Electron Microscopic Observation of the Primary Cilium in the Pancreatic Islets*

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Summary. A primary cilium often projected from the Golgi region of pancreatic A, B and D cells. The proximal portion of the cilium was found surrounded by a tubular invagination of the plasmalemma, and then the cilium extended into the intercellular canaliculus. The most proximal portion of the ciliary membrane exhibited periodical densities which might correspond to the ciliary necklace. The axoneme of the cilium was basically of the 9+0 pattern, i.e., nine peripheral doublets and no central singlet, though it was modified along the length of the cilium. Although a few appendages were projected from each doublet, it was difficult to identify dynein arms and nexin links. At the most proximal portion of the cilium, a "champagne-glass" structure connected each doublet with the ciliary membrane.

The distal and proximal centrioles of the diplosome were connected to each other by a striated band. The proximal centriole, which served as a basal body, had accessory structures, such as alar sheets, basal feet and rootlets.

Frequent projections of the primary cilium and its elaborate structure suggest that the cilium is not an aberrant structure but rather one which plays a certain role in the islet cell function.

The presence of a primary cilium has been reported in many cell types such as various epithelial cells, endocrine cells, mesenchymal cells, muscles, neurons, and sensory cells (reviewed by WHEATLEY, 1982). In the pancreatic islet, a cilium was first demonstrated electron microscopically by MUNGER (1958) in mouse B cells. It is the agreement of many authors that the axoneme of the cilium consists of nine peripheral doublet microtubules but no central singlet (the 9+0 pattern), though this is also sometimes recognized as an 8+1 or 7+2 pattern due to the disordered arrangement of doublets (BARNES, 1961; COUPLAND, 1965; DAHL, 1967; WHEATLEY, 1967a, b; KATAOKA, 1973; KATAOKA et al., 1982; WHEATLEY, 1982). WHEATLEY (1982) offered a review on the substructure of the primary cilium, but his description was based on a summary of many sporadic findings on primary cilia in different cell type in various animals, either in vivo or in culture. Few systematic investigations have been carried out on the primary cilium of specialized cell types including the pancreatic islet cells.

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MATERIALS AND METHODS

Islet-rich tissue blocks obtained from normal adult rats, mice and rabbits under a dissection microscope were fixed in 2.5% glutaraldehyde (cacodylate buffer, pH 7.3) and postfixed in 1% osmium tetroxide (cacodylate buffer, pH 7.3.) After dehydration through graded concentrations of ethanol, the tissue blocks were embedded in epoxy resin. Thin sections were doubly stained with uranyl acetate and lead citrate, and examined in a Hitachi H-300 or H-500 electron microscope.

RESULTS

A primary cilium was frequently recognized in the pancreatic islets of all animals examined (Fig. 1a, b, d). The cilium measured 200-230 nm in diameter and sometimes surpassed 4 μm in length. It was projected from the Golgi region of A, B and D cells into the intercellular spaces, especially into the intercellular canaliculus, i.e., microvillious intercellular channel among islet cells (FUJITA et al., 1981; KATAOKA et al., 1982). The proximal part of the cilium was usually surrounded by a ciliary sheath, or the tubular invagination of the plasmalemma. Many small vesicles were seen in the cytoplasm around the invagination. At the bottom of the invagination, the plasmalemma appeared thicker through the accumulation of felt-like dense substances along the cytoplasmic surface. The membrane then reflected to form the cilium. In longitudinal sections of the cilium, the ciliary membrane exhibited periodical densities (at intervals of about 20-30 nm) over a length of 0.1 μm at the most proximal portion of the cilium. Felt-like substances were also seen along the cytoplasmic side of the membrane in this

Fig. 1. a and b. A single cilium projected from an A cell of the rabbit (a) and a B cell of the mouse (b), respectively. Note that the distal centriole serves as the basal body. Periodical densities (arrow) are seen at the most proximal portion of the ciliary membrane. (Refer to Figure 4 for structures associated with the basal body, such as alar sheets, basal feet and rootlets.) c. Two cilia (arrows) projected from a B cell of the mouse. d. A cilium (arrow) projected from a D cell of the mouse. Although the membrane continuity at the proximal end of the cilium is not clear, the presence of rootlets (arrowheads) indicates that the cilium belongs to D cell. ×20,000
portion. The ciliary membrane continued to the tip without any apparent specialization in their fine structure.

The axoneme consisted of nine peripheral doublet microtubules at the proximal portion of the cilium, basically forming into this 9+0 pattern (Fig. 2b). A moderately dense spoke projected from each doublet toward the center of the cilium. Another appendage seemed to link adjacent doublet. In addition, a “champagne-glass” structure (DUSTIN, 1978) connected each doublet to the ciliary membrane at the most proximal portion of the cilium. At the principal portion of the cilium, one of the nine doublets tended to assume a course near the central axis of the cilium. The arrangement of the other eight doublets was more or less disordered simultaneously, so that the axoneme pattern appeared as a distorted 8+1 type (Fig. 2c). A few (two or occasionally three) appendages were projected from each doublet. Near the tip of the cilium the arrangement of doublets became more irregular, singlets took the place of some doublets, and the number of both doublets and singlets was decreased.

A diplosome, whose proximal and distal centrioles maintained a typical rectangular orientation to each other, was present at the base of the primary cilium (Fig. 1a, b). The distal centriole served as the basal body of the cilium. A striated band connected the basal body to the proximal centriole (Fig. 3b). The band consisted of striated fibers and exhibited three cross striations at intervals of 50–60 nm. As usual centrioles, nine triplet microtubules, connected with A–C linkers and embedded in amorphous substance, formed the cylindrical wall of the basal body (Fig. 2a). In the interior of the cylinder, a central dot with radiating spokes formed a “cartwheel” structure (Fig. 3a). The several accessory structures associated with the outer surface of the cylinder were alar sheets, basal feet and rootlets. Alar sheets, or transitional fibers, emerged from the distal portion of each triplet and extended toward the plasmalemma at the bottom of the ciliary sheath (Fig. 1a, b, 3a). The basal foot, which projected laterally from the middle portion of the basal body, was conical with a spherule at the apex (Fig. 1a, b, 3a–h). The maximum number of the basal feet observed was two per section; they either extended in opposite directions or were oriented at nearly right angles to each other. In a cross section of the basal body, the cone-shaped portion of the basal foot appeared to be a horseshoe-shaped structure. Several rootlets radiated from the dense substance at the proximal portion of the basal body (Fig. 3i). The rootlet was about 50 nm in diameter and 0.6–1 μm in length. It consisted of a bundle of fine filaments, and was cross-striated by dense bands at intervals of about 75 nm. Microtubules

![Fig. 2](image-url) Cross sections of the cilium at different levels. a. The basal body consists of nine triplet microtubules. Subfiber A of a triplet and subfiber C of the adjacent triplet are connected by A–C linker (arrow). b. At the proximal portion of the cilium, nine doublets are seen. Arrow: “champagne-glass” structure. L lumen formed by the plasmalemmal invagination. c. The principal portion of the cilium showing nine doublets. I intercellular space. ×90,000
radiated from the vicinity of the diplosome. Some of them were apparently projected from the spherule of the basal foot (Fig. 3f, g).

Although the cilium was usually single, as described above, two cilia were occasionally projected from an islet cell (Fig. 1c). Examples of the latter case were too
rare to ascertain whether their basal bodies were the two centrioles of a diplosome or the distal centriole of two diplosomes.

Figure 4 schematically shows the results of the present study.

DISCUSSION

Two types of cilia, primary and secondary, have been reported. The primary cilium, also known as the central or single cilium, issues from the distal centriole of the diplosome in many cell types (reviewed by Wheatley, 1982). On the other hand, secondary cilia, or kinocilia, usually project from multiple basal bodies in a specialized cell type such as ciliated cells in the respiratory tract and oviduct. The structure and function of the secondary cilia have been better understood.

The axoneme of the secondary cilium consists of nine peripheral doublet microtubules and two central singlet microtubules (9+2 pattern) (Dustin, 1978; Fawcett, 1981). The central pair of singlets are surround by the central sheath. Several appendages project from the peripheral doublets: nexin links connect the doublets to each other, two dynein arms project from the subfiber A of a doublet toward the adjacent doublet and radial spokes extend radially toward the central sheath. The present findings are consistent with previous discoveries that in the primary cilium. The
axoneme basically consists of nine peripheral doublets but no central singlet (9+0 pattern), though sometimes are found its modifications into an 8+1 or 7+2 pattern by 1 or 2 doublets shifting to the central axis of the cilium (Barnes, 1961; Coupland, 1965; Dahl, 1967; Wheatley, 1967a, b; Dingemans, 1969; Kataoka, 1973; Kataoka et al., 1982; Wheatley, 1982).

Little has been known about links and arms of the doublet microtubule in the primary cilium. Some appendages are demonstrated on the doublets in the present study. However, they are hardly identifiable with such specialized structures as the dynein arms, nexin links and radial spokes. The fact that these specialized structures could not be clearly identified in the primary cilium should not be simply attributed to their absence, since their identification under the electron microscope is extremely difficult even in such typical kinocilia as those of the tracheal epithelium. The basal plate, to which the proximal end of the central singlets attaches in the secondary cilium, could not be found in the primary cilium either in the present or previous studies (Wheatley, 1982).

At the proximal portion of the secondary cilium, the ciliary membrane has a ciliary necklace which consists of arrays of characteristic intramembranous particles, and each peripheral doublet of the axoneme links with the ciliary membrane with a “champagne-glass” structure (Dustin, 1978). Periodical densities at the proximal portion of the ciliary membrane shown in the present study seem to correspond to the ciliary necklace, though a definite identification as such must be made by observation using the freeze-fracture method. The present authors detected the necklace in the primary cilium of the intercalated duct cell during a freeze-fracture study on the exocrine pancreas (unpublished data). The “champagne-glass” structure was first demonstrated in the primary cilium in the present study.

At the base of the primary cilium, the basal body, or the distal centriole of a diplosome, usually maintains a rectangular arrangement to the proximal centriole in the pancreatic islet cells of the present study. This is consistent with findings in many other cell types (reviewed by Wheatley, 1982). The striated band, which connects the basal body with the proximal centriole, was found in zona glomerulosa cells of the adrenal cortex (Wheatley, 1967a, 1982) as well as in the pancreatic islet cells in the present study. The function of this striated band remains unknown. Its absence in the diplosome of cells which do not form the primary cilium (Wheatley, 1982) suggests that the striated band is not essential in sustaining two centrioles orthogonally but rather plays a role as an accessory structure of the basal body of the cilium. In the secondary cilia, the basal body does not generally accompany the proximal centriole and the striated band has not been reported.

The walls of the basal body of primary and secondary cilia as well as of the usual centriole, consist of nine triplet microtubules connected to each other by linkages and embedded in the amorphous substance, and has a “cartwheel” structure in the interior (Anderson, 1972; Dustin, 1978; Wheatley, 1982). Such are also the case in the primary cilium in pancreatic islet cells as shown in the present study.

Several accessory structures have been observed in the basal body of the secondary cilium: alar sheets, basal feet and rootlets (Anderson, 1972; Dustin, 1978). The alar sheets, also called transitional fibers, are nine radiating projections which extend upward and outward from the basal body and are anchored on the dense matrix along the cytoplasmic surface of the plasma membrane. In the primary cilium, the presence of the alar sheets has been reported in several cell types such as adrenocortical (Wheatley, 1982) and pancreatic islet cells (the present study). Wheatley (1982) has also described
the presence of these alar sheets on the centrioles of hepatocytes and salivary gland cells which never form cilia. As for the function of alar sheets, Wheatley (1982) suggested that they anchored the basal body to the plasmalemma and the distance between the basal body and the plasmalemma could be altered by changing the angle of the alar sheets to the longitudinal axis of the basal body.

The basal foot has been described as a single lateral process which projects in the direction of the effective stroke of the secondary cilium (Gibbons, 1961; Anderson, 1972; Hard and Rieder, 1983). On the other hand, little attention has been paid on the number and direction of the basal feet in the primary cilium. The present study revealed that one or two basal feet projected from the single basal body in a single thin section of the islet cell. In the latter case, the basal feet either extended in opposite directions or were oriented at nearly right angles to each other. Wheatley (1982) reported that four basal feet projected from one centriole of the cultured fibroblast. As in the case of pericentriolar satellites, the spherule of the basal foot seems to act as an organizing center of cytoplasmic microtubules in the secondary (Gibbons, 1961; Gordon, 1982) as well as the primary cilium (Wheatley, 1982; the present study).

The rootlet consists of a cross-striated bundle of filaments and projects from the proximal part of the basal body. In secondary cilia of the newt lung cell, a thick bundle (ciliary root) has been found to extend at an angle of 125–135° from the longitudinal axes of the basal bodies in a direction opposite to that of the basal foot, while a thin bundle (ciliary rootlet) projected toward the cell nucleus (Hard and Rieder, 1983). A single rootlet has been demonstrated in secondary cilia of the monkey oviduct (Anderson, 1972). In the primary cilium of islet cells examined in the present study, up to ten rootlets were found radiating from the basal body. Olsson (1962) suggested an anchoring function for the rootlet. The primary protein of the rootlet was extracted and called ankylin (Stephens, 1975). The presence of ATPase activity on the rootlet has been reported in the connecting cilium of the retinal rod (Matsusaka, 1967) and in the secondary cilia of the oviduct epithelium (Anderson, 1977). These findings suggest that the rootlet plays a certain role in ciliary movement as well as anchoring the basal body.

The primary cilium is usually single in most of the reported cells (reviewed by Wheatley, 1982); this is also the case in the present study. Occasionally, double cilia are found in adenohypophyseal cells (Barnes, 1961; Dahl, 1967; Wheatley, 1967b) as well as in pancreatic islet cells as shown in the present study. Multiple cilia, except for typical kinocilia, were reported only under pathological conditions: Boquist (1968) described the appearance of multiple cilia (both 9+0 and 9+2 in axoneme pattern) in the pancreatic ductular epithelium after alloxan-treatment, and Kawamata et al. (1986) found multiple cilia (9+0 pattern) in some gastric epithelial cells in gastric ulcer and cancer patients.

The function of the primary cilium, or 9+0 type cilia, remains to be elucidated. Because of the absence of dynein arms and a central pair of singlet microtubules, the primary cilium has been apt to be considered immotile. On the contrary, some evidence has supported their motility, though their movement seems not so active as in the kinocilia: some of the colcemid-induced cilia of fibroblasts beat erratically (Stubblefield and Brinkley, 1966) and primary cilia of the rabbit oviduct epithelium showed aberrant movements (Odor and Blandau, 1985). A sensory function of the primary cilium was suspected as early as in 1898 by Zimmerman. This view has been succeeded by those of many investigators, since some receptor apparatuses have been regarded as specialization of the primary cilium, such as retinal rods and cones, neuronal and glial cells of...
the saccus vasculosus in fish and sensory organs of the ascidian tadpole (Wheatley, 1982). Although no supporting evidence is available, the hypothesis that the primary cilium in the pancreatic islet facilitates the flow of the intercellular fluid by ciliary movement and/or receives certain information from the intercellular fluid and transmits it to the center of the cell seems to be both cogent and worthwhile for consideration in future studies. A similar view was espoused by Munger (1958), who first described the presence of the primary cilium in the pancreatic islet. If this hypothesis is accepted, then the localization of the primary cilium becomes appropriate in the pancreatic islet: the cilium projects to the lumen of the intercellular canaliculus, an efficient pathway for the intercellular fluid (Fujita et al., 1981; Kataoka et al., 1982; Yamamoto and Kataoka, 1984).

As reviewed by Wheatley (1982), the frequencies of primary cilia have been mostly reported by subjective impressions of the observer, and would seem to be underestimated. In considering the finding that primary cilia are usually single, the frequency in pancreatic islet cells is considerably high. It seems most reasonable to suggest that they should not be regarded as aberrant or sporadic structures but as a regular component of the cells. In concurrence with this, Karlsson (1966) showed 100% incidence of primary cilia in neurons of the rat brain by complete serial sections, and Dingemans (1969) estimated that about 70% of the cells in the mouse adenohypophysis projected a cilium.

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REFERENCES


Boquist, L.: Cilia in normal and regenerating islet tissue. An ultrastructural study in the Chinese hamster with particular reference to the $\beta$-cells and the ductular epithelium. Z. Zellforsch. 89: 519-532 (1968).


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