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ULTRASTRUCTURAL STUDY ON MULTICELLULAR COMPLEX OF CHLORIDE CELLS IN TELEOSTS

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Pung-Pung Hwang (1988) Ultrastructural study on multicellular complex of chloride cells in teleosts *Bull. Inst. Zool., Academia Sinica* 27(4): 225-233. Ultrastructure of chloride cells in branchial region of teleosts, flounder (*Kareius bicoloratus*), ayu (*Plecoglossus altivelis*), carp (*Cyprinus carpio*), and tilapia (*Oreochromis mossambicus*) was studied. The chloride cell has an apical membrane in contact directly with the outer medium. Generally, two or more neighboring chloride cells share an apical pit, forming a multicellular complex. The chloride cells in a multicellular complex, differ in their electron density of cytoplasm, development of tubular system and cell size. According to the depth of junctions at the apices of chloride cells, there are three types of zonular junction, i. e., tight junction, shallow junction and leaky junction. Tight junctions occur between neighboring pavement cells or neighboring pavement and chloride cells in both freshwater- and seawater- adapted fish. Chloride cells in freshwater-adapted fish link each other with shallow junctions, whereas chloride cells in seawater- adapted fish form intercellular digitations and leaky junctions. Muticellular complexes allow chloride cells to increase additional paracellular pathways with the shallow and leaky junctions. This junctional difference of multicellular complexes of chloride cells between freshwater- and seawater-adapted fish, may be related to the different permeability of ions in the branchial epithelia of the two groups of fishes.

Key words: Ultrastructure, Multicellular complex, Chloride cells, Teleosts.

Since the pioneering work of Keys and Willmer (1932) on the morphology of the gill of seawater eel, chloride cells have been shown to have an important role in the osmoregulation of teleosts. Many aspects have been concerning about the size and number of chloride cells, ultrastructure of mitochondria and tubular system, cytochemistry and enzyme activity of Na-K-ATPase in chloride cells, and the changes in these parameters in chloride cells upon seawater adaptation (Shirai and Utida, 1970; Karnaky *et al.*, 1976; Thomson and Sargent, 1977; Hootman and Philpott, 1979; Foskett *et al.*, 1981).

Recently, it has been reported that following seawater adaptation, "accessory cells" (possibly, an immature chloride cell) occur beside chloride cells, forming a multicellular complex in the gill, skin and operculum of teleosts (Karnaky and Kinter, 1977; Hootman and Philpott, 1979; Sardet *et al.*, 1979; Foskett *et al.*, 1981; Hwang and Hirano, 1985; Hwang, 1987). Moreover, it has been implied that neither accessory cell nor the

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multicellular complex developed in those of freshwater-adapted fish, i. e., each chloride cell is surrounded by pavement cells, and never directly joins with other chloride cells (Sardet *et al.*, 1979; Karnaky, 1980; Laurent, 1984).

However, in our previous studies, multicellular complexes of chloride cells also found in the gills or skin of several freshwater-adapted fishes (Hwang and Hirano, 1985; Hwang, 1987; Hwang, 1988). In the present work, four species of teleosts which were acclimated to freshwater or seawater were used to examine the ultrastructural differences of the chloride cells in gills, skin or operculum, and to discuss their functional significances.

MATERIALS AND METHODS

Flounder (*Kareius bicoloratus*), ayu (*Plecoglossus altivelis*), carp (*Cyprinus carpio*), and tilapia (*Oreochromis mossambicus*) were used in this study. One-day-old larval flounder which hatched from the fertilized eggs in seawater (33‰ salinity) were used. Young flounder were reared in seawater for 60 days or in freshwater for 6 days. Both young ayu (3.7-6.2 cm in total length) and tilapia (3-8 cm in total length) were reared in freshwater or seawater for over 3-4 months. Young carp (2.6-3.6 cm in total length) were reared in freshwater for about 60 days.

Fish were anesthetized with MS222. Gill arches and operculm were excised (or whole larva was treated) and immersion-fixed with mixture of 4% formaldehyde and 5% gluteraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 2 h. After a brief rinse in the buffer solutions, specimens were post-fixed in 1% osmium tetroxide-phosphate buffer (pH 7.4) at 4°C for 1.5 h, dehydrated through a series of graded acetone or ethanol and embedded either in Epon 812 or in Spurr resin. Ultra-thin sections were made by SORVALL MT-1 or REICHERT-JUNG ULTRACUT E ultramicrotome with glass knives, mounted on formvar-coated copper grids, and double stained with 5-15% uranyl acetate and lead citrate. The sections were observed with JEOL JEM-100S or ZEISS EM -109 electron microscope.

RESULTS

Basic morphology of chloride cells

In the gills, operculum and skin of the teleosts adapted to seawater or freshwater, chloride cells showed the same morphological characteristics (Figs. 1-8). Chloride cell had an apical membrane exposing to the outer medium and its basal membrane directly contacted with the basal lamina. Mitochondria varied from round or ovid to elongated in shapes were numerous and distributed throughout the cytoplasm. Well-branched tubular system was formed ramifying throughout the cytoplasm except the apical region. However, the cristae in mitochondria and the tubular system of chloride cells were more developed in seawater-adapted (Figs. 5-8) fish than in freshwater-adapted fish (Fig. 1-4).

Multicellular complex of chloride cells

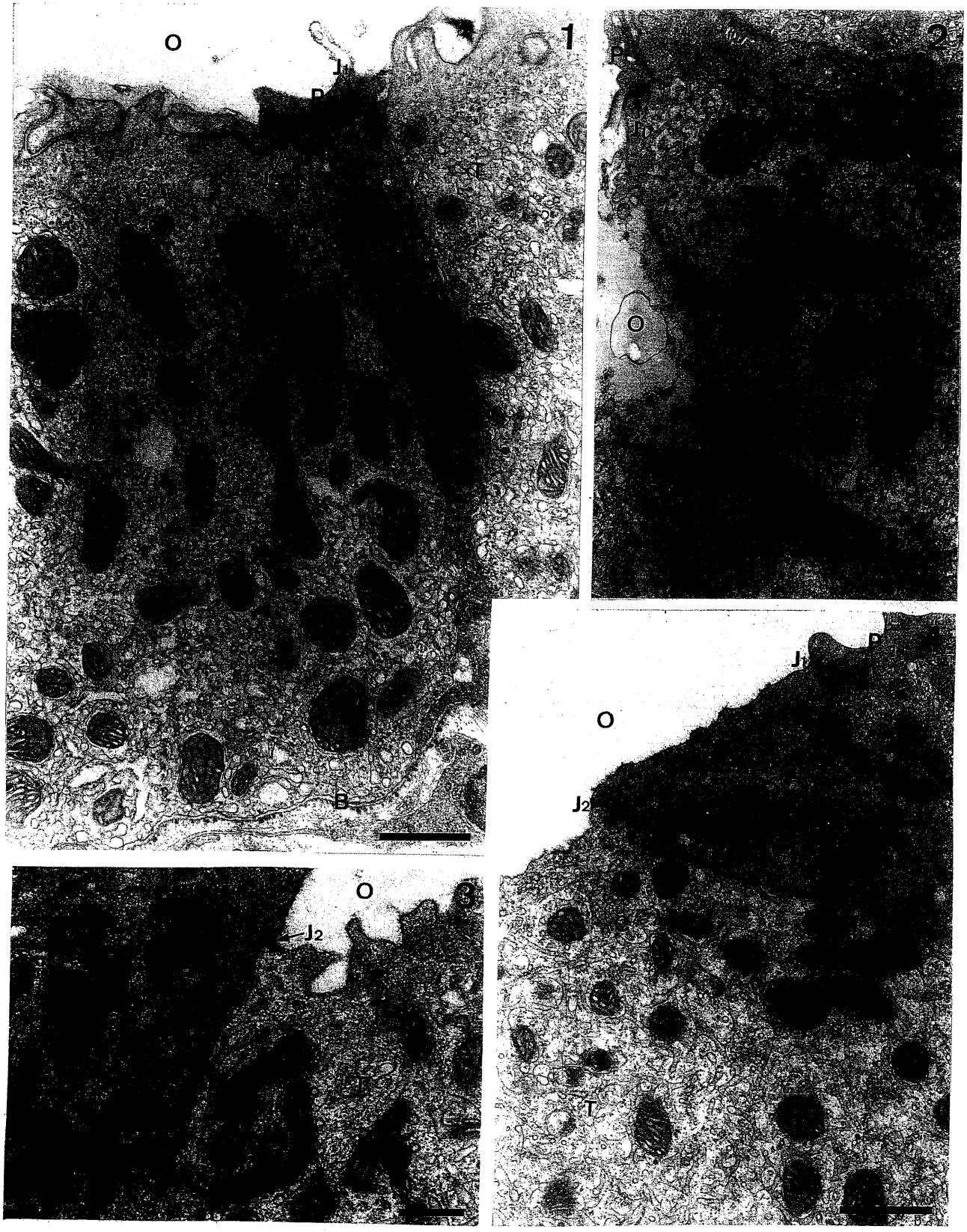
In both freshwater and seawater-adapted

Fig. 1. Ultrastructure of the multicellular complexes of chloride cells (C) in the gill of (*Kareius bicoloratus*) acclimated to freshwater for 6 days. B, basal, lamina; J1, tight junction; J2 shallow junction; M, mitochondria; O, outer medium; P, pavement cell; T, tubular system. (Abbreviations are the same in the following Figures). Scale=1 μm.

Fig. 2. Ultrastructure of the multicellular complexes of chloride cells in the gill of freshwater-adapted ayu (*Plecoglossus altivelis*). Scale=1 μm.

Fig. 3. Ultrastructure of the multicellular complexes of chloride cells in the gill of freshwater-adapted tilapia (*Oreochromis mossambicus*). Scale=0.5 μm.

Fig. 4. Ultrastructure of the multicellular complexes of chloride cells in the gill of freshwater carp (*Cyprinus carpio*). Scale=1 μm.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teleosts, two or more chloride cells frequently neighbored with each other and shared an apical pit, i. e., forming a multicellular complex of chloride cells (Figs. 1-8). Within a multicellular complex, these chloride cells were variable in their ultrastructure, particularly in the electron density of cytoplasm. Most of them revealed a cytoplasm of electron lucent, but some smaller cells more electron opacity in the cytoplasm and an expansion in the diameter of the tubular system (Figs. 1-8).

In the chloride cells of multicellular complex and their neighboring pavement cells, there are three types of zonular junctions, i. e., tight (200-500 nm in depth in case of tilapia), shallow (70-300 nm in depth) and leaky (20-40 nm in depth) junctions (Figs. 1-12). Tight junctions occured between pavement and pavement cell (Figs. 9 and 11) or between pavement and chloride cell (Figs. 1, 2, 4-6, and 9-12) in both seawater- and freshwatr-adapted fish. The chloride cells of a multicellular complex in the seawater-adapted fish extended numerous cytoplasmic processes (so-called intercellular digitation) into the apical region of neighboring chloride cells (Figs. 5-10). This character created many additional contacts beween extracellular space and outer medium via leaky junction. On the other hand, the chloride cells of a multicellular complex in the freshwater-adapted fish did not form any intercellular digitation (Figs. 1-4). Moreover, the depth of the zonular junctions between neighboring chloride cells was considerable variabe but its depth was still within a range between tight and leaky junctions (Figs. 1-4, 11 and 12).

DISCUSSION

It had been accepted that multicellular complex of chloride cells only occured in seawater-adapted fish (see introduction). However, the present results indicate that this structure is generally found not only in seawater-adapted fish but also in freshwater-adapted one. In fact, reexamining several previously published electron micrographs, it is evident that the multicellular complex of chloride cells usually appeared in the gills of freshwater fish, but was overlooked (Fig. 1 of Threadgold and Houston, 1964; Figs. 2 and 16 of Shirai and Utida, 1970; Figs. 9 and 12 of Kikuchi, 1977; Figs. 1 and 2 of Korte, 1979; Fig. 9 of Laurent and Dunel, 1980).

The most obvious differences in the ultrastructure of chloride cells between seawater- and freshwater-adapted fishes are intercellular organization and junctional structure, and these appear to reflect the different functions of the chloride cells in seawater or freshwater environment.

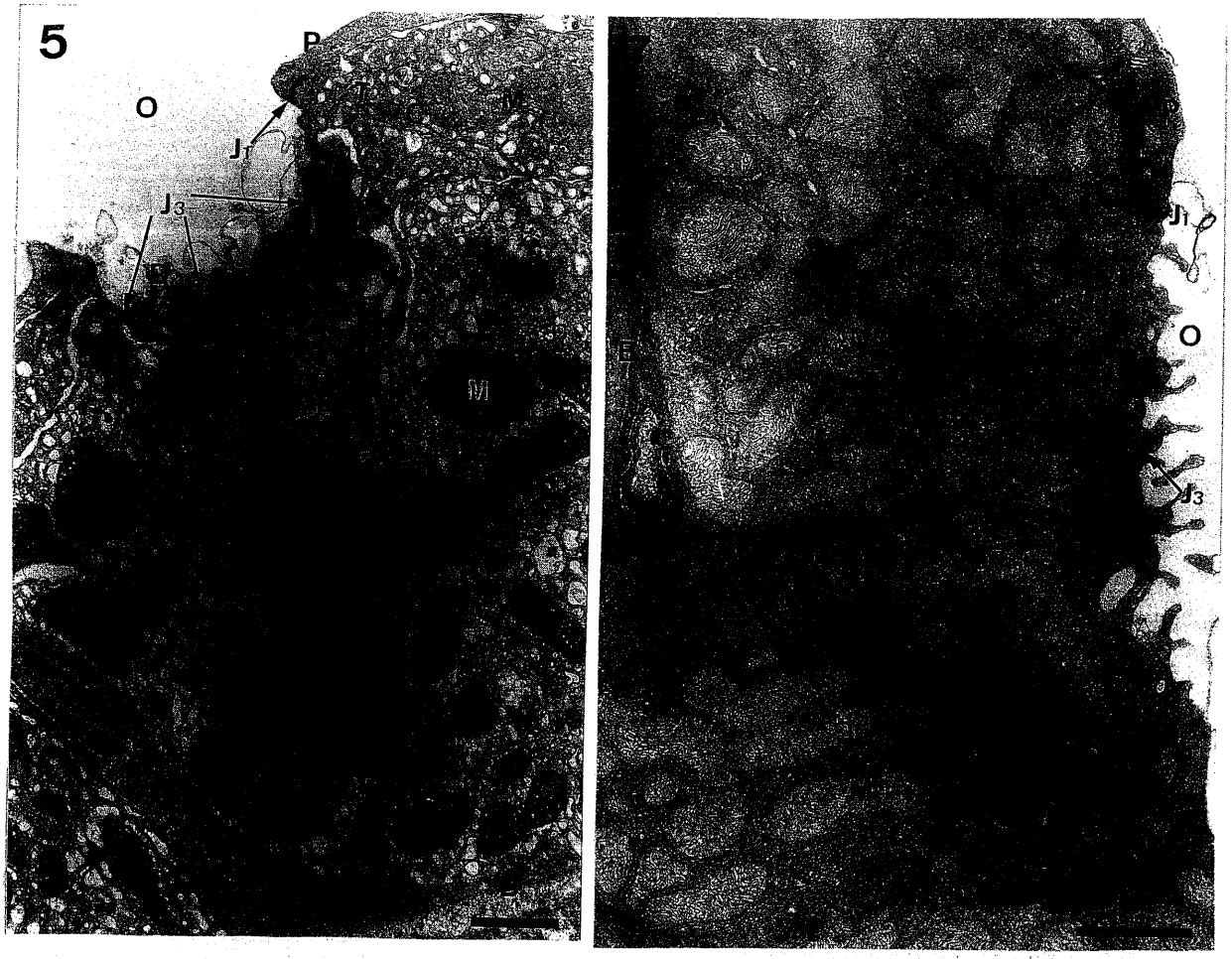
The positive correlation between the number of junctional strands and ionic permeability has been well established in many simple epithelia of mammalia and amphibia (Claude and Goodenough, 1973; Claude, 1978). In the case of branchial and opercular epithelia of the seawater-adapted teleost (Sardet *et al.*, 1979; Ernst *et al.*, 1980), tight junctions shared by neighboring pavement cells or neighboring pavement and chloride cells are five or more strands extending 300 to 500 nm in depth. On the other hand, the leaky

Fig. 5. Ultrastructure of the multicellular complexes of chloride cells (C) in the operculum of seawater-adapted flounder (*Kareius bicoloratus*). B, basal lamina; D, intercellular digitation; J1, tight junction; J3, leaky junction; M, mitochondria; O, outer medium; P, pavement cell; T, tubular system. Scale=1 μm.

Fig. 6. Ultrastructure of the multicellular complexes of chloride cells in the gill of seawater-adapted flounder (*Kareius bicoloratus*). Scale=1 μm.

Fig. 7. Ultrastructure of the multicellular complexes of chloride cells in the gill of seawater-adapted ayu (*Plecoglossus altivelis*). Scale=2 μm.

Fig. 8. Ultrastructure of the multicellular complexes of chloride cells in the gill of seawater-adapted tilapia (*Oreochromis mossambicus*). Scale=2 μm.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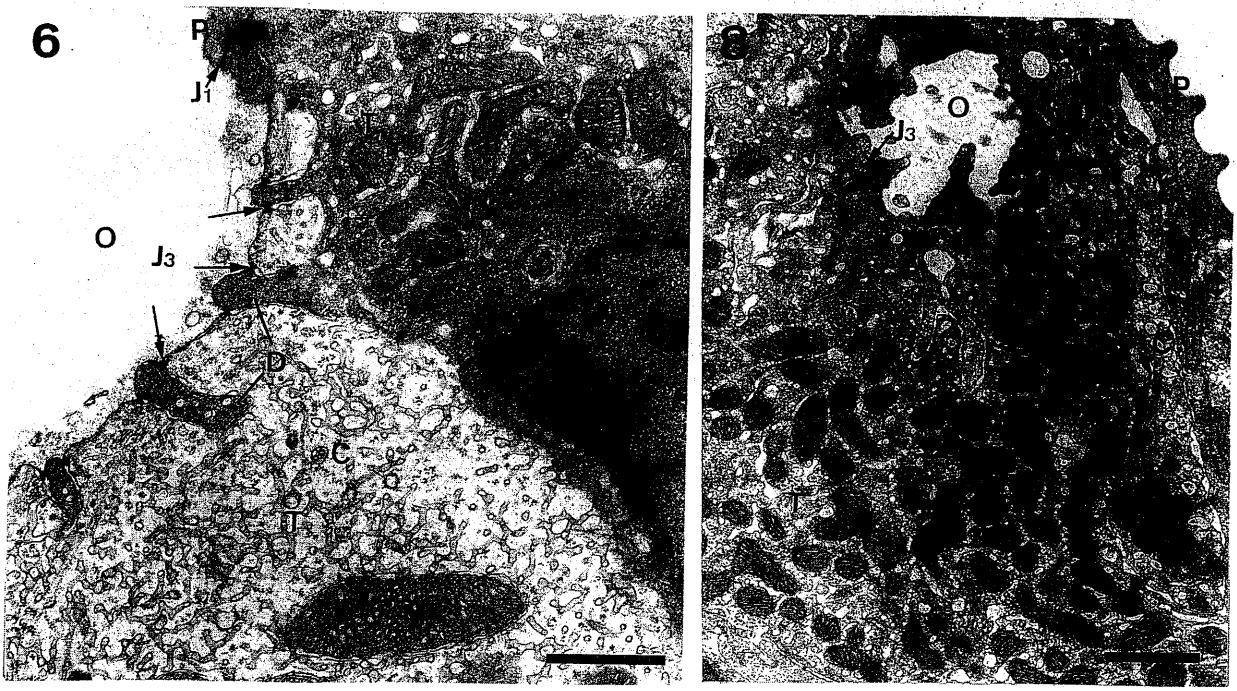
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junctions between adjacent chloride cells (and thus interdigitations) are 1 to 2 strands with a depth of 20 to 25 nm. That the junctions between pavement cells are much more electrically tighter than the apical pit of chloride cells in opercular membrane of seawater-adapted tilapia is supported by conductance measurements of the membrane with a vibrating probe (Foskett and Scheffey, 1982). Furthermore, development of leaky zonula occludens in seawater-adapted branchial epithelia may provide the morphological support for the current model, i. e., sodium paracellular permeation in NaCl secretion of chloride cells (Sardet *et al.*, 1979; Ernst *et al.*, 1980; Hwang and Hirano, 1985; Hwang, 1987; present study).

While in freshwater-adapted fish, recent studies have indicated the possible role of chloride cells in the uptake of sodium and calcium (Payan *et al.*, 1981; Avella *et al.*, 1987; Flik *et al.*, 1985; Perry and Wood, 1985). Multicellular complexes allow chloride cells of freshwater-adapted to adjoin with each other and thus to increase the number of paracellular pathways posscessing zonular junctions which are shallower than the tight junctions. These structures may increase the leakiness of the epithelia to some extent, and thus may be associated with the ionic uptake in freshwater-adapted fish (Hwang, 1988).

Leaky junctions occured in seawater-adapted chloride cells are much shallower than shallow junctions occured in freshwater-adapted chloride cells. Moreover, interdigitations which only developed in seawater-adapted chloride cells, increase the number of leaky junctions, i. e., much more leakiness of the epithelia. Therefore, it can be inferred that the great difference in ion turnover rates between freshwater- and seawater-adapted epithelia are associated with these ultrastructural differences (Sardet *et al.*, 1979; Hwand and Hirano, 1985; Hwang, 1987).

In multicellular complexes of both seawater-and freshwater-adapted fishes, some smaller chloride cells which show more electron opacity in the cytoplasm and an expansion in the diameter of the tubular system, are resemble to "acessory cells" described previously (Karanky and Kinter, 1977; Hootman and Philpott, 1979; Hwang and Hirano, 1985; Hwang, 1987; Hwang, 1988). Whether these cells are identical to acessory cells or in some physiological relation with acessory cells, more studies such as cytochemical examination of Na-K-ATPase in these cells are necessary, since this enzyme has been identified to localize in chloride cell but not in acessory cell (Hootman and Philpott, 1979).

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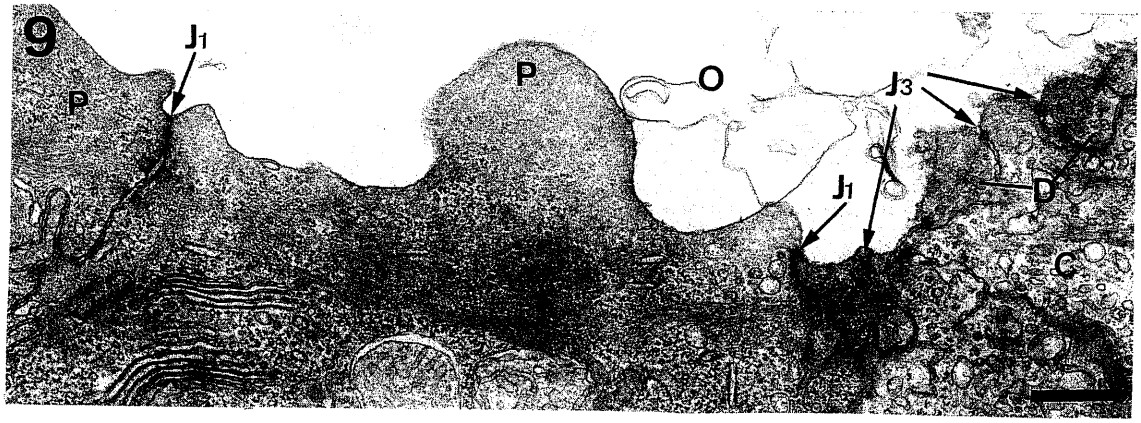
Fig. 9. Junctional structure of neighboring chloride cells (C) and pavement cells (P) in the skin of larval flounder (*Kareius bicoloratus*) hatched in seawater. D'intercellular digitation; J1, tight junction; J3, leaky junction; O, outer medium. Scale=0.5 μm.

Fig. 10. Junctional structure of neighboring chloride cells and pavement cells in the gill of seawater-adapted ayu (*Plecoglossus altivelis*). Scale=0.5 μm.

Fig. 11. Junctional structure of neighboring chloride cells and pavement cells in the gill of freshwater-adapted ayu (*Plecoglossus altivelis*). J2, shallow junction. Scale=0.5 μm.

Fig. 12. Junctional structure of neighboring chloride cells and pavement cells in the gill of freshwater-adapted tilapia (*Oreochromis mossambicus*). Scale=0.5 μm.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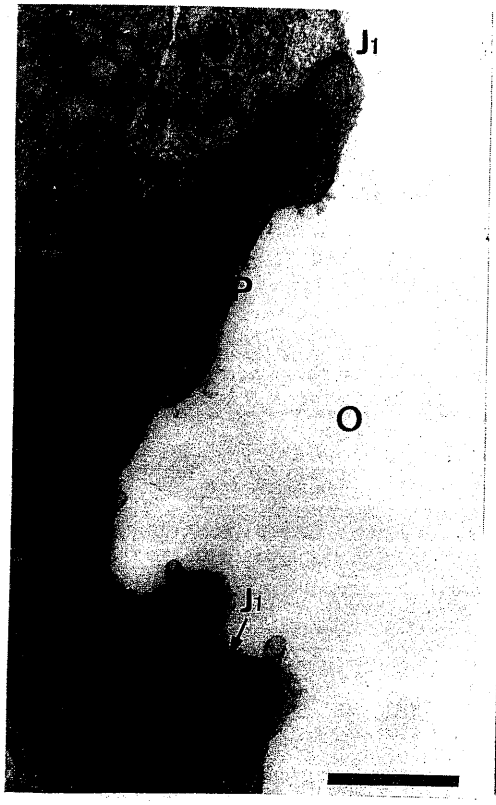
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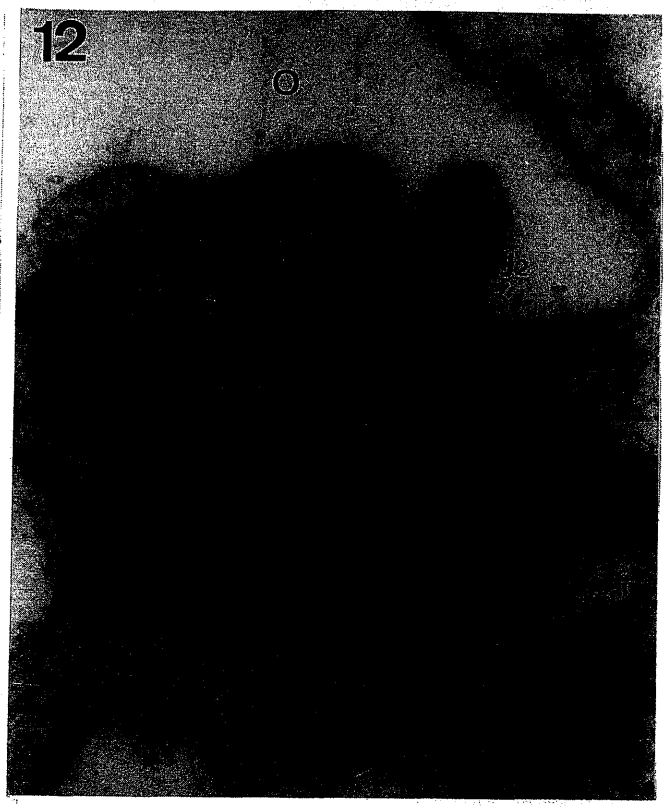
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眞骨魚鹽類細胞多細胞組合微細構造之研究

黃 鵬 鵬

本報告研究，鰈魚、香魚、鯉魚及吳郭魚鰓部鹽類細胞的微細構造。鹽類細胞頂部開口於外界；通常，兩個或兩個以上鹽類細胞相鄰，並共用一個頂部開口，形成所謂多細胞組合；多細胞組合之鹽類細胞，顯示不同之細胞質電子密度、管狀系統發達程度及細胞大小。根據細胞結合之深度，多細胞組合內有三種結合：緊密結合、淺結合及疏漏結合。在海水魚和淡水魚，相鄰之表皮細胞，或者相鄰之表皮及鹽類細胞之間，形成緊密結合。淺結合出現於淡水魚相鄰之鹽類細胞之間。而海水魚相鄰鹽類細胞之間則形成疏漏結合。多細胞組合造成鹽類細胞得以相鄰，並增加淺結合或疏漏結合之細胞間通路。因爲細胞間結合構造影響上皮的通透性，鹽類細胞多細胞組合的微細構造之差異，可能與海水魚及淡水魚鰓上皮某些物質通透性之不同有關。