



Cell Motility and the Cytoskeleton

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Article

Complex intermolecular interactions maintain a stable linkage between the photoreceptor connecting cilium axoneme and plasma membrane

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KEYWORDS

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ABSTRACT

Microtubule-membrane cross-linkers in motile and nonmotile cilia are supramolecular structures, held together by strong interactions between the constituent molecules. We have characterized these interactions in the photoreceptor connecting cilium, where cross-linkers co-fractionate and maintain their in situ location after Triton X-100 extraction of axonemes. In bovine photoreceptor cells, the transmembrane assemblage that is cross-linked to the connecting cilium axoneme contains three high molecular mass glycoconjugates of 425, 600, and 700 kDa (Horst et al., 1987). The relative amounts of the three glycoconjugates, as judged from band intensity in electrophoretograms, depend strongly on sample treatment prior to electrophoresis. The electrophoretic pattern was reproducible after several weeks of storage of the axoneme fraction in extraction buffer containing 50% sucrose. Removal of sucrose from the buffer by dialysis eliminated the 600 kDa and 700 kDa, and decreased the detected amount of the 425 kDa glycoconjugate. When samples were incubated in Laemmli sample buffer at increasing temperatures (23°, 60°, 95°C), a gradual reduction in the intensity of the three bands was observed. The quantitative reduction of high molecular mass glycoconjugates was accompanied by the appearance of novel protein species of lower molecular mass, as detected by lectin and antibody overlays of axonemal transblots. These results suggest that the previously characterized cross-linker glycoconjugates are complex, SDS-resistant multi-molecular conglomerates. We have further used fluorescent lectins to monitor the presence of glycoconjugates on whole-mounted axonemes, in conditions aimed to selectively solubilize the cross-linkers. The cross-linker complexes could not be dissociated from the axoneme by incubation with buffers containing 1 M of either Na2SO4 or NaI. The results indicate that the connecting cilium-specific cross-linker complexes are bound via high-affinity interactions to both axoneme and overlying plasma membrane. © 1994 Wiley-Liss, Inc.

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