ×3,600
Fig. 5. An ameloblast in the undifferentiated phase including two centriolar pairs located lateral to the nucleus which are probably being duplicated and separated from each other. One pair (●) is in a and the other pair (○) in b. The ciliated centriole in a is still found as a primary cilium (▲) in b. There are four serial sections (about 0.1 μm thick ×4) between a and b. The Golgi apparatus (G) and pericentriolar dense-bodies (*) are seen near the centrioles. ×12,000

Fig. 6. Centrioles located distally in an ameloblast in the differentiating phase. The ciliated (●) and the non-ciliated (○) centrioles are separated by a distance of more than 1.0 μm and linked by a rootlet-like structure (▲), near which the Golgi apparatus and pericentriolar dense-bodies (*) can be identified by observation in serial sections. ×15,000

Fig. 7. Centrioles in an ameloblast in the differentiating phase, which are located distal to the nucleus. The ciliated (●) and the non-ciliated (○) centrioles are separated by a distance of about 2.0 μm. The Golgi apparatus can be identified near the centrioles by observation in serial sections. ×15,000
The position of centrioles was usually distal in the cell (Table 1) and tended to be away from the nuclei. As seen in the previous two phases, the Golgi apparatus was proved to be always located close to the centrioles by observation in several serial sections. The centrioles of the pair were more frequently separated than in the differentiating phase (Table 2). Six pairs were separated (maximal distance; about 7.0 μm) as seen in Figure 9a, b, while the other 3 pairs were not. Microtubules around the centrioles were obscure. In one cell, a rudimentary cilium or intermediate type between a non-ciliated centriole and a cilium was found (Fig. 10), and which was regarded as a cilium in this study.

Rootlet-like structures and pericentriolar dense-bodies could not be clearly identified in this phase.

4. Secretory phase
Ameloblasts in this phase completed the formation of the Tomes’ processes and represented the characteristics of the inner enamel secretion (Warshawsky, 1968, 1974). Other features of the cells were essentially identical to those described in the previous early secretory phase.

The specimens used here were demineralized as described in Materials and Methods. The morphological constitution of the ameloblasts was well preserved.

Seven ameloblasts were examined in serial sections. The centrioles were usually distal in location (Table 1) and tended to be away from the nucleus as observed in the

| Table 1. Positions of centrioles in relation to nuclei | Table 2. Conditions of centrioles |
|------------------------------------------------------|----------------------------------|
| Proximal : Lateral : Distal                           | Separation : Close association   |
| Undifferentiated phase 1 : 9 : 1                      | Undifferentiated phase 0 : 11    |
| Differentiating phase 2 : 9 : 6                       | Differentiating phase 8 : 9      |
| Early secretory phase 0 : 0 : 9                       | Early secretory phase 6 : 3      |
| Secretory phase 0 : 0 : 7                             | Secretory phase 6 : 1            |

Fig. 8. Centrioles in an ameloblast in the differentiating phase, in which the ciliated (●) and the non-ciliated (○) centrioles are separated to the opposite sides of the nucleus. The Golgi apparatus (G) is seen near the ciliated centriole and can also be identified near the non-ciliated centriole by observation in serial sections. ×15,000
Fig. 9. The early secretory phase, which is represented by highly polarized cells. One centriolar pair (ciliated: ●, non-ciliated: ⊥), located distally away from the nucleus, is separated by a distance of about 7.0 μm, which is indicated in a and enlarged in b. The Golgi apparatus (G), as seen in the adjacent cells, can be identified by observation in serial sections near the centrioles. a: × 4,500, b: × 11,250
early secretory phase. A developed Golgi apparatus proved to be always near the
centrioles, through observation in several serial sections. In this phase, 6 out of the
7 centriolar pairs were separated (Fig. 11, Table 2). Rootlet-like structures and peri-
centriolar dense-bodies could not be clearly identified.

DISCUSSION

The occurrence and structure of the primary cilia have been described in various kinds
of tissue cells (Wheatley, 1982). In ameloblasts, however, only fragmentary informa-
tion on the cilium has been given for rats (Warshawsky, 1968; Nishikawa and Kita-
mura, 1982) and puppies (Kindaichi et al., 1985). The present study demonstrates that
each of the post-mitotic ameloblasts possesses a cilium produced from one of centrioles.

Although a 9+0 microtubular arrangement of the primary cilium could not be
morphologically identified, it seems most reasonable to identify the cilium in the rat
incisor ameloblast as a primary cilium and not as a motile cilium of the 9+2 arrange-
ment for the following reasons. First, in the ameloblasts throughout the four phases
of differentiation, a cilium was always accompanied by a non-ciliated centriole. This
finding is considered to be characteristic of a primary cilium, derived from a centriolar
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pair (Barnes, 1961; Allen, 1965). Second, some cilia in the ameloblasts were extremely rudimentary (Fig. 10) and some others were included entirely within the cytoplasm (Fig. 1). Such a variety of conditions in structure and position has been described as another feature of a primary cilium (Wheatley, 1982; Tucker, 1983). Third, a cilium in the puppy ameloblast was reported to have a 9+0 arrangement (Kindaichi et al., 1985).

The presence of this type of cilium (9+0) has been repeatedly described in various tissue cells and in some cultured cells (Wheatley, 1982). Among the various aspects of the functional roles of the primary cilium, a sensory function has been strongly suggested because of the morphological similarity between the primary cilium and specialized organelles of receptor cells in some sense organs, such as rods and cones of the retina (Wheatley, 1982). However, the functional significance of the primary cilia in vivo remains unclear.

As for cultured cells, 3T3 fibroblasts have been observed to orient the primary cilium in the direction of cell movement (Albrecht-Buehler, 1977; Albrecht-Buehler and Bushnell, 1979). In the hormone-directed differentiation of cultured granulosa cells, prolactin was shown to enhance the incidence of granulosa cells containing a primary cilium. Furthermore, granulosa cells exposed to prolactin responded to Con A by forming central surface caps containing the primary cilium. This unique positioning of the primary cilium within Con A caps has suggested a vectorializing role for the primary cilium during flow of receptors and endocytosis of caps (Herman and Albertini, 1983). Postulating that the primary cilium of an ameloblast has similar function to that of the cultured cells, the intimate localization of ciliated centrioles and Golgi apparatus observed in the present study may be of special interest since the Golgi apparatus is considered to be a critical landmark in the cell with regard to the growth, retrieval, recycling, and intracellular degradation of the plasma membrane (Farquhar and Palade, 1981).

Although it is very difficult to ascertain the number of centrioles per cell without examining the whole cellular volume by serial sections, careful observation in this study indicates that the post-mitotic ameloblasts had only one pair of centrioles per cell. In the undifferentiated phase, two cells had newly duplicated centrioles (Fig. 5a, b); these cells are likely during the S or G-2 phases in the cell cycle, since Tucker (1983) suggested that duplicated centriolar pairs were separating in cultured cells around the onset of that phase.

Pericentriolar material has been shown in some cultured cells to function as a microtubule organizing center (MTOC) (Osborn and Weber, 1976; Brooks and Richmond, 1983). Additionally, the MTOC or centrioles have been reported to play important roles in representing cell polarity in the chemotaxis of neutrophils (Malech et al., 1977; Anderson et al., 1982) and macrophages (Nemere et al., 1985), in which the MTOC and Golgi apparatus are largely oriented in the direction of the chemotactic gradient. In the natural killer (NK) cells bound to targets (Kupfer et al., 1983), the MTOC and the perinuclear Golgi apparatus inside the NK cells were shown to face the region of contact with the target cell. Concerning tissue cells in vivo, however, we have little information as to whether or not their pericentriolar matrial functions as MTOC. Recently it was demonstrated that centrioles in endothelial cells of major blood vessels in some animals showed a preferential orientation toward the heart, as an index of the cell polarity (Rogers et al., 1985). It is unlikely that pericentriolar material in ameloblasts functions as the MTOC, because in most cells microtubules around their centrioles were obscure. Karsenti et al. (1984) suggested that the centrosome in confluent density
cultured cells played a poor role in organizing microtubules, while the centrosome in a solitary cell did function as MTOC. Perhaps cell to cell contact, such as in confluent density cultured cells or epithelial cells like the ameloblasts, might attenuate the function of MTOC in pericentriolar material. Despite the poor relation to MTOC, the movement of centrioles and associated Golgi apparatus can be an index of the cell polarity of the ameloblast, as in the endothelial cells in vivo (Rogers et al., 1985).

Previous studies with light microscopes considered that, in undifferentiated ameloblasts, their centriolar pairs were located proximal to the nuclei, and during differentiation, moved past the nuclei into a distal position with Golgi apparatus (Renyi, 1933; Kaltenbach, 1971). The present study with the electron microscope, however, revealed that most centrioles in the ameloblasts in the undifferentiated phase, which included many mitotic figures, were located lateral in relation to the nuclei. During differentiation and the development of cell polarity, the centriolar pair was frequently seen distal to the nucleus; in the early secretory phase it was commonly seen in the distal part of the cytoplasm (Table 1).

The movement of the centrioles or the primary cilium has been analysed in cultured cells or in blood cells. When the chemotactic gradient was applied to neutrophils under conditions of impeded cell migration by the use of micropore filters, the centriole took a position between the nucleus and the filter. This orientation rapidly changed in response to a reversed direction of the chemotactic gradient (Malech et al., 1977). The movement of centrioles or MTOC has been also reported in migrating endothelial cells (Gotlieb et al., 1983) and fibroblasts (Kuffer et al., 1982) after scarring the confluent sheet. In those fibroblasts remote from the wound, the immunolabeled Golgi apparatus and MTOC were colocalized near the nucleus and randomly oriented in the plane of the cell layer, while in the cells at the wound edge, the MTOC and Golgi apparatus were located together forward of the nucleus in the direction of the lamellar extension of the cells. The present study has presented in detail the dynamic nature of the centroiles in tissue cells in vivo. Further, during such a dynamic movement of the centrioles, the Golgi apparatus was invariably associated with them, as in the cultured cells at the edge of an experimental wound. These findings indicate the movement of the centroiles with associated Golgi apparatus into the functional side of the ameloblasts during cell differentiation and the development of their polarity. Because of such oriented movement of centrioles and Golgi apparatus, the existence of some substance similar to the chemoattractant could be argued for this phase of epithelial-mesenchymal interactions (Slavkin et al., 1981).

One of the new findings in the present study was the separation of centriolar pairs during the course of cell differentiation. Previous reports concerning the centroiles or ciliated ones have described that two centroiles are closely associated both in vivo and in vitro (Wheatley, 1982). However, in human neutrophils, it was reported that the stimulation of randomly locomoting cells with a chemoattractant (chemokinesis) initiated the transient separation of the centroiles (Schliwa et al., 1982). Additionally in some cultured cells, the epidermal growth factor, calcium ionophore, colchicine, colcemid, nocodasol, and serum stimulation were shown to induce separation of the MTOC or the pair of centroiles (Sherline and Mascardo, 1982; Brooks and Richmond, 1983; Tucker, 1983). Tucker suggested that these cellular changes might depend on calcium-sensitive microtubules which were depolymerized by an increase in intracellular calcium localized at the centrosome. Furthermore, separation of the centroiles in tissue cells in vivo was reported in ciliated neurons in supraoptic nucleus of the hypothalamus in neonatal rats (Lafarga et al., 1980) and in paraventricular nuclei in aged hamsters.
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(SUAREZ et al., 1985). The separated centrioles were reported to be always associated with the Golgi apparatus, although the possible significance of this was not discussed. Such separation should be strictly distinguished from the separation in mitosis of centriolar pairs arising from duplication (TUCKER, 1983). Since most mitoses were believed to have been completed before the differentiating phase in the ameloblasts (WARSHAWSKY and SMITH, 1974; SMITH and WARSHAWSKY, 1975), it is quite explainable that no duplicated centriolar pairs were observed except in the undifferentiated phase. Exactly what causes the separation of the centriolar pair in the ameloblasts is not clear, but the separation of the centrioles, as well as their movement, might be related to the differentiation of the cells, the development of the cell function, and probably particular conditions, such as stimulation to the cell.

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