

# Decoupling the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ in vivo: A possible new role in the gills of freshwater fishes

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## Abstract

The literature suggests that when  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  has reduced access to its glycosphingolipid cofactor sulfogalactosyl ceramide (SGC), it is converted to a  $\text{Na}^+$  uniporter. We recently showed that such segregation can occur within a single membrane when  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  is excluded from membrane microdomains or ‘lipid rafts’ enriched in SGC (D. Lingwood, G. Harauz, J.S. Ballantyne, *J. Biol. Chem.* 280, 36545–36550). Specifically we demonstrated that  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  localizes to SGC-enriched rafts in the gill basolateral membrane (BLM) of rainbow trout exposed to seawater (SW) but not freshwater (FW). We therefore proposed that since the freshwater gill  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  was separated from BLM SGC it should also transport  $\text{Na}^+$  only, suggesting a new role for the pump in this epithelium. In this paper we discuss the biochemical evidence for SGC-based modulation of transport stoichiometry and highlight how a unique asparagine–lysine substitution in the FW pump isoform and FW gill transport energetics gear the  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  to perform  $\text{Na}^+$  uniport.

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## 1. Introduction

$\text{Na}^+ - \text{K}^+ - \text{ATPase}$  (EC 3.6.1.37) is a membrane bound enzyme that uses the energy from the hydrolysis of one molecule of ATP to transport two  $\text{K}^+$  into and three  $\text{Na}^+$  out of most animal cells. As with all lipoprotein enzymes, a membrane environment is necessary for this activity (Roelofsen and Deenen, 1973).  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  can, however, exhibit specificity in its lipid requirement, in particular for the glycosphingolipid sulfogalactosyl ceramide (SGC). Karlsson (1977, 1982) proposed that SGC is a cofactor for  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ -catalyzed  $\text{K}^+$  translocation. His cofactor model, which has subsequently been supported by extensive biochemical evidence (Gonzalez et al., 1979; Zambrano et al., 1981; Gonzalez and Zambrano, 1983; Jedlicki and Zambrano, 1985), stated that SGC functions to donate a  $\text{K}^+$  (SGC exhibits a  $\text{K}^+ > \text{Na}^+$  selective charge inter-

action via its sulfate group (Abramson et al., 1967)) to the enzyme gate site (i.e., SGC is responsible for  $\text{K}^+$  import). Furthermore, Karlsson (1982) proposed that when SGC and  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  are localized to different cellular compartments, the pump performs  $\text{Na}^+$  uniport without reciprocal  $\text{K}^+$  transport. This was based on the observation that in  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  preparations where SGC is absent there is  $\text{Na}^+$  efflux with no  $\text{K}^+$  influx (Goldin and Tong, 1974).

In a recent study we showed that this enzyme and cofactor can be functionally segregated within a single membrane:  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  localizes to SGC-enriched microdomains or ‘lipid rafts’ (see Lai (2003) for a recent lipid raft review) in the gill basolateral membrane (BLM) of rainbow trout exposed to seawater (SW) (electrolyte secreting gill epithelia) but not freshwater (FW) (electrolyte absorbing epithelia) (Lingwood et al., 2005). Additionally, we found that arylsulfatase-induced desulfation of BLM SGC reduced  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  activity in SW but not FW trout; suggesting that partitioning between SGC-enriched rafts results in a functional difference with respect to enzyme catalysis. We proposed that the raft-mediated, co-localization of enzyme and cofactor of the SW gill was

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adaptive in the sense that it helped facilitate the up-regulation of  $\text{Na}^+-\text{K}^+-\text{ATPase}$  activity necessary for trout seawater adaptation. We also suggested that the raft-mediated, segregation of enzyme and cofactor in the FW gill, decoupled the  $\text{Na}^+-\text{K}^+-\text{ATPase}$  to a  $\text{Na}^+-\text{ATPase}$ , defining a new role for this pump in this tissue. This paper is an illustration of how SGC/membrane raft-based modulation of  $\text{Na}^+-\text{K}^+-\text{ATPase}$  transport stoichiometry can work in concert with the general thermodynamic and physiological gill transport factors and specific adaptations in pump amino acid residues to facilitate transformation to a  $\text{Na}^+-\text{ATPase}$  in FW.

## 2. Decoupling the $\text{Na}^+-\text{K}^+-\text{ATPase}$

### 2.1. Modulation of transport stoichiometry by SGC

Through the manipulation of ionic medium  $\text{Na}^+-\text{K}^+-\text{ATPase}$  can be induced to perform  $\text{Na}^+$  uniport without reciprocal  $\text{K}^+$  transport:

1. Uncoupled  $\text{Na}^+$  efflux: in the absence of extracellular  $\text{Na}^+$  and  $\text{K}^+$  the  $\text{Na}^+-\text{K}^+-\text{ATPase}$  catalyzes an ouabain-sensitive ATP supported  $\text{Na}^+$  extrusion (Garrahan and Glynn, 1967a,b; Glynn et al., 1974; Karlsh and Glynn, 1974; Glynn and Karlsh, 1975; Lew et al., 1973; Glynn, 1988); the widely accepted Albers–Post scheme (Fahn et al., 1966; Post et al., 1969) predicts a transport stoichiometry of  $3\text{Na}^+_{\text{cyt}}/1\text{ATP}$  hydrolyzed (no compensating ions are returned) which has been measured in shark rectal gland (Cornelius, 1989).
2.  $\text{Na}^+/\text{K}^+$ -congener exchange: in the absence of extracellular  $\text{K}^+$  the  $\text{Na}^+-\text{K}^+-\text{ATPase}$  catalyzes an ouabain-sensitive ATP supported  $\text{Na}^+/\text{Na}^+$  exchange in which  $\text{Na}^+$  substitutes for extracellular  $\text{K}^+$  (Lee and Blostein, 1980; Blostein, 1983); transport stoichiometry is  $3\text{Na}^+_{\text{cyt}}:2\text{Na}^+_{\text{ext}}/1\text{ATP}$  hydrolyzed (Cornelius and Skou, 1987; Yoda and Yoda, 1987).

Although such decoupling events have not been thought to occur in vivo (De Weer et al., 1988), we propose that differential partitioning of  $\text{Na}^+-\text{K}^+-\text{ATPase}$  to SGC membrane subdomains or lipid rafts represents a novel physiological mechanism for similar departures from  $3\text{Na}^+_{\text{cyt}}:2\text{K}^+_{\text{ext}}$  transport stoichiometry. Several earlier studies are consistent with this concept. An ouabain-sensitive  $\text{Na}^+$  efflux rate equivalent to that obtained in a medium lacking  $\text{K}^+$  is produced from erythrocytes treated with arylsulfatase (Zambrano et al., 1981); native ouabain-sensitive  $\text{Na}^+$  efflux is only restored by SGC repletion and not  $\text{K}^+$  addition. Additionally, arylsulfatase treatment of microsomes from pig kidney medulla inhibits both ouabain-sensitive,  $\text{K}^+$ -sensitive activity (Gonzalez and Zambrano, 1983) and  $\text{K}^+$  inducible dephosphorylation of the  $\text{Na}^+-\text{K}^+-\text{ATPase}$  phospho-intermediate (Jedlicki and Zambrano, 1985); restoration in both cases only occurs following SGC repletion and not  $\text{K}^+$  addition. These data indicate that when  $\text{Na}^+-\text{K}^+-\text{ATPase}$  has limited access to SGC, it transports  $\text{Na}^+$  only. Since arylsulfatase treatment mimics the transport dynamics of systems lacking extracellular  $\text{K}^+$ , we contend that non-SGC-enriched raft  $\text{Na}^+-\text{K}^+-\text{ATPase}$  also pumps  $\text{Na}^+$  only. The resultant transport stoichiometry is not

clear and ADP stimulated  $\text{Na}^+/\text{Na}^+$  exchange (Garrahan and Glynn, 1967a,b; De Weer, 1970; Glynn and Hoffman, 1971; Cavieres and Glynn, 1979; Kennedy et al., 1986) (in absence of  $\text{K}^+$  the enzyme can catalyze a one for one  $\text{Na}^+$  exchange; it is electroneutral and net ATP hydrolysis is zero) cannot be ruled out. Nevertheless, we predict that the  $3\text{Na}^+_{\text{cyt}}:2\text{K}^+_{\text{ext}}$  coupling ratio is predominantly conserved by SGC/raft associated enzyme.

### 2.2. $\text{Na}^+-\text{K}^+-\text{ATPase}$ vs $\text{Na}^+-\text{ATPase}$

$\text{K}^+$  independent, ouabain insensitive  $\text{Na}^+-\text{ATPase}$  activity has been detected in a number of different tissues of different organisms (Proverbio et al., 1991). These systems have been generally distinguished from  $\text{Na}^+-\text{K}^+-\text{ATPase}$  by preferential inhibition by ethacrynic acid and furosemide (Proverbio et al., 1989). However, ethacrynic acid is not an ideal inhibitor because it penetrates the cell and progressively inhibits other cell functions (Epstein, 1972a,b; Gaudemer and Foucher, 1967; Gordon, 1968). Furthermore, specificity according to furosemide sensitivity should be assessed with caution as furosemide is only a general inhibitor of  $\text{Cl}^-/\text{cation}$  co-transport (Engström et al., 1991).  $\text{Na}^+-\text{ATPase}$  and  $\text{Na}^+-\text{K}^+-\text{ATPase}$  do exhibit different responses to adenosine (Caruso-Neves et al., 1997), angiotensin II (Rangel et al., 1999) and bradykinin (Caruso-Neves et al., 1999). However,  $\text{Na}^+-\text{K}^+-\text{ATPase}$  isozymes also possess considerably different kinetic properties and modes of regulation (Mobasheri et al., 2000). Moreover, differentiation according to glycoside sensitivity is questionable since  $\text{Na}^+-\text{K}^+-\text{ATPase}$  isozymes differ in ouabain sensitivity (Blanco and Mercer, 1998). Finally,  $\text{K}^+$  independence as a criterion for defining  $\text{Na}^+-\text{ATPase}$  is suspect since in the absence of  $\text{K}^+$ , the  $\text{Na}^+-\text{K}^+-\text{ATPase}$  will perform ATP-consuming  $\text{Na}^+$  transport (see  $\text{Na}^+/\text{K}^+$ -congener exchange described above). The question of whether the functionally different  $\text{Na}^+-\text{ATPase}$  and  $\text{Na}^+-\text{K}^+-\text{ATPase}$  possess distinct structures or whether they represent two different isoform expressions of the same enzyme remains unanswered (Ventrella et al., 2001).

### 2.3. The solvent capacity of water

Compared with  $\text{Na}^+$ ,  $\text{K}^+$  is relatively large and therefore has a lower charge density. This means that  $\text{Na}^+$  packs more densely with water than does  $\text{K}^+$  (Hochachka and Somero, 1984). Several workers (Hazelwood, 1979; Ling, 1979; Wiggins, 1971, 1979) have proposed that as intracellular water is highly structured due to the presence of proteins, membranes, nucleic acids and thousands of organic metabolites, the different demands of  $\text{K}^+$  and  $\text{Na}^+$  on the solvent capacity of water can govern the ease with which these two ions enter the cell. For an inorganic cation to be accommodated in such a structured solvent, it requires a relatively low demand on solvent capacity. Selective accumulation of  $\text{K}^+$  instead of  $\text{Na}^+$  may, therefore, be a physical–chemical consequence of incorporating ions into already densely packed solutions. Ling (1979) suggested that specific ATPase ion pumps may not even be needed to maintain high intracellular  $\text{K}^+$  and low  $\text{Na}^+$ . We are not advocating this extreme view of ion transport, nevertheless the different

influences of  $K^+$  and  $Na^+$  on water structure strengthen the notion of  $Na^+-K^+$ -ATPase-catalyzed  $Na^+$  uniport without reciprocal transport in vivo.

### 3. Decoupling the pump in the freshwater gill

#### 3.1. SW and FW $Na^+-K^+$ -ATPase isoforms have different transport stoichiometric capacities

Richards et al. (2003) discovered that two gill  $Na^+-K^+$ -ATPase catalytic ( $\alpha$ ) subunit isoforms are differentially expressed during rainbow trout salinity transfer. We suggested that in contrast to the FW isoform ( $\alpha 1a$ ), the isoform upregulated in SW ( $\alpha 1b$ ) had an ability to partition into SGC-enriched membrane microdomains (Lingwood et al., 2005). There are also differences in the sequences of these isoforms that contribute to a resultant decoupled  $Na^+-K^+$ -ATPase in FW.

The efficacy of ion-binding is a function of the amino acids residues that make up a transporter's binding site. With respect to  $Na^+-K^+$ -ATPase, the residues Asn<sup>324</sup> and Asn<sup>776</sup> on the  $\alpha$ -subunit have been found to act as cation coordination centres (Jorgensen and Pedersen, 2001). Specifically, ion occlusion studies on modified  $\alpha$ -subunit from the kidney of *Sus scrofa* show that single substitution of Asn<sup>776</sup> to Lys completely eliminates high affinity binding of  $Tl^+$  (a  $K^+$  analogue); replacement of Asn<sup>324</sup> to Lys also causes a large depression in high affinity  $Tl^+$  occlusion. Substitution of both residues for Lys, however, increases  $Tl^+$  binding to one  $Tl^+$  ion occluded per ouabain binding site. Energy analysis shows that the single substitution of Asn<sup>324</sup> or Asn<sup>776</sup> to Lys reduces the free energy of binding with 5.7 or 15.8 kJ/mol, respectively, while the interaction between the two Lys residues after double Lys substitution favours  $Tl^+$  binding by  $-18.3$  kJ/mol (Jorgensen and Pedersen, 2001).

Such a double Lys substitution has been found to occur in an  $\alpha$ -subunit isoform expressed in the chloride cells of the salt gland of the brine shrimp *Artemia salina* (Baxter-Lowe et al., 1989; Escalante et al., 1995; Fig. 1); additionally, this isoform is up-regulated upon increasing salt exposure (Jorgensen and Domenech, 2005). Jorgensen and Pedersen (2001) suggested that since the electrochemical work required to transport  $Na^+$  from hemolymph to the brine shrimp's high salt environment (200 to 300 ppt) is greater than the free energy of ATP hydrolysis, the double Lys substitutions facilitated a compensatory

alteration in  $Na^+-K^+$ -ATPase transport stoichiometry. Specifically the pump (activity is 10–20% with double Lys substitution) would transport one  $K^+$  and two  $Na^+$  per ATP hydrolyzed. Upon examination of the rainbow trout FW ( $\alpha 1a$ ) and SW ( $\alpha 1b$ ) isoforms it becomes apparent that in FW there is a similar Asn to Lys substitution, but only at Asn<sup>776</sup> (Fig. 1); the SW form exhibits no Lys substitutions. The replacement therefore differs from that of the shrimp in that it is in the direction of FW adaptation and is only a single substitution. Given the fact that Lys replacement at Asn<sup>776</sup> eliminates  $K^+$  binding (interestingly much more than a single substitution at Asn<sup>324</sup>, see above), and that in this case there is no recovery of  $K^+$  occlusion by an additional Lys substitution at Asn<sup>324</sup>, we can propose that the FW isoform should transport  $Na^+$  only. The nature of that stoichiometry, given the costs of  $Na^+$  across gill epithelium will be discussed later.

#### 3.2. Physiological considerations

In order to maintain ionic homeostasis, the fish gill absorbs salt in freshwater. Although the mechanism is controversial, gill chloride cells (also termed mitochondria rich cells) house much of the machinery thought to perform this function (Perry, 1997). In the current model of salt uptake (Fig. 2A; reviewed by Marshall, 2002) an apical vacuolar type  $H^+$ -ATPase generates an electrochemical gradient favourable for the passive uptake of  $Na^+$  through an epithelial  $Na^+$  channel and acidifies the boundary layer. In the apical membrane, a  $Cl^-HCO_3^-$  antiport, possibly driven by low  $HCO_3^-$  in the boundary layer, drives  $Cl^-$  into the cell. Intracellular  $H^+$  and  $HCO_3^-$  are thought to be derived from the hydration of  $CO_2$ .  $Cl^-$  probably exits the cell via basolateral  $Cl^-$  channels, while  $Na^+$  uptake is completed by the  $Na^+-K^+$ -ATPase.

Although freshwater chloride cells exhibit lower  $Na^+-K^+$ -ATPase levels than their seawater counterparts (Sakamoto et al., 2001), these levels are far greater than those possessed by their surrounding non-chloride gill cell types (Perry, 1997). Current models of salt uptake do not require  $Na^+-K^+$ -ATPase activity beyond a generic housekeeping level. The reason for heightened  $Na^+-K^+$ -ATPase in FW chloride cells therefore remains unclear (Perry, 1997; Evans et al., 2005).

$Na^+$ -ATPase is generally found in higher levels in the basal membranes of electrolyte-adsorbing epithelia (Proverbio et al., 1991). It is suggested to produce a vectorial flux of  $Na^+$  into the interstitium that is thought to facilitate salt absorption in kidney, small intestine and gill epithelium (Proverbio et al., 1991). If non-SGC/raft associated  $Na^+-K^+$ -ATPase acting as a  $Na^+$ -ATPase performs a similar function, it explains why no  $Na^+-K^+$ -ATPase was raft associated in the FW trout (Lingwood et al., 2005); rainbow trout gill  $Na^+$ -ATPase activity is higher in fish acclimated to FW than those adapted to SW (Ventrella et al., 1992). This addresses the unclear role of  $Na^+-K^+$ -ATPase in salt uptake by the gill epithelium of freshwater fishes. Based on their existence in SW adapted gill epithelia (Evans et al., 2005), current models of salt uptake in freshwater fish gill assume putative, unidentified basolateral  $K^+$  channels to account for  $K^+$  recycling (Fig. 2A). Passive  $K^+$

	324	776
<i>A. franciscana</i> (NN)	IGIIVANVPEGLL----IVYTLTSNIPPEISPFLL	IGIIVANVPEGLL----IAYTLTSKIPPEISPFLL
<i>A. franciscana</i> (KK)	IGIIVANVPEGLL----IAYTLTSKIPPEISPFLL	IGIIVANVPEGLL----IAYTLTSNIPPEISPLL
<i>O. mykiss</i> ( $\alpha 1b$ , SW)	IGIIVANVPEGLL----IAYTLTSNIPPEISPLL	IGIIVANVPEGLL----IAYTLSSKIPPEITPFL
<i>O. mykiss</i> ( $\alpha 1a$ , FW)	IGIIVANVPEGLL----IAYTLSSKIPPEITPFL	

Fig. 1. Asn–Lys substitutions in  $Na^+-K^+$ -ATPase catalytic subunit isoforms from *Artemia franciscana*, and *Oncorhynchus mykiss* (exposed to seawater (SW) or freshwater (FW)). Brine shrimp exhibit an isoform where Asn is doubly replaced by Lys at Asn<sup>324</sup> and Asn<sup>776</sup> upon increasing salinity exposure (KK) (Jorgensen and Domenech, 2005). With respect to the trout isoforms that are differentially expressed according to salinity exposure (Richards et al. (2003)), there is a Lys substitution but only at Asn<sup>776</sup> of the FW isoform ( $\alpha 1a$ ). Sequences were obtained from the SwissProt database.

leak constitutes an enormous energetic loss for absorptive epithelia (Karlsson, 1982). Under our scheme, the majority of  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  pumps  $\text{Na}^+$  only (Fig. 2B). Therefore,  $\text{K}^+$  leak at a lower level that matches housekeeping levels of  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  catalyzed  $\text{K}^+$  influx could be assumed. This does not necessarily predict that intracellular  $\text{K}^+$  is lower in FW branchial epithelium, but rather, is recycled at a lower rate.

Transforming  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  to a  $\text{Na}^+ - \text{ATPase}$  may also help facilitate  $\text{Cl}^-$  uptake in the FW gill. Experimental evidence

indicates that both uncoupled  $\text{Na}^+$  efflux and  $\text{Na}^+ / \text{K}^+$ -congener exchange are electrogenic (Cornelius and Skou, 1987; Yoda and Yoda, 1987; Cornelius, 1989). However, uncoupled  $\text{Na}^+$  efflux from red blood cell ghosts is accompanied by an ouabain-sensitive efflux of the anions present in solution ( $\text{SO}_4^{2-}$ ) (Cornelius, 1991). Additionally, an electroneutral uncoupled  $\text{Na}^+$  efflux has been observed in proteoliposomes at pH 7.0 (Goldshleger et al., 1990); the authors suggest co-transport of  $\text{Cl}^-$  or counter-transport of  $\text{H}^+$  in order to explain this. Finally, in  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  preparations where SGC is absent

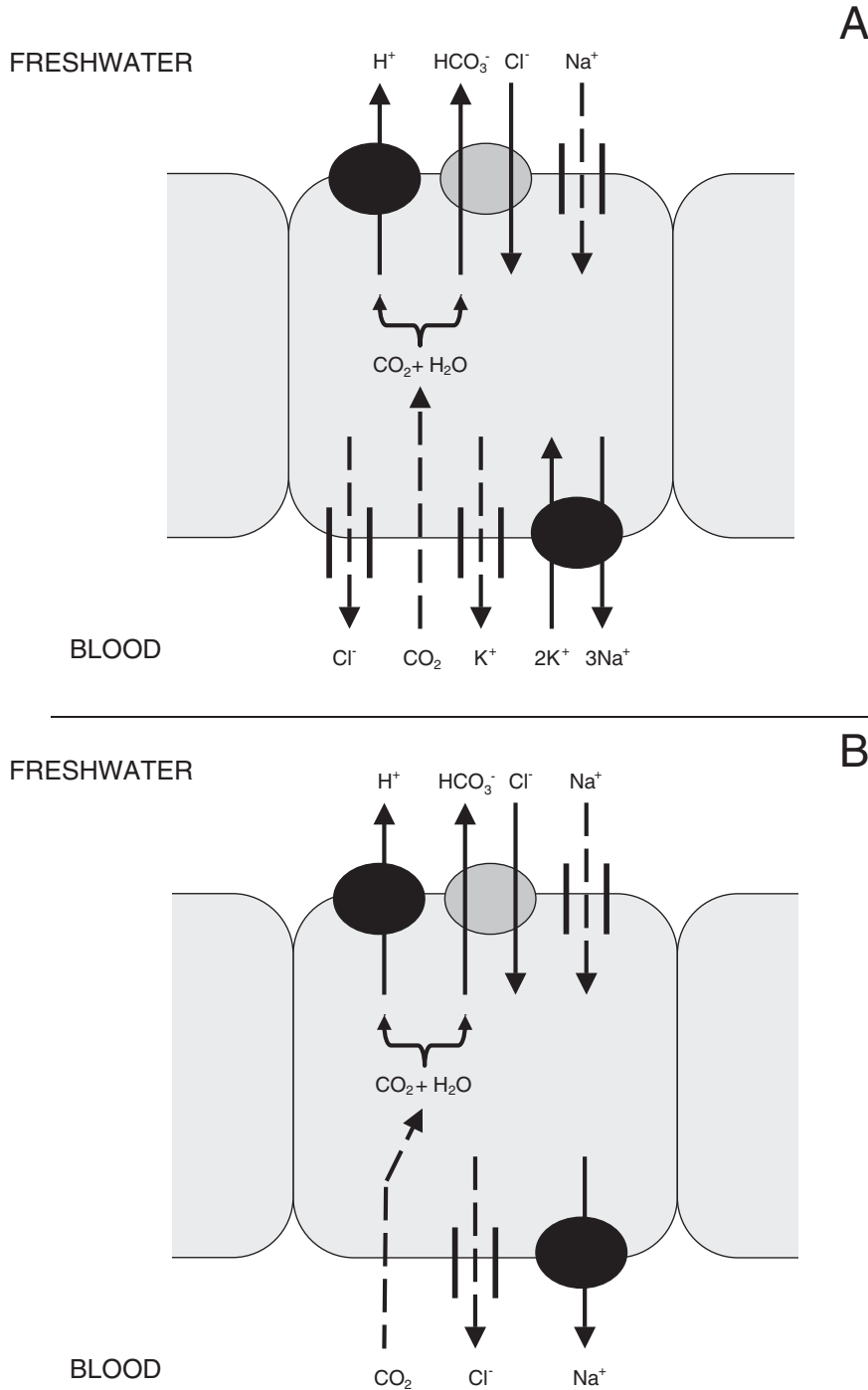


Fig. 2. Current model for freshwater gill chloride cell salt uptake (A) (Redrawn from Marshall, 2002) and proposed model with decoupled  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  (B).

Table 1  
Energetic cost of Na<sup>+</sup> transport across the gills of FW and SW adapted fish

	[Na <sup>+</sup> ] <sub>1</sub>	[Na <sup>+</sup> ] <sub>2</sub>	Δψ	Energy cost per Na <sup>+</sup>	Meaning
FW					
Apical membrane (AM <sub>FW Na<sup>+</sup></sub> )	[Na <sup>+</sup> ] <sub>cell</sub> = 66 mM (a)	[Na <sup>+</sup> ] <sub>freshwater</sub> = 1 mM (b)	-0.047 V (cell side negative) (b)	5.3 kJ/mol	Freshwater→cell active step (Na <sup>+</sup> in coupled to apical H <sup>+</sup> -ATPase)
BLM (BLM <sub>FW Na<sup>+</sup></sub> )	[Na <sup>+</sup> ] <sub>blood</sub> = 150 mM (b)	[Na <sup>+</sup> ] <sub>cell</sub> = 66 mM (a)	-0.042 V (cell side negative)	5.8 kJ/mol	Cell→blood active step (Na <sup>+</sup> out by BLM Na <sup>+</sup> -K <sup>+</sup> -ATPase)
Epithelium (EPITH <sub>FW Na<sup>+</sup></sub> )	[Na <sup>+</sup> ] <sub>blood</sub> = 150 mM (b)	[Na <sup>+</sup> ] <sub>freshwater</sub> = 1 mM (b)	-0.005 V (water reference) (b)	11.4 kJ/mol	Freshwater→blood ~ AM <sub>FW Na<sup>+</sup></sub> +BLM <sub>FW Na<sup>+</sup></sub> )
SW					
Apical membrane (AM <sub>SW Na<sup>+</sup></sub> )	N/A	N/A	N/A	N/A	No movement of Na <sup>+</sup> across AM
BLM (BLM <sub>SW Na<sup>+</sup></sub> )	[Na <sup>+</sup> ] <sub>blood</sub> = 170 mM (b)	[Na <sup>+</sup> ] <sub>cell</sub> = 37 mM (b)	-0.08 V (cell side negative)	11.2 kJ/mol	Cell→blood active step (Na <sup>+</sup> out by BLM Na <sup>+</sup> -K <sup>+</sup> -ATPase)
Epithelium (EPITH <sub>SW Na<sup>+</sup></sub> )	[Na <sup>+</sup> ] <sub>saltwater</sub> = 450 mM (b)	[Na <sup>+</sup> ] <sub>blood</sub> = 170 mM (b)	-0.025 V (water reference) (b)	-0.1 kJ/mol	Blood→saltwater passive para cellular Na <sup>+</sup> exit

Literature values used to calculate cost of Na<sup>+</sup> transport according to  $\Delta G = RT \ln([Na^+]_1/[Na^+]_2) + zF\Delta\psi$  where  $R = 8.31 \text{ J/K/mol}$ ,  $T = 283 \text{ K}$ ,  $z = +1$ ,  $F = 96.5 \text{ kJ/mol}$ ,  $\Delta\psi$  = electrical difference across barrier BLM<sub>FW Na<sup>+</sup></sub> Δψ calculated from difference between the Δψ of AM<sub>FW Na<sup>+</sup></sub> and Δψ of EPITH<sub>FW Na<sup>+</sup></sub> BLM<sub>SW Na<sup>+</sup></sub> Δψ calculated from the Goldman Equation  $\{\Delta\psi = (RT/F) \ln(([\text{K}^+]_{\text{in}} + 0.01[\text{Na}^+]_{\text{in}})/([\text{K}^+]_{\text{out}} + 0.01[\text{Na}^+]_{\text{out}}))\}$ ; a = values obtained from Morgan et al. (1994); b = values obtained from Wright (1991).

(Karlsson, 1977), Na<sup>+</sup> translocation (with no reciprocal K<sup>+</sup> transport) is accompanied by a co-efflux of Cl<sup>-</sup>, presumably by a Cl<sup>-</sup> transporter (Goldin and Tong, 1974). Current models of salt uptake by the FW gill predict that Cl<sup>-</sup> absorption into the blood is completed by a Cl<sup>-</sup> channel in the gill basolateral membrane (Fig. 2A); a translocation that would therefore be favourable in the presence of decoupled Na<sup>+</sup> transport (Fig. 2B).

### 3.3. Decoupled Na<sup>+</sup> transport energetics

Decoupled Na<sup>+</sup> transport itself appears more thermodynamically plausible in the FW gill. Using available literature values (Wright, 1991; Morgan et al., 1994) we calculated the energy barriers for Na<sup>+</sup> transport across whole epithelium, apical membrane and BLM of FW acclimated (Na<sup>+</sup> absorbing) and SW adapted (Na<sup>+</sup> secreting) gill epithelia (Table 1). Assuming no junctional potentials these values are reliable because energy is conserved in FW (EPITH<sub>FW Na<sup>+</sup></sub> ~ BLM<sub>FW Na<sup>+</sup></sub> AM<sub>FW Na<sup>+</sup></sub>) and EPITH<sub>Na<sup>+</sup></sub> is less than zero in SW (a negative value is indicative of the passive paracellular Na<sup>+</sup> extrusion driven by the transepithelial potential associated with apically concentrated Cl<sup>-</sup> (Evans et al., 2005)). BLM<sub>Na<sup>+</sup></sub> represents the cost of Na<sup>+</sup> transport by the basolaterally restricted Na<sup>+</sup>-K<sup>+</sup>-ATPase in SW and FW, whereas the cost associated with AM<sub>FW Na<sup>+</sup></sub> is consistent with the energy consumption by the apical H<sup>+</sup>-ATPase modeled for salt uptake in FW (Evans et al., 2005). Although total energetic costs to Na<sup>+</sup> movement appear similar in FW and SW (EPITH<sub>FW Na<sup>+</sup></sub> ~ BLM<sub>FW Na<sup>+</sup></sub> + AM<sub>FW Na<sup>+</sup></sub> ~ BLM<sub>SW Na<sup>+</sup></sub> + EPITH<sub>SW Na<sup>+</sup></sub> ~ 11 kJ/mol), the FW gill divides this energy into two active steps (Table 1) making it energetically cheaper to transport Na<sup>+</sup> across its BLM (BLM<sub>FW Na<sup>+</sup></sub> < BLM<sub>SW Na<sup>+</sup></sub>). Conservatively, if we assume Na<sup>+</sup>-K<sup>+</sup>-ATPase operates at <60% efficiency (Kirschner, 2004), the energy released by ATP hydrolysis in a cycle (~ -63 kJ (Civan

et al., 1983)) could support a novel 6–7Na<sup>+</sup><sub>cyt</sub>/1ATP stoichiometry (5× BLM<sub>FW Na<sup>+</sup></sub>) in FW but only the traditional 3Na<sup>+</sup><sub>cyt</sub>/1ATP stoichiometry in SW (3× BLM<sub>SW Na<sup>+</sup></sub>). We would therefore contend that decoupling Na<sup>+</sup>-K<sup>+</sup>-ATPase to a Na<sup>+</sup>-ATPase is more favourable in the Na<sup>+</sup> absorbing system.

If such decoupling is conserved across the animal kingdom, we would predict variability in the transport stoichiometry of other absorptive tissues: a 5Na<sup>+</sup><sub>cyt</sub>:1K<sup>+</sup><sub>ext</sub> antiport ratio has been measured in dog kidney medulla (Anner et al., 1977); a 3Na<sup>+</sup><sub>cyt</sub>:1K<sup>+</sup><sub>ext</sub> stoichiometry associated with greater Na<sup>+</sup> reabsorption has been observed in renal proximal tubules of Dahl salt-sensitive rats (Orosz and Hopfer, 1996); and the Na<sup>+</sup> to K<sup>+</sup> coupling ratio can exceed the value of 3Na<sup>+</sup><sub>cyt</sub>:2K<sup>+</sup><sub>ext</sub> during periods of enhanced pump activity in amphibian renal proximal tubules (Sackin and Boulpaep, 1983). The extent to which coupling plasticity is linked to Na<sup>+</sup>-K<sup>+</sup>-ATPase SGC/raft partitioning and/or adaptations in amino acid signature warrants future investigation.

## 4. Future directions

To summarize, we have proposed a thermodynamically and physiologically viable mechanism whereby Na<sup>+</sup>-K<sup>+</sup>-ATPase may be transformed to a Na<sup>+</sup>-ATPase via its separation from SGC due to partitioning into different membrane subdomains. This concept is of wide interest since it relates to the functioning of the main ion transporter in most cells with applications in understanding the regulation of all epithelial electrolyte absorbing/transport processes; particularly those of the FW gill where it is complemented by adaptations in amino acid residues and gill transport energetics. The challenge now is to consistently account for altered Na<sup>+</sup>-K<sup>+</sup>-ATPase transport stoichiometry both as a function of cation binding residues and pump position relative to SGC microdomains within the membrane.

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