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Ionic Transport in the Fish Gill Epithelium

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ABSTRACT The gill of fishes is modified for gas exchange, thereby providing a site for net movement of salts and water down their respective gradients. Specialized cells in the gill epithelium are joined by tight junctions of variable depth and express a variety of transporters and channels. These cells mediate NaCl extrusion in marine fishes and NaCl uptake in freshwater fishes. These transport steps also provide pathways for the extrusion of ammonia and acid vs. base equivalents. *J. Exp. Zool. 283:641–652, 1999.* © 1999 Wiley-Liss, Inc.

The fish gill, like any gas exchanger, is modified to: (1) maximize the surface area available for diffusion of O_2 and CO_2; (2) minimize the diffusion distance between the external medium and the blood; and (3) maximize the perfusion of the tissue. The gill evolved from the surface epithelium of the branchial basket of protovertebrates, which was used in filter feeding, and probably appeared about 550 million years ago in the Pteraspid agnathans (Gilbert, '93). Evolutionary modification of a surface epithelium to facilitate gas exchange is not without physiological cost: in an aquatic environment it exacerbates any diffusional movements of solutes or water into or out of an organism that is not iso-osmotic to the medium. Ancestors to the vertebrates were iso-osmotic/ionic to their marine habitats, but, for reasons that are still debated (e.g., Griffith, '87; Evans, '93), the vertebrates (except the hagfishes) are not. So aquatic gas exchange presents osmoregulatory problems to fishes. Although osmoregulation in fishes is mediated by a suite of structures including the gastrointestinal epithelium and kidney, the gill is the major site of ion movements to balance diffusional gains or losses. The marine elasmobranchs have evolved a rectal gland that provides for ion extrusion (see Shuttleworth, this volume), but there also is evidence that gill ionic extrusion mechanisms exist (see below). This review examines current models for these transport steps in the gill epithelium in fishes and shows how they are pivotal in acid-base regulation and nitrogen excretion. For recent reviews of these subjects, see Perry ('97); Claiborne ('98); Karnaky ('98); Marshall and Bryson ('98); Walsh ('98). For the purpose of this review, we will focus on teleost fishes, with some references to the elasmobranchs, and do not include gill transport steps for Ca^{2+} regulation, which have been reviewed recently by Flik and his coworkers ('95, '96). For a discussion of what little is known about gill function in the agnatha (hagfishes and lampreys), see reviews by Evans ('93) and Karnaky ('98).

FISH GILL STRUCTURE

Each branchial arch in the teleost is elaborated into multiple filaments, which are further subdivided into thousands of lamellae, the sites of gas exchange (Fig. 1a). The flow of water that irrigates the gills is counter-current to the flow of blood that perfuses the lamellae, maximizing gas, ionic, and osmotic gradients that exist. This facilitates gas exchange, but also enhances net ionic and osmotic movements that the fish must counter to maintain osmotic homeostasis. The filaments and lamellae are perfused via a complex series of vessels (Fig. 1b), which receive the entire cardiac output through the ventral aorta. In the past few years, it has become clear that the perfusion of the gill epithelium (both in the filaments and lamellae) is under the control of a variety of endocrine and paracrine factors (e.g., Olson, '97), which may play a role in controlling the permeability of and ionic transport steps in the fish gill. For instance, the opposing surfaces of the individual lamellae are held apart by pillar cells, which are thought to contain actomyosin (Laurent, '84). It is generally accepted that alteration of pil-

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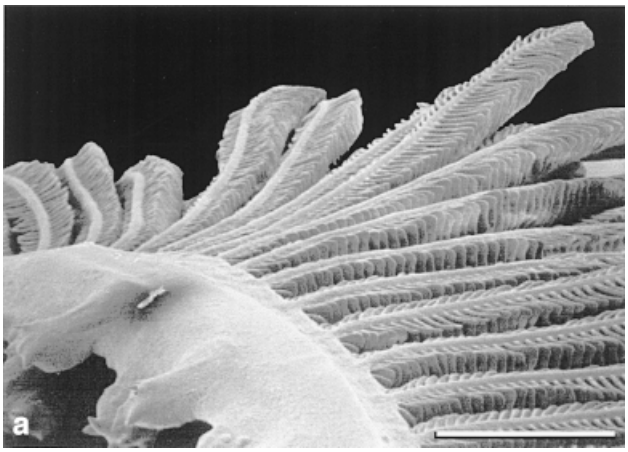

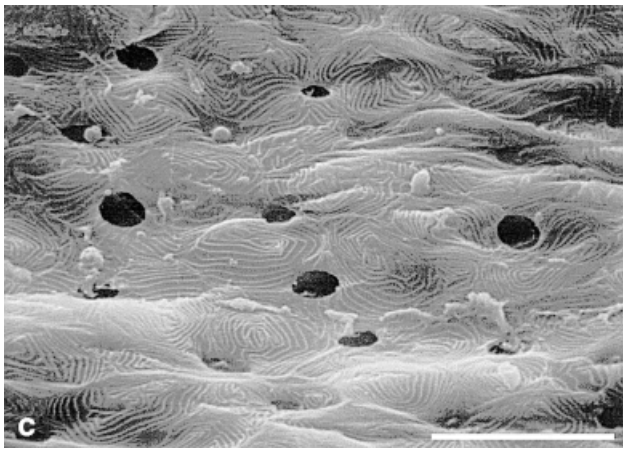

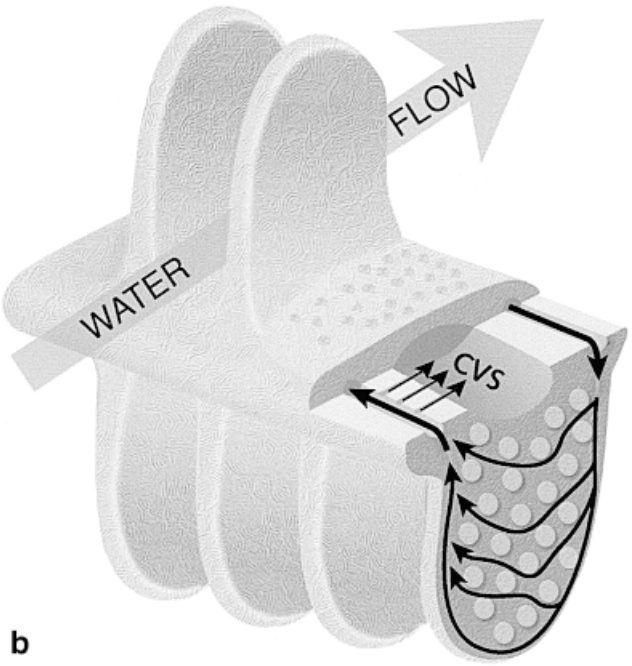

Fig. 1. **a:** Scanning electron micrograph of a single gill arch from the gulf toadfish (*Opsanus beta*) showing 15 filaments that contain scores of lamellae. Reference bar is 100 μm. **b:** Diagram of a single filament and six lamellae showing the direction of water flow (irrigation) between the lamellae and blood flow within the filament and a single lamella. The afferent filamental artery perfuses single lamellae via a small pre-lamellar arteriole. Blood flow in the lamella is around individual pillar cells, or around the peripheral channel, through the post-lamellar arteriole and into the efferent filamental artery. Significant post-lamellar blood can be shunted into the central venous sinus (CVS) of the filament under certain conditions. The external surface of the filament and lamellae is characterized by the microridges of the PVCs, with interspersed, infrequent pores into the apical surface of MRCs (shown as small, gray circles in the figure). See text for details (redrawn from Pisam et al., '87). **c:** Scanning electron micrograph of the surface of a gill filament from *O. beta*, showing the characteristic pattern of the apical surface of PVCs and pores leading to MRCs. Reference bar is 20 μm.

lar cell thickness could play a major role in the pattern of blood flow through the lamellae, thereby controlling gas exchange, and possibly ion regulation. There appear to be endothelial cells lining the marginal channel of the lamellae (Hughes, '84; Perry and Laurent, '90), and recent studies have shown that a variety of endothelium-derived substances are vasoactive in fishes (e.g., Evans et al., '96; Evans and Gunderson, '98). In fact, Sundin and Nilsson ('98) have found that the endothelium-generated peptide endothelin redistributes blood flow through the lamellae of trout (*Oncorhynchus mykiss*) gills by contracting pillar cells.

The fish gill epithelium is characterized by the presence of three cell types of interest to this review: (1) pavement cells (PVCs); (2) mitochondrion-rich cells (MRCs); and (3) accessory cells (ACs), in addition to mucous cells that are usually on the leading or trailing edge of the filament (e.g., Laurent, '84). More than 90% of the gill surface epithelium, and usually all of the lamellar surface, is characterized by PVCs. These cells may be columnar, but are generally squamous, and contain moderate numbers of mitochondria and a well-developed Golgi and rough endoplasmic reticulum, indicative of "intense metabolic activity" (Laurent and Dunel, '80). The tight junctions between adjacent PVCs are generally termed "deep" because of numerous interconnecting strands between the cells (Sardet et al., '79; Karnaky, '92). When viewed on the surface of the gill, PVCs appear to have "fingerprints" composed of circular microridges on the mucosal surface

(Fig. 1b, c). Although these cells, especially on the lamellae, are assumed to be the site of transepithelial gas transfer, recent evidence suggest they may also play a role in ion and acid-base regulation (see below).

Mitochondrion-rich cells (often termed "chloride cells") are found interspersed with the PVCs, especially in the interlamellar epithelium and often on the trailing edge of the filament (e.g., Laurent, '84; Van Der Heijden et al., '97). MRCs are not necessarily confined to gill epithelia; they are also found, with PVCs and ACs, in the inner surface of the operculum of the killifish *Fundulus heteroclitus* (e.g., Degnan et al., '77) and tilapia, *Oreochromis mossambicus* (Foskett et al., '81), as well as the jaw skin of the goby *Gillichthys mirabilis* (Marshall and Nishioka, '80). The MRCs are characterized by a relatively high metabolic activity compared to PVCs (Perry and Walsh, '89). MRCs (Fig. 2) contain sub-apical vesicles and elaborate basolateral infoldings that produce an extensive intracellular tubular system, associated with numerous mitochondria (e.g., Laurent, '84) and the transport enzyme Na/K-ATPase (Karnaky et al., '76). Tight junctions between MRCs and adjacent PVCs are also considered "deep" because of multi-strand connections (Sardet et al., '79; Sardet, '80; Karnaky, '92) (Fig. 2). In marine species, the mucosal surface of the MRCs are usually sunk below the PVCs, which produces "pores" between PVCs (Figs. 1b, c and 2). In marine teleosts, or euryhaline teleosts acclimated to seawater, the MRCs usually display multi-cell complexes, a more elaborate intracellular tubular system, and an apical crypt (Fig. 2; also Hossler et al., '79 and Laurent, '84). Importantly, an AC develops next to the MRC in seawater teleosts, and the two cells share a single-strand, shallow junction (Fig. 2), suggesting that a "leaky" paracellular pathway is present between the cells (e.g., Laurent, '84). The same is true for adjacent MRCs. This is thought to be the morphological basis for the relatively high

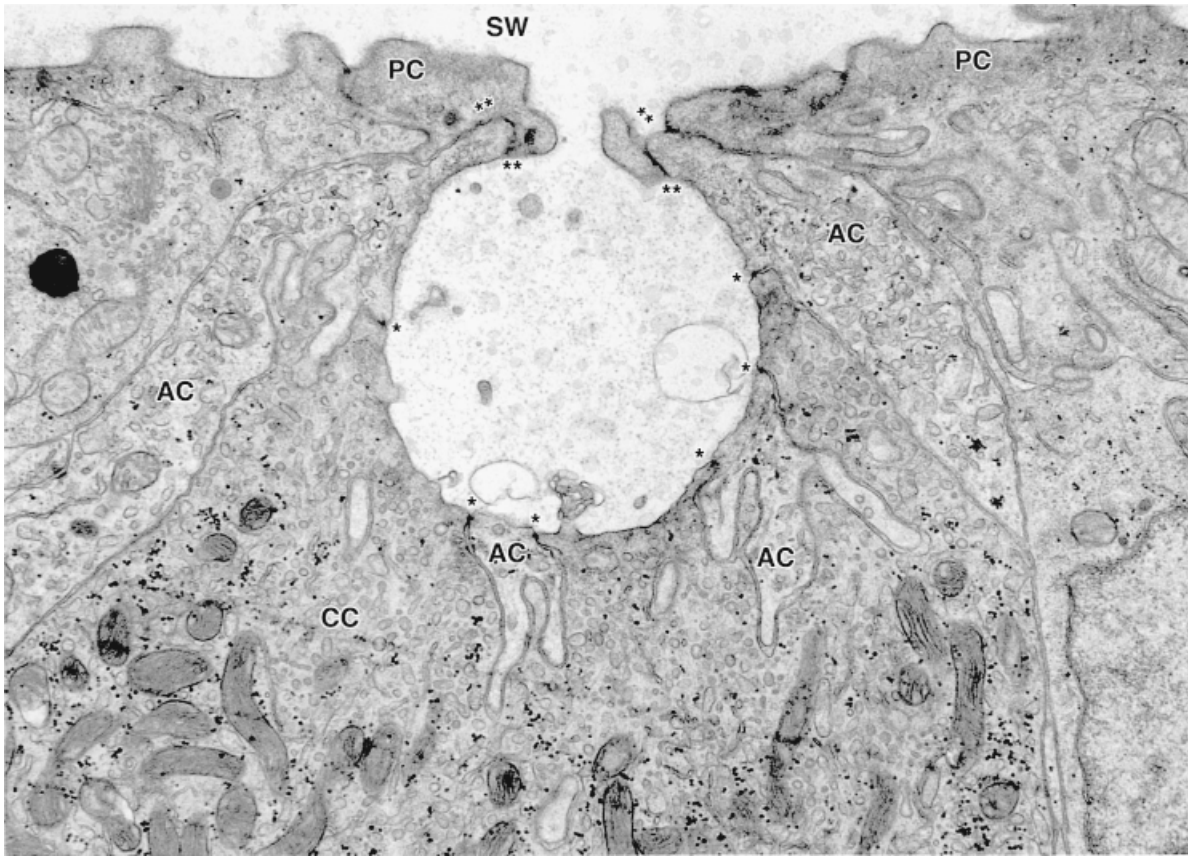

Fig. 2. Electron micrograph of gill tissue from the killifish (*Fundulus heteroclitus*) acclimated to 100% seawater (SW). A single mitochondrion-rich cell (labeled CC) shares an apical crypt with an accessory cell (AC). The CC/AC tight-junctions (*) are shallow, whereas AC/PVC tight-junctions (**) are deep. Note the much higher density of mitochondria in CC than PVC or AC. (Micrograph kindly supplied by Dr. Karl Karnaky.)

ionic permeability of the marine teleost gill (e.g., Karnaky, '92). Although ACs are usually found only in marine species, they also were found in the gill epithelium of euryhaline salmonids in fresh water (Pisam et al., '89), as well as two species of tilapia (Cioni et al., '91). It has been proposed that ACs are merely young stages in MRC development because of similar cytoplasmic structures (e.g., Wendelaar Bonga and van der Meij, '89), but their function is still unknown. Pisam and coworkers ('87) also have described two types of MRCs, termed α and β, present in freshwater species and euryhaline species in fresh water. The α form is thought to be the homologue of the MRC in marine teleosts and actually transforms into the MRC in the filamental epithelium of euryhaline fishes acclimated to seawater (Pisam et al., '87, '95). The β form exists only in fresh water teleosts. It is not known whether any functional differences exist between the two freshwater types of MRCs.

In freshwater teleosts, MRCs are generally singular on the gill epithelium, lack an apical crypt, have extensive tight junctions with adjacent cells, and usually have their mucosal surface above the adjacent pavement cells. Tilapia seems to be an exception, with an apical crypt below the surface of the pavement cells (Van Der Heijden et al., '97). In addition, the basolateral tubular system often is less well developed. Freshwater MRCs react with antibodies to Na/K-ATPase, demonstrating that the transport enzyme is present (e.g., Uchida et al., '96; Witters et al., '96). There are multicellular complexes in some freshwater species (Hwang, '88), and the opercular skin of the killifish acclimated to fresh water has clustered MRCs that share deep, apical tight junctions (Marshall et al., '97). As in seawater, the MRCs are relatively abundant on the filamental epithelium, but they may also appear on the lamellar epithelium in some freshwater species (e.g., Uchida et al., '96; Perry, '97). Freshwater MRCs display apical microvilli, which presumably increases mucosal surface area (Huang, '88; Perry et al., '92; Marshall et al., '97). Fishes in very dilute or soft freshwater display more extensive proliferation of MRCs on the lamellar epithelium (e.g., Perry and Laurent, '93; Perry, '97).

MRCs are also found in the gill epithelium of elasmobranchs (e.g., Laurent, '84; Wilson et al., '96). Although the cells are located in a similar filamental location as marine teleost MRCs, their ultrastructure is quite different. Instead of an intracellular tubular system, elasmobranch MRCs have numerous basolateral infoldings that are less extensive, long apical microvilli, and usually lack an apical crypt (Laurent, '84). As in teleosts, elasmobranch MRCs express relatively large amounts of Na/K-ATPase compared with adjacent PVCs (Conley and Mallatt, '88). There is no evidence published for the presence of ACs in the branchial epithelium of elasmobranchs.

ELECTROCHEMICAL GRADIENTS ACROSS FISH GILLS

The fact that the Na^+ and Cl^- concentration of fish plasma differs from that of either seawater or freshwater has been known for more than 100 years (see Holmes and Donaldson ('69) for the most complete tabulation). The large Na^+ or Cl^- gradients across the freshwater fish gill could not be maintained by the electrical potentials that have been measured across the gills of freshwater fishes (e.g., Evans, '80a; Potts, '84; Potts and Hedges, '91), so it is clear that electrochemical gradients exist that must produce a net diffusional loss of NaCl from these fishes. This adds to the renal loss and must be countered by some sort of NaCl uptake system. The case is not so clear for marine teleosts, where the measured transepithelial electrical potential (TEP; ca. + 20 to 30 mV, plasma relative to seawater) may equal or slightly exceed the Nernst potential for at least Na^+ ($E_{Na} \approx 28$ mV) in some, but certainly not all, species (e.g., Evans, '80a; Potts, '84). A TEP higher than the E_{Na} would not only counter any diffusional uptake of Na^+, it would provide an electrochemical gradient to extrude Na^+ taken up across the intestinal epithelium secondary to the oral ingestion of seawater. This ingestion is necessary for marine teleosts to maintain osmotic balance (e.g., Karnaky, '98). However, 21 species of marine teleosts display TEPs less than +20 mV, with at least six species generating a TEP that is negative (plasma relative to seawater; Evans, '80a; Potts, '84). This is approximately 50% of the relatively large number of species that have been studied! There is no systematic difference in techniques published that can explain these TEPs that are decidedly different from E_{Na} In no case is the TEP close to the E_{Cl} (≈ -35 mV), and in marine teleosts where it approaches E_{Na}, Cl^- will be drawn inward by both the diffusional gradient and electrical potential. In these species, both Na^+ and Cl^- are out of electrochemical equilibrium and, therefore, must be actively extruded by the gill epithelium. This idea is important because the current model for ionic transport across

the MRC in the marine teleost gill assumes that Na^+ is in electrochemical equilibrium (see below).

The very few measurements of elasmobranch species have found that their TEP is slightly negative (Evans, '80a), suggesting that both Na^+ and Cl^- are out of electrochemical equilibrium across their gill epithelium. The elasmobranch rectal gland extrudes both Na^+ and Cl^- (see Shuttleworth, this volume), but elasmobranchs can ion regulate even when the rectal gland has been removed (Burger, '65; Evans et al., '82). Therefore, the elasmobranch gill epithelium may extrude both ions, although details of the process are lacking. No TEP measurements have been published for freshwater elasmobranchs, but it is doubtful that either Na^+ or Cl^- are in electrochemical equilibrium across the gill in these species.

To summarize, many teleosts maintain a TEP close to that necessary to keep Na^+ in passive equilibrium across the gill; but many other teleosts, and the few elasmobranchs so far examined, have a lower positive or indeed negative TEP such that both Na^+ and Cl^- must be actively transported across the gill.

GILL IONIC TRANSPORT MECHANISMS

The complexity of the fish gill dictates that some direct experimental approaches are not possible, so much of what we know about the transport steps in the epithelium is derived from indirect studies, model systems, and, most recently, molecular techniques. For instance, the complexity of the anatomy of the gill has precluded the use of isolated sheets of epithelial tissue to allow a rigorous electrophysiological approach to the mechanisms of membrane transport. In addition, the pattern of water flow across the elaborate external surface, as well as the complexity of the blood flow through the branchial vasculature, has reduced the efficacy of irrigation and perfusion of isolated heads, branchial arches, or gill fragments to study transport steps (e.g., Evans et al., '82). Nevertheless, whole animal studies (see Evans, '79, for a review of the early literature), use of skin epithelial sheets that contain MRC cells (e.g., Zadunaisky, '84), and, most recently, patch-clamp and molecular techniques (reviewed in Marshall, '95; Karnaky, '98; Marshall and Bryson, '98) together have contributed greatly to understanding the ionic transport pathways in the fish gill.

NaCl extrusion by fishes in seawater

It became clear in the 1970s that the fish gill epithelium expressed large quantities of the well-known transport protein, Na/K-ATPase, whose activity was usually, but not always, proportional to the external salinity (reviewed in de Renzis and Bornancin, '84; McCormick, '95) Despite early physiological data that suggested the enzyme was located on the apical membrane (e.g., Maetz, '69; Evans and Cooper, '76), histochemical techniques demonstrated uneqivocally that it is on the basolateral infoldings of the MRC (Karnaky et al., '76; Hootman and Philpott, '79). At the same time, Silva and his colleagues showed that injection of ouabain (the standard inhibitor of Na/K-ATPase) into intact eels (*Anguilla rostrata*) inhibited the efflux of radio-labeled Na^+ and Cl^-, with a much smaller effect on the efflux of tritiated water (Silva et al., '77). In that seminal paper, the authors suggested that the basolateral Na/K-ATPase generated an electrochemical gradient for Na^+ from the plasma to the cytoplasm of the MRC, which drove both Na^+ and Cl^- inward across the basolateral membrane. This proposition was supported by a contemporary study of the opercular membrane from the killifish, which demonstrated, under short-circuited conditions, that the net Cl^- extrusion rate (serosal to mucosal) was equal to the short-circuit current (SCC), but there was no net extrusion of Na^+ (Degnan et al., '77). Both ouabain and furosemide (an inhibitor of coupled Na-Cl cotransport) inhibited the SCC and net extrusion of Cl^-. Elegant electrophysiological studies, using the vibrating probe technique, demonstrated that the MRC was the site of the ionic extrusion mechanism (Foskett and Scheffey, '82; Foskett and Machen, '85). More recent research has fine-tuned the model resulting from these studies (e.g., Zadunaisky, '84); the current model for NaCl extrusion by the teleost gill epithelium (Fig. 3) is best described in a recent review by Marshall ('95). The Na^+ gradient produced across the basolateral membrane by Na/K-ATPase-driven extrusion of Na^+ from the cell, drives Na^+ into the cell coupled to Cl^- and K^+, via a common transport protein. K^+, which enters via the basolateral Na/K-ATPase and basolateral Na-K-2Cl cotransport, is thought to exit the cell via K channels in both the apical and basolateral membranes. Cl^- exits the cell via an apical Cl^- channel, which generates a serosal-side positive TEP that moves Na^+ through the leaky paracellular pathway between adjacent MRCs and ACs. It should be noted that the experiments that support this model are confined to teleosts where the TEP $\geq E_{Na}$. This model alone does not account for Na^+ extrusion from fishes in which the TEP $\leq E_{Na}$.

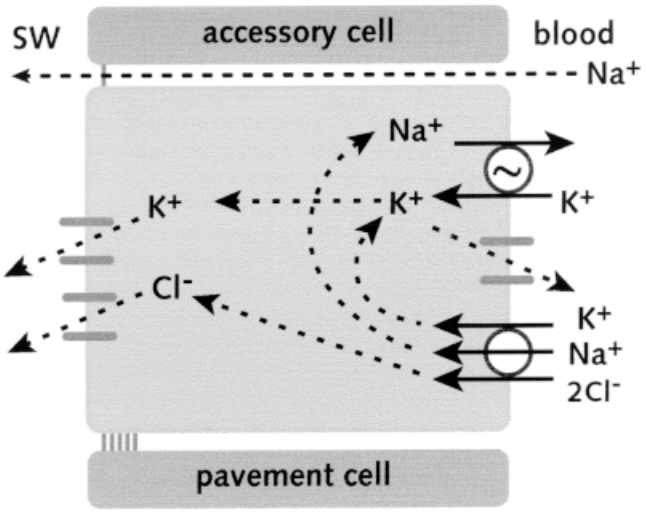

Fig. 3. Current model for NaCl extrusion by the marine teleost MRC. Note the deep-tight junction between the MRC and PVC, and shallow-tight junction between the MRC and AC. See text for details. (Redrawn from Marshall '95.)

The furosemide sensitivity of basolateral NaCl uptake suggested that the basolateral carrier was in the Na-K-2Cl cotransporter family (e.g., Payne and Forbush, '95; Kaplan et al., '96) rather than the thiazide-sensitive Na-Cl cotransporter, and electrophysiological studies of isolated opercular epithelia support this conclusion (Eriksson and Wistrand, '86; Marshall, '95). In addition, a recent study has shown upregulation of the cotransporter in seawater-acclimated rainbow trout, with a Western Blot using monoclonal antibodies to the shark rectal gland Na-K-2Cl cotransporter (Behnke et al., '96). Both the α and β subunits of the gill Na/K-ATPase have been cloned for the white sucker (*Catostomus commersoni*) and European eel (*Anguilla anguilla*) (see Karnaky, '98). Addition of Ba^{2+} to the serosal surface of the opercular membrane inhibited Cl^- secretion, consistent with a basolateral K^+ channel (Degnan, '85). The short-circuited skin of *G. mirabilis* secreted K^+ into the mucosal bath, which suggests an apical K^+ channel (Marshall and Bryson, '98). The presence of an apical Cl^- channel appears to be well established. Its electrical characteristics and stimulation by cyclic AMP (Marshall et al., '95) indicate that it is a member of the mammalian cystic fibrosis transmembrane conductance regulator (CFTR) family. Recent cloning of a killifish CFTR-like gene (which shares only 59% homology with human and shark rectal gland CFTR) supports this proposition (Singer et al., '98).

Surprisingly, a recent study (Avella and Ehrenfeld, '97) demonstrated that PVCs may be a site of Cl extrusion, in addition to the MRCs. Pavement cells from the sea bass (*Dicentrarchus labrax*), cultured in sea bass serum, displayed a SCC, TEP, and resistance characteristic of the seawater gill epithelium, and extruded Cl^- in the serosal to mucosal direction via a bumetanide-(inhibitor of Na-K-2Cl cotransport) and ouabain-sensitive (applied serosally) pathway. In addition, the net Cl^- secretion was inhibited by application of Cl^- channel inhibitors, applied to the mucosal surface. The authors conclude that "the chloride secretion through these cultures of respiratory-like cells makes it necessary to reconsider the previously accepted seawater model in which the chloride cells are given the unique role of ion transport through fish gills" (Avella and Ehrenfeld, '97). The current measured in this study was less than 10% of that generated by the opercular preparation (e.g., Degnan et al., '77), but PVCs comprise more than 90% of the epithelial surface, so the total NaCl extrusion by PVCs and MRCs may be equivalent. On the other hand, it is possible that these cultured PVCs are expressing transport steps not expressed in vivo.

Despite the fact that the gill epithelium of elasmobranchs contains MRCs, which express Na/K-ATPase (see above), the evidence for NaCl extrusion by the elasmobranch gill is circumstantial at best. The evidence is largely based upon the finding in a variety of studies that removal of the rectal gland does not impair regulation of plasma NaCl in sharks (e.g., Evans et al., '82; Wilson et al., '96). In addition, ammonia excretion by the perfused head of the dogfish shark pup was inhibited by addition of bumetanide to the perfusate (Evans and More, '88). This suggests that a basolateral Na-K-2Cl cotransporter exists because this carrier protein has been shown to be sensitive to NH_4^+ at the K site (e.g., O'Grady et al., '87). Further study of the mechanisms of NaCl transport across the gill epithelium of the elasmobranch would be quite interesting. Molecular techniques may now give us this opportunity.

NaCl uptake by fishes in freshwater

The large electrochemical gradients for both Na^+ and Cl^- between fish plasma and the medium in freshwater dictate that mechanisms must exist for extraction of both of these ions from the very dilute environment. Because Na^+ or Cl^- can be taken up independently of each other by fishes, Krogh ('38) suggested that the extrusion of internal counter ions, such as NH_4^+, H^+, or HCO_3^-, was probably chemically-coupled to Na^+ and Cl^- up-

take in order to maintain reasonable electrical gradients across the gill. This hypothesis was supported by a variety of studies over the next 50 years (see Evans, '75; Evans et al., '82; McDonald et al., '89 for reviews), but more careful analysis of the electrochemical gradients involved suggested that uptake of Na^+ is most probably via an apical Na^+ channel (Fig. 4), down an electrical gradient generated by an apical H^+ pump (Avella and Bornancin, '89; Lin and Randall, '91; Potts, '94). Lin and Randall ('93) originally proposed that the H^+-ATPase was the P-type (plasma membrane) because of the inhibitor profile, but more recent immunological studies (see below and Lin and Randall, '95) support the proposition that the transporter is probably vacuolar or V-type, H^+-ATPase. In addition, Lin and Randall ('93) showed that relatively specific inhibitors of H^+-ATPase inhibited the ATPase activity in gill homogenates of rainbow trout, and hypercapnia increased the activity of this H^+-ATPase by 70%. Proton extrusion by an H^+-ATPase accounts for Na^+ uptake in a variety of "tight" epithelia, including the frog skin (e.g., Ehrenfeld et al., '85; Harvey, '92), and the freshwater fish gill is generally considered to be "tight" because of deep junctions between adjacent pavement cells and MRCs and pavement cells (see above and Sardet, '80). This hypothesis

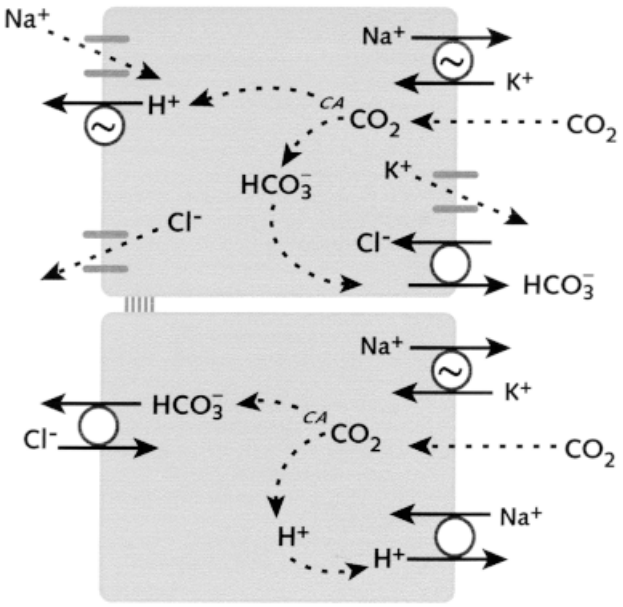

Fig. 4. Current model for the uptake of NaCl by the freshwater fish gill epithelium. Two cells are depicted for clarity and because some evidence suggests that Na^+ and Cl^- uptake may be through separate cells. See text for details. (Redrawn from Claiborne, '98.)

is supported by the fact that the unidirectional flux of isotopically labeled Na^+ or Cl^- across freshwater fishes is generally less than 5% of that across marine species (e.g., Karnaky, '98). The electrical resistance of a cultured freshwater gill cell preparation, which is largely pavement cells, is 3.5 $k\Omega \cdot cm^2$, even greater than other cultured "tight" epithelia (Wood and Pärt, '97). Basolateral transit of Na^+ into the extracellular fluids is assumed to be via the Na/K-ATPase present in MRCs in the freshwater gill (e.g., Witters et al., '96), presumably on the basolateral membrane. The putative basolateral K^+ channel in these cells has not been identified.

It is generally assumed that apical Cl/HCO_3 exchange mediates Cl^- uptake across the gill in freshwater; for example, inhibitors of this exchange lower Cl^- uptake and produce a metabolic alkalosis in fishes, as does removal of external Cl^- (reviewed in Perry, '97; Goss et al., '98). It is unclear how this exchange is driven, since it is unlikely that there is a chemical gradient favoring uptake of Cl^- from freshwater, and the true apical HCO_3^- gradient is unknown. Presumably, net Cl^- movement across the gill is mediated via a basolateral Cl^- channel, driven by the inside-negative membrane potential, but this has not been identified as yet.

Intracellular generation of the H^+ and HCO_3^- necessary for these apical extrusion mechanisms is probably from hydration of CO_2, since carbonic anhydrase has been localized in MRCs of the killifish opercular epithelium (Lacy, '83), and inhibition of carbonic anhydrase by acetazolamide reduced proton excretion by the trout gill (Lin and Randall, '91). Another study found carbonic anhydrase on both the MRCs and PVCs in the gill epithelium, but it appeared to be on the outer surface of the cells (Rahim et al., '88), probably functioning in the acidification of the apical unstirred layer, which may play a role in nitrogen excretion (e.g., Gilmour, '98).

There is some debate about the cellular site of the uptake mechanisms for Na^+ and Cl^- (e.g., Lin and Randall, '95; Sullivan et al., '96; Perry, '97; Goss et al., '98). It is generally accepted that Cl^- uptake is via the MRCs because morphological changes in the number and structure of MRCs correlate well with rates of Cl^- uptake (e.g., Perry and Laurent, '89; Goss et al., '94), and the opercular skin of freshwater-adapted *F. heteroclitus*, which has a high density of MRCs, has a net uptake of Cl^- when exposed to Ringer's on both surfaces (Wood and Marshall, '94; Marshall et al.,

'97). In addition, X-ray microanalysis has determined that injection of acetazolamide or exposure to thiocyanate (which is presumed to inhibit apical Cl/HCO_3 exchange) lowered the Cl^- content of MRCs, but not pavement cells, in the brown trout (Morgan et al., '94; Morgan and Potts, '95). Finally, Sullivan et al. ('96) recently used in situ hybridization to localize the message for the Cl/HCO_3 exchanger on both the filament and lamellar epithelium of the rainbow trout; but the largest signal was in the interlamellar region of the filament where the MRCs are most abundant. Somewhat surprisingly, cultured branchial epithelium from the trout (composed almost exclusively of pavement cells) appears to actively take up Cl^-, and not Na^+, from the mucosal surface (Wood et al., '98), suggesting that either these cultured pavement cells are expressing transporters that don't exist in vivo, or else we should revisit the conclusion that Cl/HCO_3 exchange is the exclusive domain of the MRC.

The cellular site of the H^+-ATPase and Na^+ channel is even less clear (Lin and Randall, '95; Goss et al., '98). Unexpectedly, PVCs were implicated as a site when it was shown that hypercapnic acidosis in the bullhead (*Ictalurus nebulosus*) produced a significant increase in the apical surface area, and the density of mitochondria and apical vesicles of the PVCs, with a concomitant and quite significant reduction in the apical surface area of the MRCs (Perry and Laurent, '93; Goss et al., '94). At the same time, this group (Laurent et al., '94) showed (using ultrathin sections and high-magnification transmission electron microscopy) that the PVCs, and not the MRCs, displayed cytoplasmic, vesicular "studs," similar to those described in the intercalated cells of the mammalian kidney and turtle urinary bladder, which have been shown to contain H^+-ATPase (e.g., Brown et al., '87). X-ray microanalysis studies (Morgan et al., '94; Morgan and Potts, '95) found that the intracellular Na^+ content of the PVCs, but not MRCs, was proportional to the external Na^+ concentration. More recent immunohistochemical and in situ hybridization studies of the rainbow trout gill (Sullivan et al., '95, '96) have demonstrated that the H^+-ATPase signal is in PVCs on the lamellae, although some signal appeared in the interlamellar region of the filaments (where MRCs are mixed with PVCs). Hypercapnia stimulated the expression of the pump in both studies, confirmed by a Western Blot of gill tissue (Sullivan et al., '95; Goss et al., '98). These studies used an antibody raised against a 31 kDa subunit of bovine renal V-type ATPase. Interestingly, the immunoreactivity for the H^+-ATPase was localized to a specific subpopulation of PVCs on the lamellae, suggesting that not all PVCs function in ionic uptake (or acid-base regulation; see below).

These data are to be contrasted with the study of Lin et al. ('94) which used antibodies raised against a 70 kDa subunit of bovine V-type ATPase and demonstrated (via in situ hybridization) that expression of the H^+-ATPase was uniformly distributed on both lamellae and filaments of the trout gill, suggesting that both PVCs and MRCs were sites of proton extrusion and Na^+ uptake. Perry's recent review of chloride cell structure and function (Perry, '97) displays electronmicrographs of immunolocalization studies from both groups: one demonstrates MRC localization of the H-ATPase (J. Wilson, in Perry, '97); and the other specific PVC, but not MRC, localization (Sullivan et al. in Perry, '97). Since the same species was used in these and earlier studies, one can only conclude that either differences in the composition of the tapwater (Ottawa vs. Vancouver, Canada), trout population, or antibody must have provided the conflicting results.

Clearly, more species should be examined using the variety of techniques now available to sort out the cellular site of NaCl uptake (and H^+ and HCO_3^- extrusion) across the fish gill. However, the hypothesis that separate cells may mediate these transport steps is intriguing. Such a cellular separation of acid vs. base extrusion has been described to exist in the collecting duct of the mammalian kidney (e.g., Al-Awqati, '96).

Acid-base regulation

The foregoing should suggest that the branchial mechanisms that extract Na^+ and Cl^- from freshwater must play an important role in acid-base regulation by fishes. This appears to be the case, since hypercapnia or other alterations of the pH of fish blood are associated with changes in the relative effluxes of H^+ vs. HCO_3^-, influxes of Na^+ vs. Cl^-, and morphology of PVCs vs. MRCs (most recently reviewed by Claiborne, '98; Goss et al., '98). Indeed, in many studies it has been acid-base perturbations that allowed the examination of the mechanisms for NaCl uptake (e.g., Goss et al., '95, '98). It is important to note that it is these gill transport mechanisms (rather than changes in respiratory or renal acid-base excretion) that play a major role in the response of fishes to acid-base disturbances (reviewed by Evans, '86; Heisler, '93). Since these transporters, antiports, and channels

play multifunctional roles, one might ask whether they evolved for ion regulation or acid-base regulation (Evans, '75). The fact that physiological (e.g., Claiborne et al., '97) and molecular (Wilson et al., '97) data indicate that even marine species (including the hagfish, which is iso-osmotic to seawater; Evans, '84) express these systems, suggests that control of plasma/cell pH rather than ion concentration may have been the selective force (Evans, '84). In fact, current evidence suggests that marine teleosts may extrude H^+ via apical Na^+/H^+ exchange (see Claiborne, '98) for a review of the recent data). If marine species use Na^+ and Cl^- uptake systems to regulate their pH, what sort of ionic load does this present in relation to the diffusive and intestinal uptake of these ions? One study (Evans, '80b) suggested that the salt load produced by acid-base regulation in a marine species may be relatively small; however, more species, especially elasmobranchs (where diffusional uptake of NaCl is small), need to be studied.

Excretion of nitrogen

The fish gill is also the site of excretion of excess nitrogen in the form of ammonia, and current models suggest that the pathways outlined in Fig. 4 play vital roles (e.g., Evans and Cameron, '86; Wilkie, '97; Walsh, '98). The relative importance of paracellular vs. cellular pathways and mode of excretion (NH_3 vs. NH_4^+) appear to depend on whether the fish is in seawater or fresh water.

The marine teleost gill has leaky-tight junctions between MRCs and ACs (see above) that theoretically permit the diffusional loss of either NH_3 or NH_4^+. NH_3 also can diffuse across the cells themselves, although the lipid solubility of ammonia gas is significantly below that for CO_2 (Knepper et al., '89). However, intracellular pH is far below the pK of NH_3/NH_4^+ (ca. 9.3), so any ammonia in the cell is mainly ionic NH_4^+. Basolateral transporters such as Na/K-ATPase and Na-K-2Cl can substitute NH_4^+ for K^+ (e.g., Wilkie, '97) and the concentration of NH_4^+ (and hence of NH_3) should be higher in the cell than the plasma. In any event, intracellular NH_4^+ must then be extruded across the apical membrane, presumably via a Na/H antiporter, with NH_4^+ substituting for the H^+ (Wilkie, '97). NH_3 may diffuse across the apical membrane. As Wilkie ('97) has pointed out, many of the published inhibitor and ionic substitution experiments do not allow a critical distinction between the putative pathways; hence, more definitive studies are required.

The multistrand nature of the junctions between adjacent cells in the freshwater fish gill presumably hinder paracellular diffusion of NH_4^+, and apical H^+-ATPase will not extrude NH_3. Because of these limitations, it is generally assumed that the dominant, and possibly sole, mode of ammonia extrusion across the freshwater fish gill is via paracellular diffusion of NH_3 (e.g., Wilkie, '97). Interestingly, acidification of the apical unstirred layer by either proton extrusion or carbonic anhydrase-mediated hydration of expelled CO_2 (see above) would facilitate NH_3 diffusion by reducing the NH_3 to NH_4^+. This would maintain the partial pressure gradient for NH_3 diffusion across the gill (e.g., Wilkie, '97). However, it is important to note that, once again, critical experiments to support the hypothesis are lacking.

SUMMARY

The gill epithelium of fishes possesses a suite of transport steps for Na^+ and Cl^- that also function in the excretion of acid vs. base and ammonium ions. Unfortunately, the structural complexity of the gill precludes some of the electrophysiological techniques that have been so important in the study of ionic transport across other epithelia. Recent application of molecular and immunological techniques has allowed the confirmation of earlier models, and, in some cases, added to our confusion. It is hoped this review stimulates some readers to bring new techniques and ideas to the study of this very interesting tissue.

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