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Localization and Ultrastructure of Mitochondria-Rich Cells in the Inner Ears of Goldfish and Tilapia

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Abstract The distribution and ultrastructure of mitochondria-rich cells in the inner ears of goldfish (*Carassius auratus*) and tilapia (*Oreochromis niloticus*) were examined by light and transmission electron microscopes. The mitochondria-rich cells were found in patches in the ampullae of three semicircular canals, sinus superior, utriculus and sacculus in both species. The cells were also found in the transverse canal in goldfish and the lagena in tilapia. They were absent in the goldfish lagena. The cells were characterized by a large number of mitochondria and an associated network of the smooth endoplasmic reticulum. Fenestrated capillaries were distributed in the connective tissue beneath the patches of the mitochondria-rich cells. The distribution and fine structure of these cells suggested their involvement in the ionic control of endolymph.

The morphology of the inner ear in teleosts has been studied by many investigators (Flöck, 1964; Hama, 1969; Lewis and Nemanic, 1972; Saito, 1973; Dale, 1976; Popper, 1976, 1977; Jenkins, 1977, 1979; Coombs and Popper, 1982; Platt, 1983; Popper and Platt, 1983; Saidel and Popper, 1983). However, most of these previous works have been carried out from a neuro-physiological point of view. Enger (1964) reported that endolymph in the teleost inner ear showed high potassium and low sodium concentrations compared with the perilymph in the cranial cavity. However, no morphological basis on the mechanism of ionic regulation of endolymph has been presented.

The author recently reported that a number of mitochondria-rich cells are localized in the saccular epithelium in tilapia (Saitoh and Yamada, 1989). In the present study, the localization and ultrastructure of mitochondria-rich cells in the teleostean labyrinth were examined with emphasis on a comparative aspect in ostariophysian (goldfish) and non-ostariophysian (tilapia) fishes.

Materials and methods

Juveniles of goldfish (*Carassius auratus*) and tilapia (*Oreochromis niloticus*), both approximately 20 mm in total length, were used. The animals were decapitated, and the heads were immersed immediately in a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4 (Karnovsky, 1965). After 30 min the heads were cut along the median line (tilapia) or trimmed (goldfish), and then fixed for another 5 h at room temparature. The head pieces were rinsed overnight in a cold buffer solution (0.1 M cacodylate buffer containing 7% sucrose, pH 7.4), and then decalcified in 10% EDTA, pH 7.4, for 3 days at 4°C. They were again rinsed overnight again in the cold buffer solution, then postfixed with cold 1% OsO_4 in 0.1 M cacodylate buffer, pH 7.4 (secondary fixative) for 1 h, and then dehydrated in ethanol. The specimens were embedded in Epon epoxy resin following the routine procedure (Luft, 1961). Thin sections were cut with glass knives using an ultramicrotome Porter-Blum MT-1, stained with uranyl acetate (Sjöstrand, 1967) and lead citrate (Reynolds, 1963), and then observed with a Hitachi H-300 electron microscope operated accelerating voltage at 75 kV. Semithin sections were also made, and stained with 1% toluidine blue for light microscope examination.

Observations

Gross anatomy of the membranous labyrinth. The three semicircular canals, the sinus superior and the utriculus in goldfish (Fig. 1A) and tilapia (Fig. 1B) are almost similar in gross structure. In goldfish, the utriculus, sacculus and lagena are connected with each other through broad openings, and the right and left sacculi are joined by a duct, the transverse canal. The sacculus takes a slender and elongated

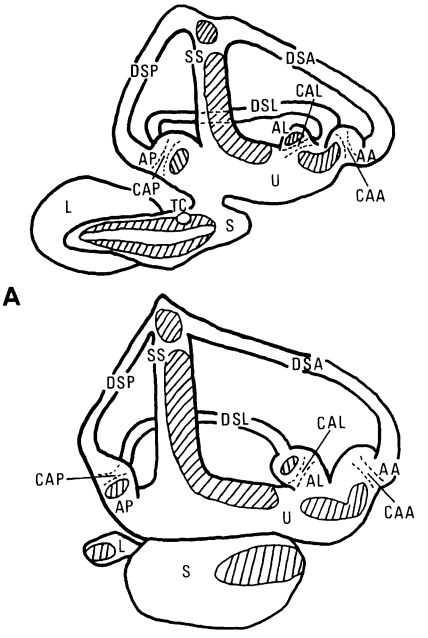

Fig. 1. Schematic representations of a median view of the left membranous labyrinth of goldfish (*Carassius auratus*) (A) and tilapia (*Oreochromis niloticus*) (B). The hatched areas indicate those occupied by mitochondria-rich cells. AA, ampulla anterior; AL, ampulla lateralis; AP, ampulla posterior; CAA, crista ampullaris anterior; CAL, crista ampullaris lateralis; CAP, crista ampullaris posterior; DSA, ductus semicircularis anterior; DSL, ductus semicircularis lateralis; DSP, ductus semicircularis posterior; L, lagena; S, sacculus; SS, sinus superior; TC, transverse canal; U, utriculus.

shape and the lagena is relatively large. In tilapia, on the other hand, the sacculus is a simple ovoid sac completely separated from the utriculus, and the small lagena is attached to the posterior end of the sacculus.

Localization of mitochondria-rich cells. In both goldfish and tilapia, mitochondria-rich cells are distributed in patches in the three semicircular canals, the sinus superior and the utriculus in a similar pattern (Fig. 1). Mitochondria-rich cells in the ampullae of all the semicircular canals are always found in the epithelium adjacent to the crista ampullaris (Fig. 2). In the anterior ampulla, the cells are located almost completely surrounding the portion of the opening to the utriculus. In the lateral ampulla, they are located on both the utricular and the canal sides of the crista in two groups; one extending to the utricular opening and the other forming an oval patch in the canal base. In the posterior ampulla, the cells are located only on the utricular side. In the middle part of the utriculus (Fig. 3), the mitochondria-rich cells are found along the lateral wall, independently of those in the anterior part. The lateral wall of the sinus superior is occupied by a large elongated patch of mitochondria-rich cells extending from the middle part of the utriculus to the upper part of the sinus superior. Separated from this large patch, a small patch is found on the top of the lateral wall of the sinus superior.

The distribution of the mitochondria-rich cells in the sacculus and the lagena in goldfish is different from that in tilapia. In goldfish, the cells can be seen in the transitional epithelium around the macula of the sacculus (Fig. 4) and on the basal wall of the trasverse canal which is continuous with the transitional epithelium of the sacculus (Fig. 5). The cells cannot be seen in the lagena. In tilapia, on the other hand, the mitochondria-rich cells are observed in the lateral wall of the sacculus. They are also observed in the lateral wall of the posterior part of the lagena in a pattern similar to that in the sacculus.

Ultrastructure of mitochondria-rich cells. The most conspicuous cytological characteristics of the mitochondria-rich cells are the presence of abundant mitochondria and dilated cisternae of the smooth endoplasmic reticulum. The cells in both goldfish and tilapia are basically similar in ultrastructural features.

In the ampullae they are columnar, about 10 μm in height, and have an oval nucleus (Fig. 6). Some vacuoles are found in the cytoplasm. Medullated fibers run through the connective tissue beneath the cells. Epithelial cells have electron-dense cytoplasm with poorly developed cell organells; they are often seen between the mitochondria-rich cells.

Mitochondria-rich cells in the utriculus and the sinus superior often show their nuclei located in the apical portion of the cells. The cell surface is relatively smooth, but occasionally it is equipped with poorly developed microvilli, and the cell boundaries are generally indistinct (Fig. 7). Some of the cells, however, reveal indented cell boundaries with a few desmosomes in the apical and the basal regions (Fig. 8). Remarkably, dilated cisternae of the smooth endoplasmic reticulum are seen in the cytoplasm. Numerous glycogen granules are sometimes detected

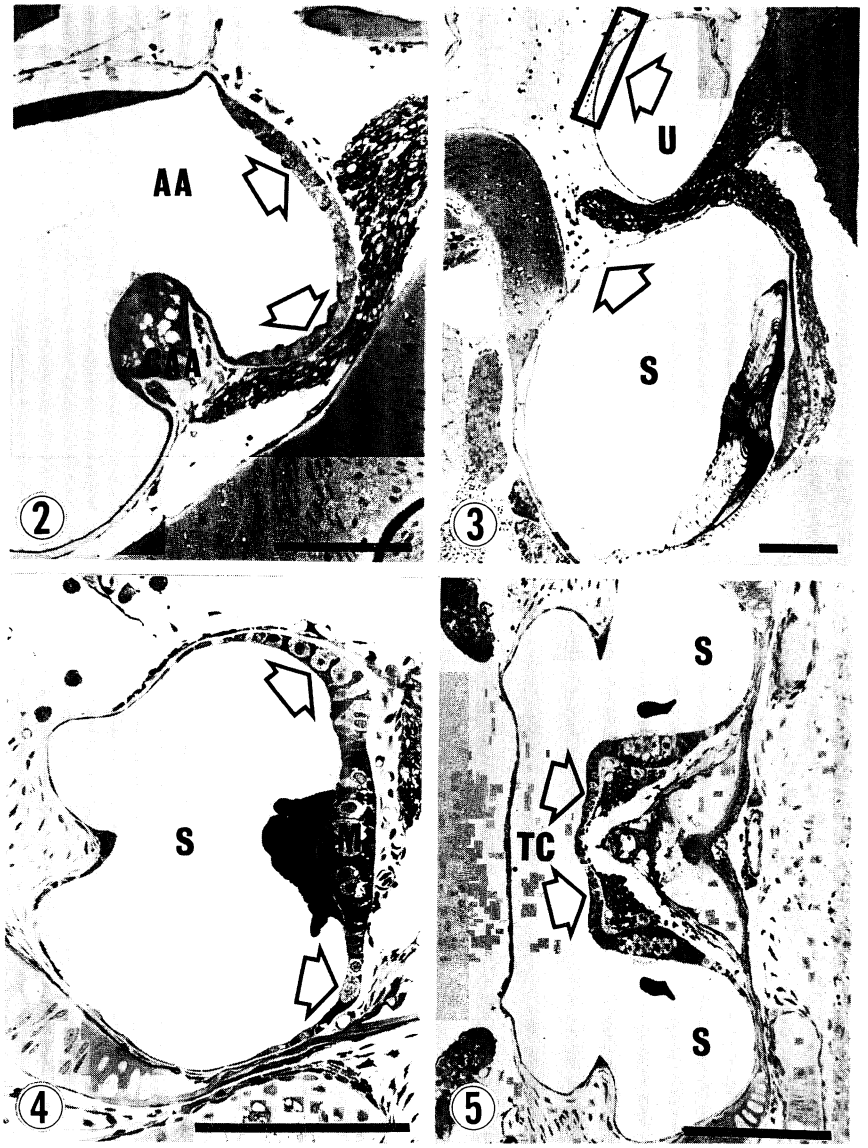

Fig. 2. Light micrograph of the ampulla anterior (AA) of tilapia. Note mitochondria-rich cells (arrows) located near the crista ampullaris anterior (CAA). Bar=100 μm.

Fig. 3 Light micrograph of the sacculus (S) and the utriculus (U) of tilapia. Mitochondria-rich cells (arrows) are seen in the lateral walls of the sacculus and the utriculus. A higher magnification of the encased area is shown in Fig. 7. Bar=100 μm.

Fig. 4. Light micrograph of the middle part of the sacculus (S) of goldfish. Mitochondria-rich cells (arrows) are seen in the transitional epithelium of the macula (M). Bar=50 μm.

Fig. 5. Light micrograph of a section through the right and left sacculi (S) and the transverse canal (TC) of goldfish. Note mitochondria-rich cells (arrows) in the basal wall of the transverse canal. Bar=50 μm.

in clustered or scattered among mitochondria (Fig. 9). Capillaries with the fenestrated endothelia are noticed in the connective tissue beneath the mitochondria-rich cells (Fig. 10).

In the sacculus and the lagena of tilapia, the mitochondria-rich cells are basically similar in ultrastructural features to those in the utriculus and the sinus superior. They are generally squamous or

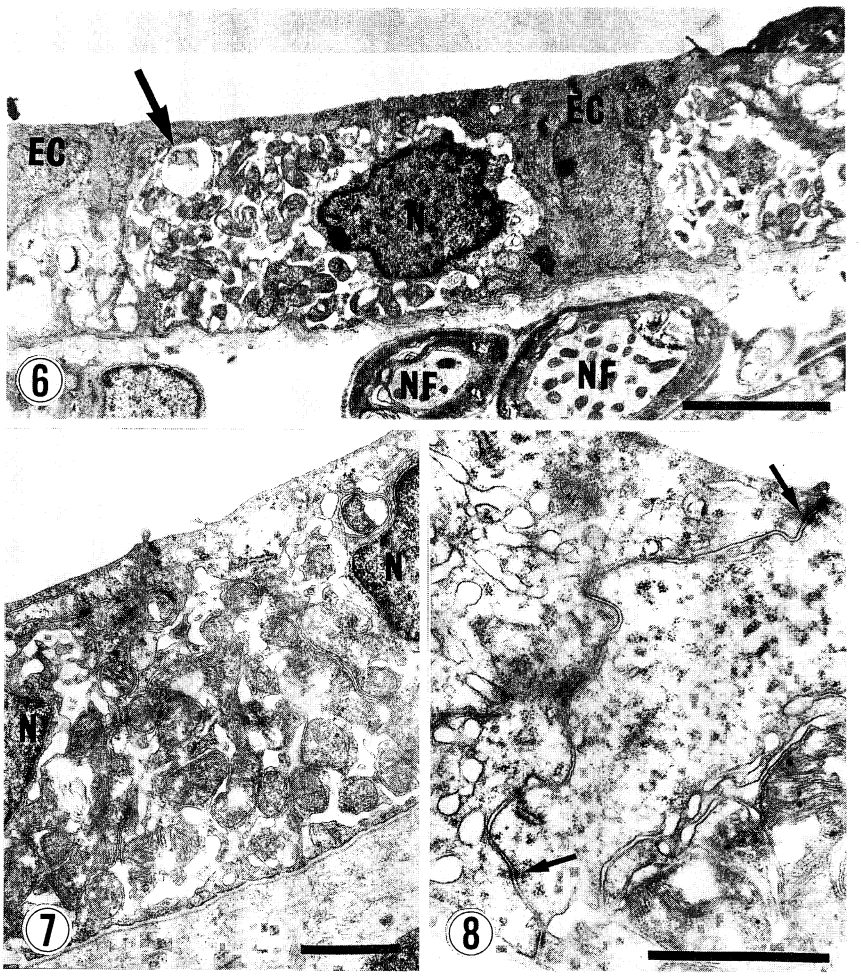

Fig. 6. Transmission electron micrograph (TEM) of mitochondria-rich cells in the ampulla anterior of tilapia. Note large vacuoles (arrow) and invaginated nucleus (N) in the cytoplasm. Medullated nerve fibers (NF) are seen in the connective tissue. EC, epithelial cell. Bar=5μm.

Fig. 7. TEM of two mitochondria-rich cells in the middle part of the utriculus of tilapia. The cell boundary is not evident. N, nucleus. Bar=1μm.

Fig. 8. TEM of mitochondria-rich cells in the middle part of the utriculus of tilapia. Desmosomes (arrows) are apparent at the apico-lateral and baso-lateral regions. Dilated smooth endoplasmic reticulums are seen near the cell boundary. Bar=1μm.

cuboidal, 3 to 5μm in height, as are other epithelial cells.

The mitochondria-rich cells in the sacculus (Fig. 11) and the transverse canal (Fig. 12) of goldfish are similar to those in the ampullae in size and shape, but their cell surfaces are provided with many microvilli, and the indentations of the cell boundaries between them are less complicated.

Discussion

The present study revealed for the first time the distribution and ultrastructure of mitochondria-rich cells in the teleost inner ear. They are possibly involved in the active transport of inorganic components of the inner ear fluids (endolymph) considering their characteristic morphology. Similar ion transporting cells have been found in the kidney (Rhodin, 1962), salt secreting glands (Bulger, 1963),

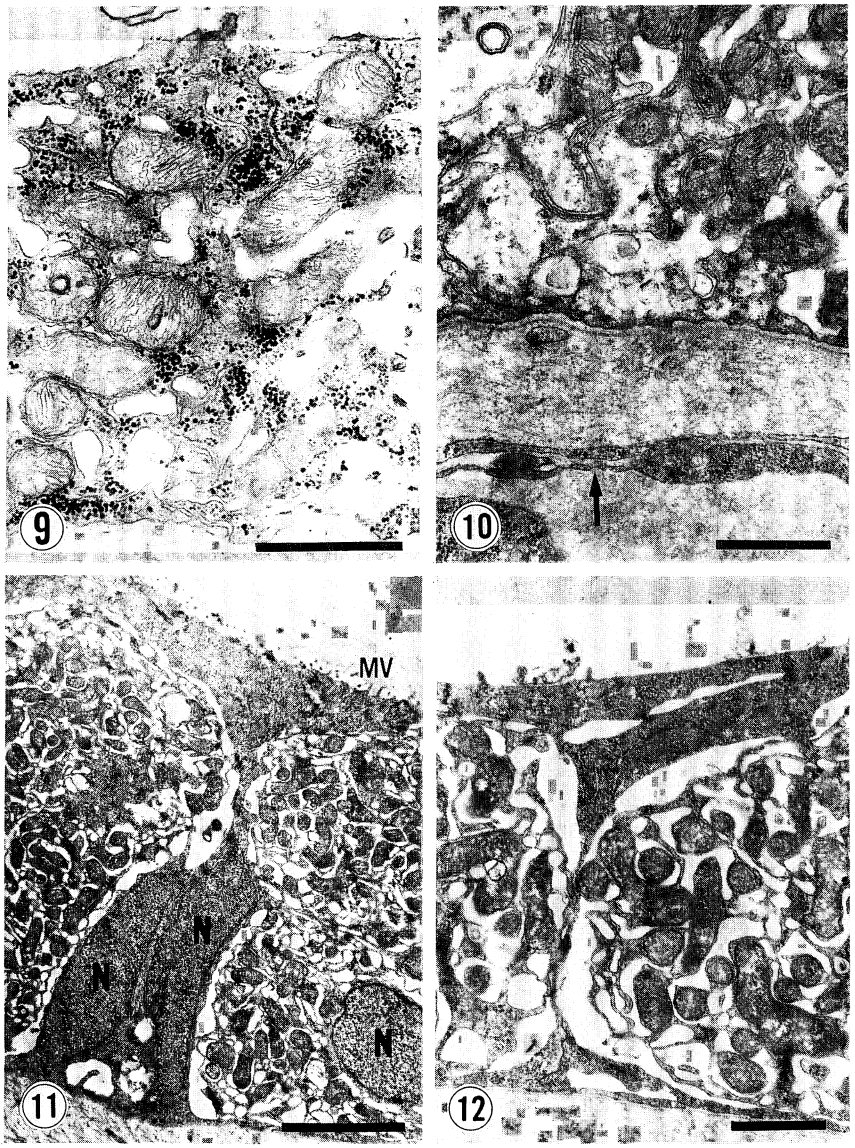

Fig. 9. Transmission electron micrograph (TEM) of a mitochondria-rich cell in the middle part of the utriculus of tilapia. Numerous glycogen granules are seen in the cyotoplasm. Bar=1 μm.

Fig. 10. TEM of a mitochondria-rich cell in the middle part of the utriculus of tilapia. Fenestrated endothelium (arrow) of a capillary is seen in the connective tissue. Bar=1 μm.

Fig. 11. TEM of mitochondria-rich cells and intervening epithelial cells in the perimacular epithelium of the sacculus of goldfish. Note many microvilli (MV) on the epithelial cell surface. N, nucleus. Bar=5 μm.

Fig. 12. TEM of mitochondria-rich cells in the transverse canal of goldfish. A few microvilli are seen on the cell surface. Bar=1 μm.

ciliary body of the eye (Tormey, 1963), choroid plexus (Pease, 1956), and gills (Copeland, 1948) in various animals.

In the mammalian inner ear it is generally acknowledged that the stria vascularis of the cochlea has functional importance in the production of endolymph (Smith, 1957; Yamamoto and Nakai, 1964; Johnson and Spoendlin, 1966; Nakai, 1969; Reale et al., 1975). On the other hand, not only the stria vascularis but also the "dark cells" of the utriculus

and ampullae have been assumed to be engaged in the production of endolymph (Dohlman and Ormerod, 1960; Lundquist et al., 1963; Dohlman, 1964; Kimura et al., 1964; Hamilton, 1965; Nakai and Hilding, 1968; Kimura, 1969; Kawamata et al., 1985). The dark cells are characterized by the presence of numerous microvilli, concentration of many mitochondria, many short cisternae of the endoplasmic reticulum, a developed vacuolar system and extensive intercellular and basal plasma membrane infoldings. A network of abundant capillaries with fenestrated endothelium extends beneath the cells. These characteristic features of the dark cells are consistent with those of the mitochondria-rich cells in the goldfish and tilapia, although infoldings of the basal plasma membrane were scarcely observed.

High potassium and low sodium concentrations of endolymph have generally been observed in the cochlea of higher vertebrates. The sensitivity of receptor cells have been considered to depend on this ionic situation (Tasaki, 1960). Enger (1964) also reported that the potassium concentration is much higher than the sodium concentration in teleostean saccular endolymph, and therefore, the endolymph has a nature of intracellular fluid. Maintenance of the potassium and sodium concentrations of endolymph can be attributed to ion transport activities of the mitochondria-rich cells. Fenestrae of the endothelium of the capillaries may be a feature responsible for the fluid transport (Rhodin, 1962).

The distribution of mitochondria-rich cells in the ampullae, the sinus superior and the utriculus was similar in both goldfish (ostariophysian) and tilapia (non-ostariophysian), and also to that of dark cells in those utricular organs in mammals (Kimura, 1969). However, their distribution in the sacculus and lagena was different between the two species according to the structural difference of the labyrinth. In mammals, dark cells are absent in the sacculus and the cochlea (Kimura, 1969). It is of interest to note that the utriculus and the sacculus are completely separated sacs in tilapia, while those in mammals are joined by a duct called ductus utriculosaccularis (Bloom and Fawcett, 1975) through which endolymph can flow freely. In goldfish, mitochondria-rich cells were not found in the lagena, which was connected with the sacculus by a broad opening, a similar situation between the utriculus and the sacculus in mammals.

In goldfish, mitochondria-rich cells were found in the basal wall of the transverse canal and the perimacular transitional epithelium of the sacculus. The sacculus of tilapia lacks the tansverse canal, and the cells were observed at the lateral wall of the sacculus and lagena. This different distribution of mitochondria-rich cells may be attributed to the structural difference of the labyrinth between ostariophysian and non-ostariophysian fishes.

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キンギョとティラピアの内耳におけるミトコンドリアに富む細胞の分布と微細構造

齊藤節雄

キンギョとティラピアの内耳におけるミトコンドリアに富む細胞の分布と微細構造を，光学顕微鏡と電子顕微鏡で観察した．この細胞は両種ともに，三半規管の膨大部，通のう及び小のうに密集して存在していた．また，キンギョの横行管及びティラピアの壺においても認められた．しかし，キンギョの壺には観察されなかった．微細構造上の特徴としては，細胞質は核以外ほとんどがミトコンドリアとそれを取り巻く滑面小胞体で占められ，細胞基底部の下の結合組織中には内皮に小孔を有する毛細血管が多数存在していた．これらの形態的特徴から，ミトコンドリアに富む細胞は，おそらく内リンパ液のイオン調節に関与していると考えられる．

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