Ultrastructural & Analytical Methods in Life Sciences

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Ultrastructure of plasma membrane and membrane-microtubule linker of the connecting cilia in rat retinal rod cell examined by rapid-freeze deep-etch technique

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Mutations in human CEP290 cause cilia-related disorders. It has been recently shown that CEP290 is a component of radial Y-shaped structures observed between nine microtubular doublets of the axoneme and plasma membrane in transverse section of the connecting cilia and transitional zone of motile cilia (1). In spite of special attention to the Y-shaped cross-linker, its detailed structure and its manner of association to the plasma membrane have not been clarified yet.

In the present study, the plasma membrane and internal architecture of the connecting cilia in fresh rat retinal rod cell were examined with rapid-freeze deep-etch technique. The extracellular surface (ES) of the ciliary plasma membrane was decorated with large protrusions (11-15nm in diameter) in the form of periodic transverse strands. (Figs. 1A, B). Center to center distance of the adjacent arrays was about 25nm. The arrays were at an angle of 5-10° with respect to the transverse axis of the cilium. The spacing of ES protrusions in line (about 25nm) was similar to that of the necklace particles seen on the E-face, suggesting that both of them represent aspects of single transmembrane protein (necklace protein). About thirty-six necklace proteins were located along a perimeter of the cilium.

The cross-fractured and deep-etched cilia revealed cross-linkers between microtubules and plasma membrane (Figs. 1C, D). Each cross-linker consists of a proximal stem and a peripheral branching part, which seems to be composed of four feet, perpendicular to each other. The distance between adjacent feet-membrane contact sites was about 25nm. Stereo view showed that the cross-linkers were spirally lined up along the longitudinal axis of the cilium. Based on these observations, three-dimensional model about ciliary membrane-microtubule linkage is proposed (Fig. 1E). In this model, each foot in the cross-linker is shown to be connected to each necklace protein. Two feet of a cross-linker are connected to two necklace proteins in an array and the other two feet of the same cross-linker are attached to two necklace proteins in the neighboring array. Then, thirty-six necklace proteins along a perimeter of an array are linked by feet from eighteen cross-linkers. The parallel necklace arrays are supposed to be portions of continuous spiral array. These results may suggest that the necklace proteins, lined along spiral arrays and connected to microtubules by four-legged cross-linkers, may play an important role in conveying opsin molecules from the cell body toward the rod outer segment.

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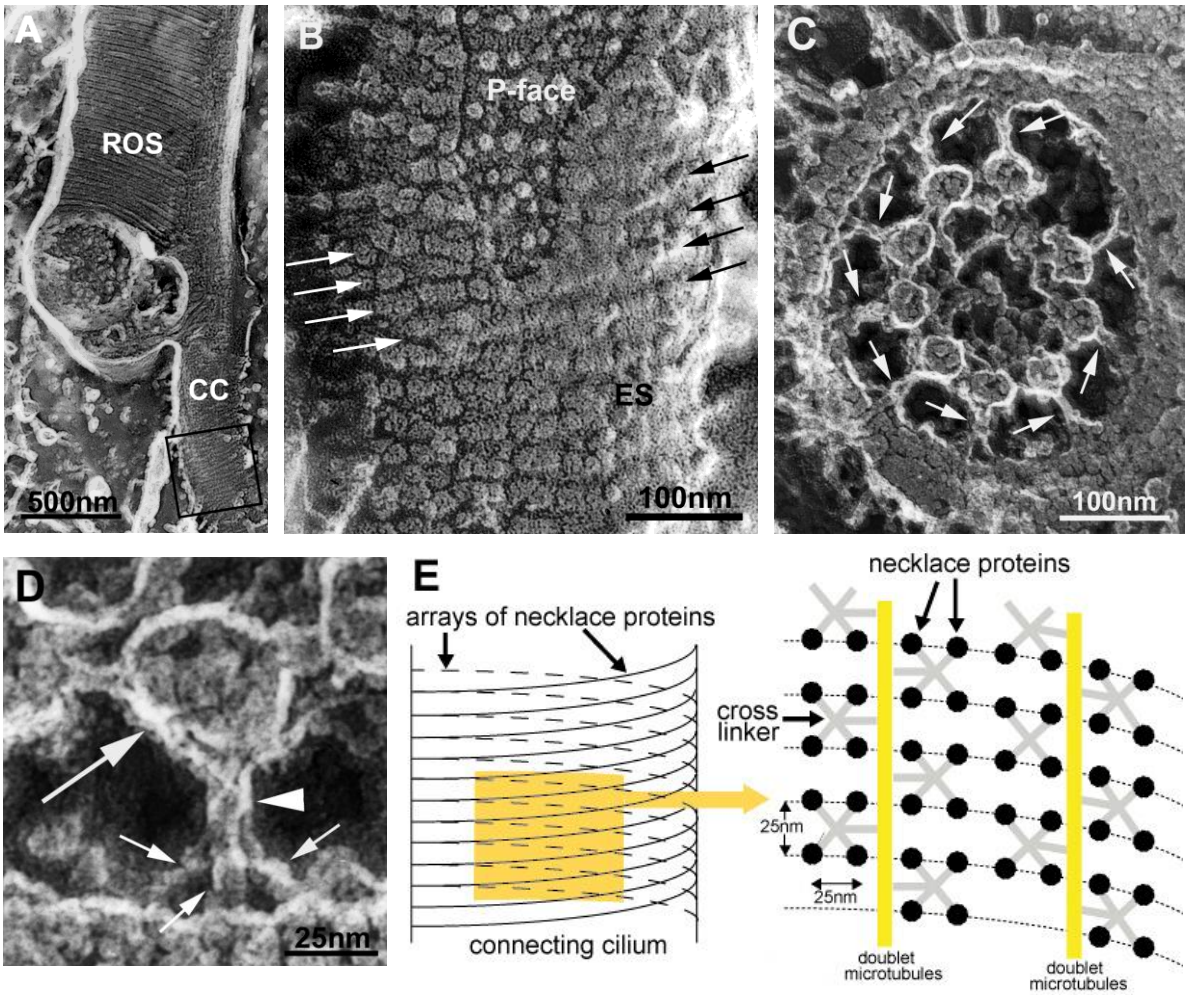

Figure 1. (A) Rod outer segment (ROS) and connecting cilium (CC) of rat retinal rod cell visualized by rapid-freeze deep-etch technique. (B) Higher magnification of the connecting cilium. On the extracellular surface (ES) of the plasma membrane, protrusions are arranged in a form of parallel arrays (between arrows). (C) Cross-fractured and deep-etched view of connecting cilium. Nine doublet microtubules are connected to the plasma membrane by nine cross-linkers (arrows). (D) Higher magnification of a cross-linker. Each cross-linker is composed of a proximal stem (arrowhead) and peripheral feet (short arrows) perpendicular to each other. Long arrow indicates doublet microtubules. (E) Schematic diagram of internal architecture of the connecting cilium. The spiral array of necklace proteins is shown in the left, and linkage between the four-legged cross-linker and necklace proteins is in the right. Each necklace protein is linked to each foot of the cross-linker in this model.