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Development of striated rootlets during ciliogenesis in the human oviduct epithelium

Haruo Hagiwara[1], **Takeo Aoki**[1], **Nobuo Ohwada**[2], **Toyoshi Fujimoto**[1]

[1] Department of Anatomy and Cell Biology, Gunma University School of Medicine, 3-39-22, Showa-machi, Maebashi 371, Japan
[2] Department of Obstetrics and Gynecology, Gunma University School of Medicine, 3-39-22, Showa-machi, Maebashi 371, Japan

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Abstract. Striated rootlets in ciliated cells are conical banded structures composed of longitudinally aligned filaments. The formation of striated rootlets during ciliognesis in the human oviduct epithelium was studied by electron microscopy. Primitive rootlets appeared at the proximal side of basal bodies before or at the same time as ciliary budding. After the formation of several striations, the tip of the rootlets extended deeply toward the interior of the cell and became differentiated into two distinct parts, viz., the proximal conical part connected to the basal body and the distal fibrillar part. The periodicity of the striations in the fibrillar part was 68.5±2.95 nm, about 5 nm longer than that of the conical part (63.9±2.25 nm). The dark band in the striation was thicker in the fibrillar part than in the conical part. Since the fibrillar part was not observed in the mature cilium, this part was considered as being either degraded or changed into the conical part during ciliogenesis.

Key words: Ciliogenesis – Striated rootlets – Oviduct – Ciliated cells – Ultrastructure – Human

Introduction

Cilia are motile hair-like processes extending from the basal bodies located in the apical region of ciliated cells. Several accessory structures, including striated rootlets, basal feet, and transitional fibrils, are attached to the basal body (Pitelka 1974). The striated rootlet, which is composed of longitudinally aligned filaments, is a cross-banded structure with a conical profile that extends from the proximal end of the basal body toward the interior of the cell.

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Correspondence to: H. Hagiwara (Fax: +81-027-2207906)

Ciliogenesis, i.e., the formation of the ciliary apparatus, is divided into the following four stages: (1) duplication of centrioles; (2) migration of centrioles to the apical cell surface to become basal bodies; (3) elongation of cilia containing the axoneme; (4) formation of accessory structures of basal bodies. The term "ciliogenic cells" includes cells of the four phases. Previous studies of ciliogenesis have mainly focused on the first three stages (Sorokin 1968; Kalnins and Porter 1969; Dirksen 1971; Brenner and Anderson 1973; Chang et al. 1979; Youson 1982; Loots and Nel 1989; Hagiwara et al. 1992). Little attention has been paid to the development of structures associated with basal bodies during ciliogenesis.

We have previously described in detail the ultrastructural aspects of basal body replication in the human oviduct epithelium during the normal menstrual cycle (Hagiwara et al. 1992). The present study has been undertaken to elucidate the developmental process of striated rootlets during ciliogenesis. The terminology recommended by Brenner and Anderson (1973) has been used in this study to describe the structures appearing in the course of ciliogenesis.

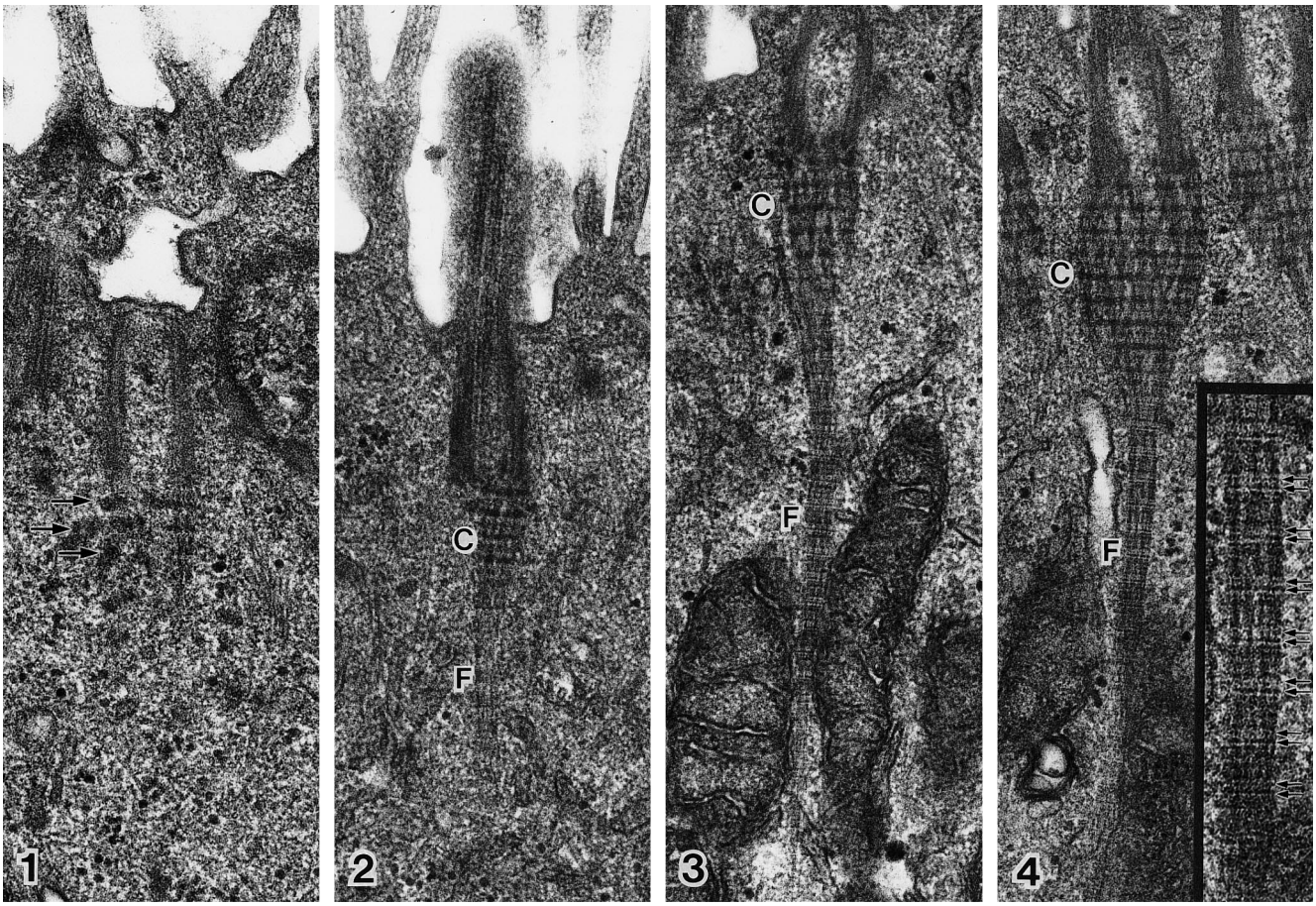
Materials and methods

Oviducts

Human oviducts were obtained from 19 women undergoing hysterectomy and bilateral salpingo-oophorectomy, because of squamous cell carcinoma of the uterine cervix. The age of the women varied between 32 and 45 years, with a mean age of 39 years. Twelve oviducts were from the proliferative phase and 7 from the secretory phase.

Electron microscopy

Immediately after removal of the uterus and its appendages, ampullary segments of oviducts were cut into small pieces and fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4° C or at room temperature. The tissues



Figs. 1–5. Developmental course of striated rootlets. *C*, Conical part; *F*, fibrillar part

Fig. 1. Fibrous granules (*arrows*) are aligned linearly at the level of dense bands of a primitive rootlet. *Bar:* 0.2 µm. ×52000

Fig. 2. A rootlet extends deeply toward the nucleus, and the fibrillar part with thickened dense bands is formed at the distal half. *Bar:* 0.2 µm. ×48000

Fig. 3. The fibrillar part is apposed to the membrane of the smooth endoplasmic reticulum and mitochondria. *Bar:* 0.2 µm. ×48000

Fig. 4. The number of striations in the conical part is increased to nine on maturation of the rootlets. Two traverse lines (*arrows*) are seen in each light band of the fibrillar part (*inset*). *Bar:* 0.2 µm. ×48000. *Inset:* ×100000

Fig. 5. The fibrillar part is not observed in striated rootlets after completion of ciliogenesis. *Bar:* 0.2 µm. ×41000

were washed overnight in the same buffer containing 7% sucrose, post-fixed in 1% osmium tetroxide buffered with 0.1 M phosphate buffer for 90 min at 4° C, dehydrated in an ascending series of ethanol, and embedded in Epoxy resin. Semithin sections were cut at a thickness of 1 µm on an LKB 8800 Ultratome III and stained with toluidine blue for light-microscopic observation. Ultrathin sections (80 nm thick) were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined at 80 kV in a JEOL electron microscope 200 CX.

Measurement of the periodicity of striated rootlets

In the oviduct of the late proliferative and early secretory phases (5 cases), ciliogenic cells and/or newly formed ciliated cells containing developing striated rootlets were photographed at a magnification of ×18000. More than 15 cells were photographed in each case. Photographs were magnified at ×90000, and the distance between neighboring bands of striations in the conical and fibrillar parts was measured in a total of 442 rootlets.

Table 1. Periodicity of striations in the rootlets during two menstrual phases and in the two distinct parts of the developing rootlets

	No. of analyses	Periodicity of striations (nm±SD)
Striated rootlets in the proliferative phase	1308	63.6±2.69
Striated rootlets in the secretory phase	763	63.5±2.44
Conical part of the developing rootlets	442	63.9±2.25
Fibrillar part of the developing rootlets	442	68.5±2.95

The periodicity of banding in striated rootlets of ciliated cells in the proliferative (12 cases) and secretory phases (7 cases) was compared. A total of 1308 rootlets were studied in the proliferative phase oviduct, and 763 in the secretory phase oviduct.

Results

Morphogenesis of striated rootlets

Various profiles of ciliogenesis were observed in the oviduct epithelium from the mid-proliferative to early secretory phases. As we reported previously (Hagiwara et al. 1992), new centrioles were duplicated via both centriolar and acentriolar pathways. In the acentriolar pathway, which was a major course of ciliogenesis, centrioles were formed by fusion of fibrous granules in contact with electron-dense deuterosomes measuring 100–110 nm in diameter. Duplicated centrioles subsequently migrated to the apical surface, aligned in rows with their longitudinal axes oriented perpendicular to the apical cell surface, and developed into basal bodies.

Before or at the same time as ciliary budding, primitive rootlets with a few striations appeared at the proximal side of the basal bodies (Fig. 1). Fibrous granules of 50–80 nm in diameter were frequently encountered inside or in close proximity to the rootlets. They were arranged linearly and appeared to be continuous with the dense bands of the rootlets (Fig. 1). After the formation of 4–5 striations, the rootlets became longer and subsequently divided into two distinct parts, viz., the proximal conical part connected to the basal body and the distal fibrillar part descending into the cytoplasm (Fig. 2). In more than half of the rootlets, the fibrillar part adhered laterally to the membranous cell organelles, such as mitochondria, smooth endoplasmic reticulum, and small vesicles (Fig. 3). The number of striations in the conical part increased from 4–5 to 8–10 during the development of striated rootlets (Fig. 4). The longitudinal filaments composing the fibrillar part and arranged in parallel were 6–12 nm in diameter, whereas those of the conical part was thicker and 8–15 nm in diameter. The thickness of dark striations in the fibrillar part was 45–50 nm, about twice as thick as that of the conical part (Fig. 4). Two dark lines traversed the light bands in the fibrillar part.

When ciliogenesis was completed, striated rootlets appeared as pen-tip-like structures composed of filaments 8–15 nm in diameter (Fig. 5). The number of striations in each rootlets was mostly 7–10. The thickness of their dark striations was 20–25 nm, equivalent to the conical part of developing rootlets.

Periodicity of striations of rootlets

The periodicity of striations in the rootlets was measured, and the result of the statistical analysis is summarized in Table 1. The periodicity in the fibrillar part of developing rootlets was 68.5±2.95 nm, about 5 nm longer than that in the conical part. There was no difference in the periodicity between ciliated cells of different menstrual phases.

Discussion

We have observed that the formation of striated rootlets in the human oviduct epithelium starts around the time of ciliary budding, a result that is consistent with previous reports (Brenner and Anderson 1973; Lemullois et al. 1987; Gaillard et al. 1989). Fibrous granules have been assumed to be involved in rootlet formation in the ciliated epithelium of the rat (Sorokin 1968), monkey (Brenner and Anderson 1973), and quail (Lemullois et al. 1987). The relationship appears to be the same in the epithelium of the human oviduct.

The developing rootlets are divided into two distinct parts, viz., the conical part and the fibrillar part. The periodicity of striations in the fibrillar part is 5 nm longer than that of the conical part and is also longer than that of the rootlets of the mature cilium. Because the fibrillar part was rarely seen in the rootlets of mature cilia, we presume that it is a transient structure during ciliogenesis. The observations that the fibrillar part first appears when 4–5 striations can be recognized in the primitive rootlets, and that the number of striations in the rootlets of matured cilia is 7–10, suggest that the fibrillar part is probably transformed into the conical part during ciliogenesis.

It is well known that the basal feet that project laterally from the mid-region of basal bodies are all oriented in the same direction in a ciliated cell. On the other hand, during ciliogenesis, the direction of the basal feet is random (Boisvieux-Ulrich et al. 1985). For coordinated movement of cilia, the basal feet must be rearranged in the same direction by rotation of the basal bodies (Boisvieux-Ulrich et al. 1985). This rearrangement is thought to occur in early stages once the cilia are formed, and the apical cytoskeletal networks have been proposed to play an important role in the rotation (Boisvieux-Ulrich and Sandoz 1991). In this study, the fibrillar part can be recognized in the developing striated rootlets and it extends deep into the cytoplasm only during ciliogenesis. The fibrillar part, by connection to cell organelles such

as mitochondria and the smooth endoplasmic reticulum, may anchor the basal bodies during their rotation.

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