Functional morphology of the crista ampullaris: with special interests in sensory hairs and cupula: a review

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Abstract The functional significance of the ciliary interconnections and cupula has been reviewed. The ciliary interconnecting systems are divided into 2 types, i.e. side links and tip links. The side links acts to maintain the regular distance between the cilia thereby keeping the geometrical arrangement of the entire sensory hair bundle intact as well as to prevent close contact between neighbouring cilia. The tip links, stretching upwards from the tips of the shorter stereocilia to their taller neighbouring shafts, are actually involved in mechanoelectrical transduction. The cupula is composed of the cupula and subcupular meshwork. The subcupular meshwork consists of long branching filaments cross-bridged to one another. The cupula would function as a rigid plate and equally distribute the shear force of the cupula to all the ciliary bundles. The subcupular meshwork may play a role in the transmission of the shear strain force of the cupula to the ciliary bundle and may also exert an additional damping effect in order to prevent unwanted vibrations.

Key words: cupula, crista ampullaris, tip-link, SEM

Introduction
A crista is located in the ampulla of each of the lateral, anterior and posterior canals. Three cristaes ampullares register angular acceleration in the same plane as the semicircular duct that is irritated. The crista ampullaris contains the neuroepithelium, the cupula, dark and transitional cells, connective tissue, blood vessels, and nerve fibers. The epithelium in the crista ampullaris is made up of hair cells, specialized receptor cells for the sense of equilibrium, and supporting cells. Above the epithelium of the crista ampullaris a mass of extra cellular, highly specialized material (the cupula) is located, extending from the epithelial cell surface to the roof of the ampulla (Hunter-Duvar & Hinojosa, 1984; Anniko & Takumida, 2001). Recently, great interest has been focused on the surface structure of the crista ampullaris. The mechanical qualities of the cupula and its relationship to the sensory hair bundles in the crista provide a basis for their specialized mechanical transduction functions. Analysis of their fine structure and microarchitecture provides important complementary information to the often more difficult direct mechanical and other physiological measurement procedures.

Fine structure of the glyocalyx and ciliary interconnections
It is a well-known fact that all epithelial cells have a carbohydrate-rich surface coat, a so-called glyocalyx. The glyocalyx is present on the free surface of the cells and is most highly developed on the luminal surface of certain epithelial cells. In the inner ear, it has been known that luminal surface of the crista possesses glyocalyx. In order to visualize the inner ear glyocalyx, several histochemical methods have been used. Ionized carboxyl and sulfate groups of the acid polysaccharides give the glyocalyx a strong negative charge, which causes it to binding dyes such as cationic ferritin, alcian blue and ruthenium red (RR). By using a RR staining technique, the glyocalyx could be easily visualized. The glyocalyx appeared to consist of a short fuzzy filamentous layer, which emerged from the outer layer of the plasmamembrane. Filaments, projecting the surface of the glyocalyx interconncted neighbouring stereociliary shafts (Takumida, et al., 1988; Anniko & Takumida, 2001).

Scanning electron microscopy (SEM) revealed two types of ciliary interconnections. Interconnections of the first type were so-called ‘side links’ between the hair cell cilia, looking like a web-like network that closely apposed a cilium with its neighbours. Ciliary membrane showed a rather rough appearance. The fine filamentous structures emerging from the surface of the cilia tightly interconnected the neighbouring ones. These membrane roughness and side links are suggested to be a part of glyocalyx, since the membrane roughness noticed by SEM is well corresponded to the outline of glyocalyx observed by TEM and the side links showed morphological similarities (Fig. 1) (Takumida, 1989, Anniko & Takumida, 2001).

Interconnections of the second type are the so-called ‘tip links’. These tip links stretched upwards from the tips of the shorter stereocilia to their taller neighbouring shafts and consisted of a single thin fibre about 10-20 nm in diameter. Each stereocilium gave rise to only one tip link. These tip links were arranged in a direction parallel to the kinocilium (Fig. 1) (Takumida, 1989, Anniko & Takumida, 2001).
Function of the glycocalyx and ciliary interconnections

It has been known that the glycocalyx and ciliary interconnections are affected in some pathological conditions such as after treatment with ototoxic drugs. After intratympanic injection of gentamicin, the first detectable change was a general disarrangement of the cilia. The hair bundle did not show any signs of fusion or loss of cilia. However, the distance between the neighbouring cilia varied from near contact to widen. The distance between neighbouring stereocilia varied greatly from almost direct contact to a large distance. The interconnections, which connected the neighbouring ciliary shafts, were reduced in number and had completely vanished in some cases. The surface of the cilia showed a rather irregular appearance with small blebs and protrusions. The next phase in the degeneration process was a complete contact or fusion between neighbouring cilia. The decrease in or disappearance of the side links may indicate a general decrease in glycocalyx. This was suggested to induce plasma membrane contacts resulting in fusion of neighbouring sensory hairs. These findings altogether, support the fact that the glycocalyx (side links) acts to maintain the regular distance between the cilia thereby keeping the geometrical arrangement of the entire sensory hair bundle intact as well as to prevent close contact between neighbouring cilia (Takumida et al., 1989).

In order to clarify the function of tip links, morphological and functional relationships after the selective alteration of the ciliary interconnections have been investigated (Takumida et al., 1993). Elastase produced dramatic effects upon hair bundles. At low magnification, the arrangement of the hair bundle is quite normal. At the higher magnification, however, almost all tip links disappear and the tips of the stereocilia have lost their characteristic rounded shape and become conical, just as if the tip links have torn off and shrunk. The side links show less damage with slight elongation and fragmentation, and the surface of the stereocilia show a slight decrease in granularity. The action potentials fall remarkably over 50% and more marked in smaller stimuli (Fig. 2). These findings, that elastase caused severe damage to tip links resulting in a remarkable decrease in compound action potential, may support the idea that the tip links are actually involved in mechanoelectrical transduction (Takumida, et al., 1993).

Cupula

Above the epithelium of the crista, the cupula is located, extending from the epithelial cell surface to the roof of the ampulla. In order to observe the fine structure of the cupula, the special freeze cracking method have been utilized. By using this technique, it becomes to be possible to investigate the fine structures, which form the cupula, and is frequently shrunken during conventional preparation technique (Takumida, et al., 1992).

The cupula is divided into two parts. One is the cupula in the narrow sense and the other is the subcupular meshwork. The cupula in general consists of closely arranged filaments 40-60 nm in diameter cross-bridged by thinner filaments. It appears like a homogeneous matrix of consistently densely packed and cross-linked filaments. The filaments have a beaded appearance similar to the filaments of the subcupular meshwork. The subcupular meshwork consists of long branching filaments 50-70 nm in diameter. The filaments, in most cases, are directional oriented parallel to the main axis of the stereocilia. The filaments display a beaded appearance, cross-bridged to one another by thinner filaments, giving the impression of a network (Fig. 3). The subcupular meshwork filaments
are continuous with those of the cupula on one side and with the surface of the epithelium. This subcupular meshwork fills the space between the cupula and the surface. At the tip of the sensory hair bundle, the cilia are more tightly connected to the subcupular meshwork and in some cases; the top of the longer stereocilia or the kinocilium was directly inserted to the cupula and tightly connected to the densely arranged filaments of cupula (Fig. 3).

**Functional relationship between the sensory hairs and the cupula**

The highly cross-linked isotropic texture of the cupula indicates that this layer could function as a rigid plate and equally distributes the force of inertia by the large bulk of cupula to all sensory hair bundles. The cupula form a functional unit, which is coupled to the kinocilium and the long stereocilia. The cupula may present seismic masses that are spring-loaded to the kinocilium and some of the long stereocilia. The subcupular meshwork may function as a second kind of spring to dampen the system and help to isolate it from unwanted vibration. Shear stress due to relative acceleration of the cupula results in shear strain of the cupula. This energy is being transmitted to the sensory hairs directly or indirectly through the subcupular meshwork, which is continuous with the surface glycocalyx of the sensory hairs resulting in displacement of the stereocilia. Even if it only occurs in a few cilia in the early phase, all cilia will be displaced together as a bundle mediated by the action of the ciliary interconnections. The shearing force exerted on the cilia stretches the tip links may result in opening of the gated channels of the ciliary tip. The subcupular meshwork may play a role in the transmission of the shear strain force of the cupula to the ciliary bundle. It may also exert an additional damping effect in order to prevent unwanted vibrations (Fig. 4).

**References**


