

Structure of Chloride Cell in *Telaji* (Cyprinidae, Teleost) of Caspian Sea

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Abstract: The gills are an important organ in fishes, with specific functions and is highly sensitive to many factors. Chloride cell in gill's epithelia has the main role in these functions. These cells exhibit the features of ion's transporting cells. An ultra structural study was performed on chloride cells of euryhaline *telaji* in south of Caspian Sea. The chloride cells are distributed in the interlamellar region of filaments. They are oval to elongated form with an apical positioned nucleus, expanded tubular system and heteromorphic mitochondria. These cells are surrounded by pavement cell and accessory cell. A small depressed surface formed by pavement cells and chloride cells that are in contact with the aquatic milieu. There are also channel systems in accessory cells that are in continuity with intercellular area. There is only one type of chloride cell in *telaji*, that's resembled to B-cell. This feature and also the broad loose network of tubular system seem to chloride cell of freshwater species.

Key words: Gill • Filament • Chloride cell • *Telaji*

INTRODUCTION

The *telaji* (in Mazandarani), belongs to cyprinidae family is one of the most important endemic species of fishes in south of Caspian Sea.

This species is eurytopic, living in rivers, streams, lakes, reservoirs and fresher parts of seas. Because of the variability in structure of tissues among different fish species [1], clarification of the normal histology of a particular species is important to interpret the morphological and functional modifications occurring under pathological conditions. The gills are exposed to direct contact with pathogenic agents that can enter the aquatic milieu as a result of natural occurrence or anthropic action [2] and caused by ventilatory movements, a particularly high water volume bypasses the large gill surface. Therefore, the gills are one of the most frequently affected organs under these circumstances [3,4]. Since relevant physiological processes take place in the gills, such as gas exchange, osmoregulation, excretion of nitrogenous waste products and acid-base balance, disturbance of the structure of this

organ compromises the survival of fish. In addition, the gills represent an appropriate model for the study of the potentiation environment effects on the fish organism and, indirectly an indicator of degree of environmental contamination.

The aim of the present study was to study using transmission microscopy, the structure of chloride cells of gill epithelia in R.r.Caspicus under seawater conditions in south of Caspian Sea to a salinity of 12/48-12/64 gr/lit.

MATERIALS AND METHODS

Six specimens of *rutilus rutilus caspicus* ranging in body mass from 800-1500gr were caught from south of Caspian Sea. The gill arches were dissected out and fixed by immersion in 2% phosphate-buffered glutaraldehyde for 24h. After rinsing with 0/1 M PB, the specimens were postfixed with 1% osmium tetroxide in 0/2 M PB for 1h. After rinsing with PB and dehydration with ethanol and acetone, the specimens were infiltrated and embedded in resin. Ultrathin sections were cut with glass or diamond knives in an ultra microtome.

Sections were mounted on grids, double stained in Uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM, LEO).

RESULTS

The chloride cells were most frequently observed in interlamellar regions of the filament and at the base of the lamellae.

These cells are oval to elongated in shape and shown an elliptical euchromatic nucleus, apical positioned and an abundant cytoplasm. They are surrounded by the pavement cells and accessory cells (Fig. 1). The apical plasma membrane of CL is covered by lateral cytoplasmic projections of adjacent pavement cell. So a small area of the cell is exposed to the aquatic milieu (Fig. 2). The most

important feature of chloride cells is the great abundance of mitochondria and intracellular tubular system. The profile of mitochondria varied from round or ovoid to elongate with electro-dense matrix and abundant tubular cristae (Fig. 2, 3). The extensive tubular system consisting of a network of tubules connected with basolateral plasma membrane and also mitochondria (Fig. 3). An accessory cell is also next to chloride cell, and the two cells share a shallow junction. The most distinctive feature of AC is the presence of some widened channels. These channels which are originated from the plasma membrane appeared to be in continuity with intercellular area (Fig. 4).

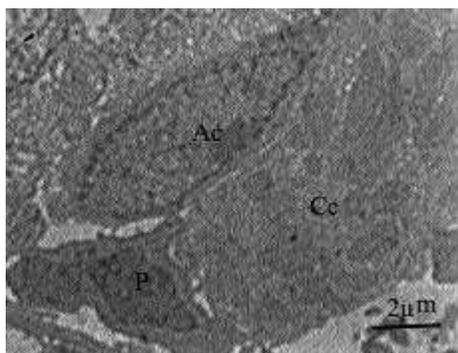


Fig. 1: General Appearance of chloride cell (Cc) covered by pavement cell (P) Accessory cell (Ac) is in close contact with chloride cell

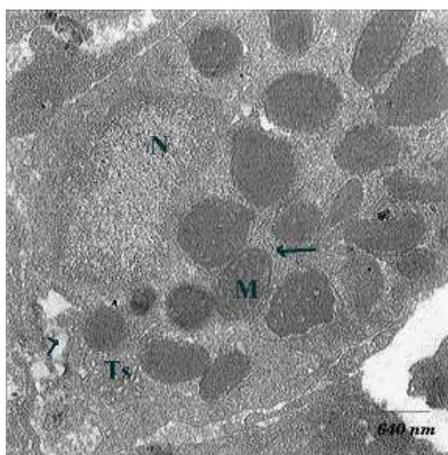


Fig. 2: An electron micrograph of a chloride cell. An euchromatic nucleus positioned apical cell. Note the tubular network (Ts) and apical crypt in this cell (arrowhead)

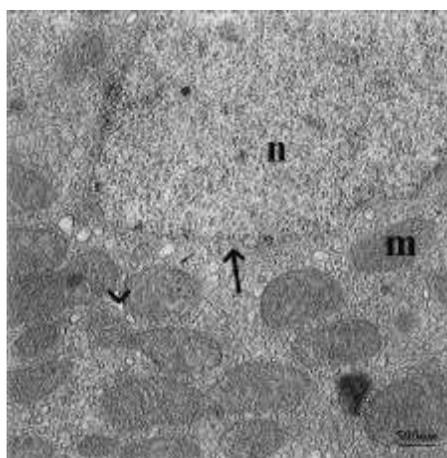


Fig. 3: Tubulo-vesicular network comes into close contact with mitochondria (arrowhead) and nucleus (arrow)

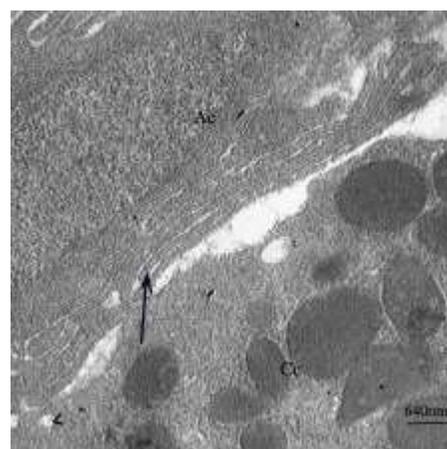


Fig. 4: The channels of accessory cells forming intercellular channel system (arrow). Note the relation between vesicles of chloride cell and intercellular area (arrowhead)

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DISCUSSION

The gills in fishes are the main osmoregulation organ that is highly sensitive to many factors, including changes in salinity, pollution and stress [5,6]. Importance of the role of gills on gas exchange [7], ion and acid-base equilibrium [8] and nitrogen excretion [9] is proportional to their susceptibility to changes in the environment.

Gill chloride cells, appear mostly in the epithelia of lamellae, and when necessary may appear on lamellae [10]. In *R.r.Caspicus*, also this cell appears mostly on the interlamellar region and near the base of the secondary lamellae. This has been observed in many other teleosts [1] of both seawater (Sw) and freshwater (Fw) adapted fishes. However, in many species, *Brachdanio rerio* [11], *Oreochromis mossambicus* [5], *Solea solea* [12], *Onchorhynchus mykiss* [13] and euryhaline fish [10,14] it is on both in the filament and lamellar epithelia. The occurrence of lamellar chloride cell is thought to satisfy the physiological demand of ion uptake in some euryhaline teleosts [15,16] but not in others [17].

Chloride cells, are mitochondrial rich cells exhibited the ultrastructural features typical of this cell population which is mainly involved in the active transport of ions [18]. For this function, they are endowed with a large amount of mitochondria, an extensive network of tubules and vesicles and invaginations of basolateral membrane for widening ionic exchange area. The apical surface of this cell has different features in species. In this study chloride cells have crypts and invaginations in contact with the free surface. Although this character is typical for marine teleost [19, 20, 21], this can occur when a euryhaline species passes from fresh to saltwater [22, 23].

Another feature typical of these cells indicates that there is one type of chloride cell. Ultrastructural studies of other fishes, have found more than one type of chloride cell, distinguished by different electrodensity of their

cytoplasm [24] or by their different in apical membrane characteristics [25]. The larger with lower mitochondria seemed this cell in telaji to B-cell and this can be consistent with this finding that B-cell is specifically important in freshwater environment [26]. Cytoplasmic tubules associated with numerous mitochondria, have been considered important for the osmoregulatory function, because they have represented sodium pump proteins involved in active ion excretion. These reticulated cytoplasmic tubules which anastomosed with each other to form a network, often continuous with the basolateral plasma membrane and their lumen open to the intercellular space. This has been frequently observed in many ion-transporting cells [27] and is probably involved in active ion excretion for the regulation of body fluid osmolarity. The membranous system in *R.r.Caspicus* seems to tubular system in freshwater species and formed a broad and loose network [28]. Although accessory cells are usually found in marine species, they also were found in the gill epithelium of euryhaline Salmonids in freshwater [29], as well as two species of tilapia [30]. It has been proposed that accessory cells are merely young stages in chloride cell development [23], but their function is still unknown. Importantly accessory cell and chloride cell share a single-strand shallow junction, suggesting that a leaky paracellular pathway is present between the cells. This is thought to be the morphological basis for the relatively high ionic permeability of the gill [31].

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REFERENCES

1. Wilson, J.K. and P. Laurent, 2002. Fish gill morphology. *J. Exp. Zool.*, 293: 192-213.
2. Austin, B., 1999. The effects of pollution on fish health. *J. Appl. Microbiol.* 85: 234-242.
3. Lindesjoo, E. and T. Jhulin, 1994. Histopathology of skin and gills of fish in pulp mill effluents. *Dis. Aquat. Org.*, 18: 81-93.
4. Vigliano, F.A., N. Aleman, M.I. Quiroga and J.M. Nieto, 2006. Ultrastructural Characterization of gills in Juveniles of the Argentinian Silverside. *Anat. Histol. Embryol.*, 35: 76-83.
5. Kuitz, D., K. Jurs and L. Jones, 1995. Cellular and Epithelial Adjustments to Altered Salinity in the gill and opercular Epithelia of a Cichlid Fish. *Cell Tissue Res.*, 279: 65-73.

6. Uchida, K., T. Kaneko, K. Yamauchi and Y. Hirano, 2000. Morphometrical analysis of chloride cell activity in the gill filament and lamellae and changes in Na⁺, K⁺-ATPase activity during seawater adaptation in chum salmon fry. *J. Exp. Zool.*, 276:193-200
7. Hughes, G.M., 1984. General anatomy of the gills. In: *Fish Physiology*. Vol. XA. New York: Academic Press, pp: 1-72.
8. Evans, D.H., 1980. Kinetic studies of ion transport by fish gill epithelium. *Am. J. Physiol.*, 238: 224-230.
9. Haswell, M.S., D.J. Renald and S.F. Perry, 1980. Fish gill carbonic anhydrase: acid-base regulation or salt transport. *Am. J. Physiol.*, 14: 1-25.
10. Sakamoto, T., K. Uchida and S. Yokota, 2001. Regulation of the iontransporting mitochondria-rich cell during adaptation to teleost fishes to different salinities. *Zool. Sci.*, 18: 1163-1174.
11. Isisag, S. and H. Karakisi, 1998. Fine structure of the chloride cell in the gill epithelium of *Brachydonio rerio* (Cyprinidae, teleostei). *J. Vet. Ani. Sci.*, 22: 431-436.
12. Dunel, Erb. S and Laurent, 1980. Ultrastructure of Marine teleost gill epithelia: SEM&TEM study of chloride cell apical membrane. *J. Morphol.*, 165: 175-186
13. Bindon, S.D., J.C. Fenwick and S.F. Perry, 1994. Branchial chloride cell proliferation in the *Rainbow trout*. *Onchorhynchus Mykiss*. *Can. J. Zool.*, 72: 1395-1402
14. Varsamos, S., J.P. Diaz, G. Charmantier, G. Flik, C. Blasco and R. Connes, 2002. Branchial chloride cells in sea bass (*Dicentrarchus labrax*) adapted to freshwater, seawater, and dubly concentrated seawater. *J. Exp. Zool.*, 293: 12-26.
15. Sasai, S., T. Kaneko, S. Hasegawa and K. Tsukamoto, 1998. Morphological alteration in two types of gill chloride cells in Japanese eel (*Anguilla japonica*) during catadromous migration. *Can. J. Zool.*, 76: 1480-1487.
16. Hirai, N., M. Tagawa, T. Kaneko, T. Seika and M. Tanaka, 1999. Distribution changes in branchial chloride cells during freshwater adaptation in Japanese sea bass *Iteolabrax Japonicus*. *Zool. Sci.*, 16: 43-49.
17. Lin, H.C. and W.T. Sung, 2003. The distribution of mitochondria-rich cells in the gills of air breathing fishes. *Physiol. Biochem. Zool.*, 76: 215-228.
18. Laurent, P. and S. Perry, 1991. The effects of cortisol on gill chloride cell morphology and ionic uptake in the freshwater trout. *Salmo gairdneri*. *Cell Tissue Res.*, 259: 429-442.
19. Laurent, P. and S. Dunel, 1980. Morphology of gill epithelia in fish. *Am. J. Physiol.*, 238: 147-159.
20. Olson, K.R., 1996. Scanning electron microscopy of the fish gill. In: *Fish Morphology: horizons of new research* (Dutta, H.M. and J.S.D. Mushi (Eds.). Lebanon: Science Publishers, pp: 31-45.
21. Olson, K.R., 2000. Respiratory system. Gross functional anatomy. In: *The Laboratory Fish* (Ostrander, G.K. (Ed.)). London: Academic Press, pp:151-159.
22. Fishelson, L., 1980. Scanning and transmission electron microscopy of the squamous gill-filament epithelium from fresh and seawater adapted Tilapia. *Environ. Biol. Fish.*, 5: 161-165.
23. Wendelaar Bonga, S.E. and J.C.A. Van der Meij, 1989. Degeneration and death by apoptosis and necrosis of the pavement and chloride cells in the gills of the teleost. *Cell Tissue Res.*, 255: 235-243.
24. Wendelaar Bonga S.E., G. Flik, Balm P.H.M. Van der and J.C.A. Meij, 1990. The ultrastructure of chloride cells in the gills of the teleost *Oreochromis mossambicus* during exposure to acidified water. *Cell Tissue Res.*, 259: 575-585.
25. Pisam, M., Moal Le, C. Aurperin, P. Prunet and A. Rambourg, 1995. Apical structures of mitochondrial-rich alpha and beta cells in euryhaline fish gill. *Anat. Rec.*, 241: 13-24.
26. Chris, K., C. Wong and D.K.O. Chan, 1999. Chloride cell subtypes in the gill epithelium of Japanese eel *Anguilla Japonica*. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.*, 227: 517-522.
27. Berridgo, M.J. and Oshman, 1972. Ion transporting type epithelial cells of the gills. *J. Biol. Chem.*, 247: 7218-7223.
28. Pisam, M., 1981. Membranous systems in the chloride cell of teleostean fish gills, their modifications in response to the salinity of the environment. *Anat. Rec.*, 200: 401-414.
29. Pisma, M., A. Caroff and A. Rambourg, 1989. Two types of chloride cells in the gill epithelium of a freshwater adapted euryhaline fishe: *Lebistes reticulates*; their modifications during adaptation to saltwater. *Am. J. Anat.*, 179(1):40-50.
30. Cioni, C., D. Merich, E. Cataldi and S. Sataudella, 1991. Fine structure of chloride cells in freshwater and seawater adapted *Oreochromis niloticus* and *Oreochromis mossambicus*. *J. Fish Biol.*, 39: 197-209.
31. Karnaky, K.J., 1992. Teleost osmoregulation: Changes in the tigh junction in response to the salinity of the environment. In: *Tigh junctions*. Erejjido, M. (Ed.). Boca Raton: CRC Press, pp: 175-185.