Journal of Photoscience (2002), Vol. 9(2), pp. 284–286

Three-dimensional analysis of the arrangement of microtubules of the outer segment in the ciliary-type photoreceptor cell in the *Onchidium* dorsal eye

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The inverted retina of the *Onchidium* dorsal eye (DE) is composed only of ciliary-type photoreceptor cells (CC's). The outer segment (OS) of the CC is a concentric lamellar structure consisting of many modified ciliary membranes and stains positively with anti-ß-tubulin antibody. Near the base of the OS there are about 30 basal bodies each connecting individually to a cilium. The cilia are rod-shaped at the base, progressing upwards to a flattened sheet-like shape with increasing surface area. Three-dimensional analysis on serial sections demonstrates the ladle-shape of a modified cilium. Many modified cilia wrap around each other like the leaves of a cabbage. Nine pairs of microtubules (MT's) are located regularly in a ring at the base of the cilium, gradually losing their regular arrangement towards the periphery, where they separate into two subgroups that are contained within two swollen portions of a modified cilium. Within the CC of the *Onchidium* DE, MT's in the modified cilium exist as two poles extending longitudinally in a thin expanded ciliary membrane. This arrangement may support the photoreceptive OS and serve to maintain its structural integrity.

Keywords; ciliary-type photoreceptor cell, outer segment, microtubule, dorsal eye, *Onchidium*.

INTRODUCTION

Photoreceptor cells have a specialized membrane that remarkably increases the photoreceptive surface area. The photoreceptive membrane may be derived from the membrane of the cilium or the cell surface, resulting in ciliary-type or rhabdomeric-type photoreceptor cells respectively [1]. Although invertebrate eyes display a great diversity in structure, most vertebrate eyes have only the ciliary-type photoreceptor cell. In the inverted retina of the vertebrate eye, the outer segment (OS) of photoreceptor cells (rods and cones) is derived from a single, specialized ciliary membrane. The OS of a rod contains numerous closely packed membranous discs. Nine doublets of microtubules (MT's) are located at one side of the OS like a flagpole [2]. A ring of nine regularly spaced MT's is maintained from the base to the tip of the OS.

Onchidium has two kinds of eyes. The stalk eye is similar to other gastropod eyes in fine structure; in particular the visual cell is a typical rhabdomeric-type. The dorsal eye (DE) contains typical ciliary-type photoreceptor cells (CC's) and is different from the stalk eye in its structure and response to light [3]. The DE is known as the 'vertebrate-type eye' because of its structural similarity. The distal portion of the CC shows a concentric lamellar structure regardless of whether it is sectioned horizontally or longitudinally. The three-dimensional arrangement of the

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lamellar structure in the OS of the CC in the *Onchidium* DE has not previously been determined. We performed serial sections of the CC and studied electron micrographs to try and clarify the relationship between the lamellar sheet in the OS and the connecting cilia that are located near the base of the OS. As MT's were found within two portions of a lamellar sheet in the OS, the arrangement of MT's in the modified cilium appears to be different from that seen in rods and cones [2]. There are two possible arrangements of MT's in the modified cilium; A U-turn configuration with a group of 9 paired MT's that are regularly arranged, depending on morphological changes within the cilium or an arrangement of two subgroups of MT's extending upward. The arrangement of MT's in the modified cilia was reconstructed in detail.

MATERIALS AND METHODS:

Ocellus papillae bearing DEs were isolated from the dorsal mantle of *Onchidium* sp. (Mollusca, Gastropoda). The specimens were fixed with 2% osmium tetroxide in 0.2 M phosphate buffer at pH 7.6 for 2hr at 4 °C, dehydrated in a graded ethanol series and embedded in Epon 812. Sixty serial ultrathin sections (0.1 μm thick) were stained with 2% aqueous uranyl acetate and lead citrate, and examined with a Hitachi H-700 electron microscope at 75 kV. Electron micrographs with a final magnification of x7,500 were prepared to identify the positions of the CC's in the retina, those with a magnification of x17,000 were prepared to examine the shape and the position of the cilium in the OS, and those with a magnification of x45,000 were used to reconstruct the arrangement of MT's in each cilium. Using semitransparent papers on the electron micrographs, we traced the outline of 4 out of 29 cilia and mapped the position of MT's in the ciliary matrix. The contextual relationship of the targets was checked by overlapping the traced figures in turn. Three-dimensional analyses were performed using the computer-aided Rise OZ-95-32 3D Reconstruct System. The upper part of the cilium, after becoming a ring on cross-section, was not reconstructed. This was because this part displayed a concentric lamellar structure that made it very difficult to specify a target lamella from many other similar lamellar structures.

RESULTS:

DE's are located in the ocellous papillae that are distributed on the dorsal mantle. The DE is an open vesicle type (200 μm in diameter) and comprises a cup-shaped pigment cell layer, an inverted retina and a lens, but lacks a cornea. The retina is composed of a single columnar epithelium of CC's. The distal OS, which makes up one third of the CC, is ellipsoidal in shape and stains positively with anti-ß-tubulin antibody. Near the base of the OS, there are about 30 basal bodies each connecting individually to a cilium. When the connecting cilium is sectioned horizontally at its base, it displays a fine structure that is typical of the cilium of photoreceptor cells. It contains nine paired peripheral doublets of MT's and lacks the two central ones. Various sections showed that the middle part of the OS has a concentric lamellar structure. Each lamella of the OS can be traced like a contour line in a map. In longitudinal section, a direct connection between a connecting cilium and a lamellar sheet can be occasionally observed. The reconstruction study through serial sections revealed that a sheet of lamellae is derived from the modified membrane of a single cilium. The number of connecting cilia is consistent with the number of lamellar sheets forming the OS.

Each cilium transforms from a round shape at the base to a flattened sheet-like shape more distally, eventually becoming a ring in cross-section. The modified ciliary membranes expand laterally, fuse at their edges on both sides and form a ladle-like shape higher up (Fig. 1A). Multiple cilia with increased surface area overlap each other like the leaves of a cabbage and show a concentric lamellar structure. Nine pairs of MT's are arranged regularly at the base of the cilium. These gradually lose their regular arrangement and separate into two subgroups or poles (Figs. 1A, 1B). MT's in each subgroup run parallel within the ciliary matrix. The lamellar sheet of the OS is not uniform in thickness, containing two swollen portions which each contain a subgroup of MT's. The swollen portions of neighboring cilia are located adjacent to each other.

DISCUSSION:

This 3D-reconstruction study demonstrates the structural changes of the cilia in the OS of the CC and the

arrangement of MT's in the ciliary matrix in the *Onchidium* DE. Within the *Onchidium* DE, incident light passes through the epidermis, the lens and the proximal portion, and reaches the distal OS of the CC. The DE has an inverted retina similar to vertebrate eyes. The distal OS of the CC corresponds to the OS of rods and cones in vertebrate eyes. The photoreceptive OS of the CC in the *Onchidium* DE displays lamellar structure [3]. Each lamellar sheet in the OS is derived from a modified ciliary membrane with notably increased surface area. The OS of typical a CC (e.g. rods) shows a stack of numerous specialized membranous discs that are derived from a single cilium. The lamellar structure of the OS in the *Onchidium* DE is composed of a number of expanded modified ciliary membranes overlapping each other. Each modified cilium has a ladle-like shape and is a member of the OS consisting of a number of compactly overlapped lamellae. Consequently, the surface area for photoreception is markedly enhanced. The morphology of the modified cilia in the CC of the *Onchidium* DE that we have demonstrated here is unique, differing from that seen in the photoreceptive sites in the CC of other vertebrate and invertebrate eyes. The modified cilia in the OS of CC in the *Onchidium* DE contains MT's showing a 9+0 pattern that is similar to those of CC's in vertebrate and some invertebrate eyes. In rods and cones, MT's are located at one side of the OS similar to a flagpole, and may relate to metabolism for photoreception and support of the OS [2]. There are few reports on the arrangement of MT's in photoreceptive sites in the CC's of other invertebrate eyes. The arrangement of two poles containing 4 or 5 MT's has never been reported in other CC's. In the *Onchidium* DE, MT's of the CC may act to mechanically support the expanded, thin sheet-like modified ciliary membranes of the OS and help to maintain its structural integrity.

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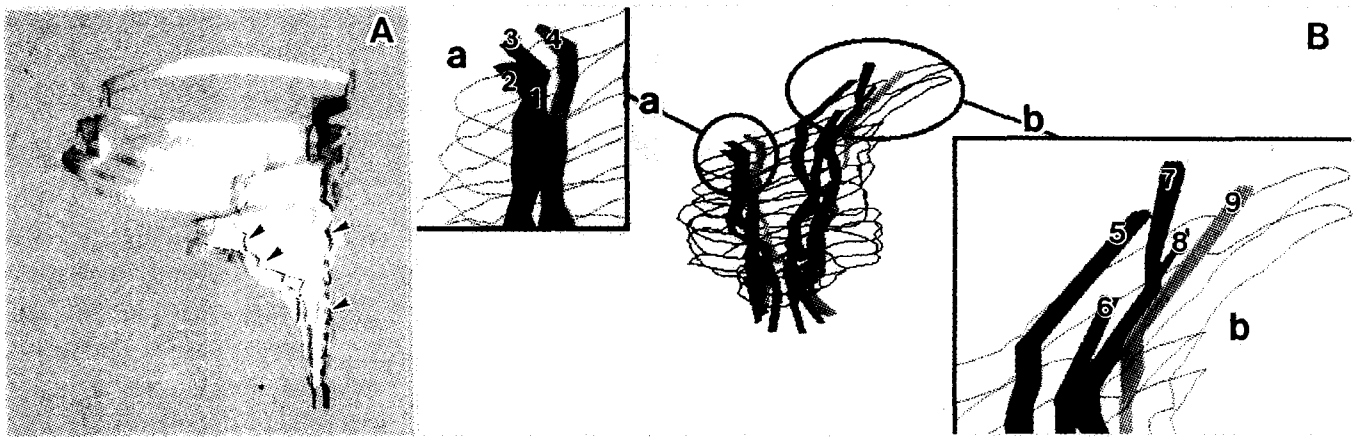

Figure 1A. Smooth-overlap graphic of a reconstructed cilium containing MT's. The smooth-overlap graphic displays the inter-relationship between the shape of a cilium and the arrangement of MT's. The arrangement of MT's in the cilium can be identified by controlling the degree of transparency in color of the smooth-overlap graphic. **1B.** The arrangement of microtubules in a three-dimensionally reconstructed cilium. High magnification of a wire-frame graphic of a cilium displays that MT1-MT4 and MT5-MT9 separate into 2 subgroups. The outline of the cilium is round at the base and an irregular, flattened sheet higher up.