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[REVIEW]

Cl^--ATPases: Novel Primary Active Transporters in Animals

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INTRODUCTION

Four mechanisms of Cl^- transport across plasma membranes have been widely documented and they are: Na^+/Cl^- symport, H^+/Cl^- symport, Cl^-/anion antiport and a passive electrochemical coupling process (Gerencser, 1996). Although there have been numerous reports of primary active transporters for Cl^- (Cl^--ATPase) existing in numerous tissues, the evidence for their actual existence and functional role(s) have been, for the most part, indirect and suspect. This review will highlight relatively new evidence supporting the hypothesis that Cl^--ATPase exists and that it mediates the transport of Cl^- across animal plasma membranes by the hydrolysis of ATP.

Since the time Durbin and Kasbekar (1965) first observed anion-stimulated ATPase activity in a microsomal fraction of frog gastric mucosa in the mid-1960's, there has been little question as to the existence of, at least, the biochemical manifestation of this enzyme. The distribution of anion-stimulated ATPase activity appears to be as widely distributed throughout biology as the number of different plants and animals studied (DePont and Bonting, 1981; Gerencser, 1989; Stekhoven and Bonting, 1981).

Anion-stimulated ATPase activity, and therefore possibly Cl^- pump existence, has been demonstrated in both microsomal and mitochondrial fractions of many tissues (Table 1) in which net ion transport occurs, suggesting a transport function for this enzyme. DeRenzis and Bornancin (DeRenzis and Bornancin, 1977) demonstrated the existence of a Cl^-/HCO_3^--stimulated ATPase in goldfish gill epithelia. It was not until this documentation in 1977 that Cl^--stimulated ATPase activity was linked with possible primary active Cl^- transport, because Cl^- stimulation of this enzyme had not been previously demonstrated.

Since then, observations of plant cell membranes (Gradmann, 1984; Hill, 1984) have yielded Cl^--pump activity and associated Cl^- accumulation which are inconsistent with the four models for Cl^- transport described previously (*vide supra*). Perhaps, the strongest and most compelling evidence for an Cl^--ATPase primary active transport mechanism resides with the observations of Gerencser (Gerencser, 1990; Gerencser and Zelezna, 1993) and Inagaki (Inagaki *et al*., 1985; Shiroya *et al*., 1989) who have characterized Cl^--ATPase activity and ATP-dependent Cl^- transport in the same plasma membrane system as well as reconstituting these activities in a liposome system (Gerencser, 1990; Zeng *et al*., 1994). Indeed, the speculation by Frizzell (Frizzel *et al*., 1979), and DePont and Bonting (1981) that Cl^--stimulated ATPases are not involved in biological Cl^- transport may have been too premature considering the recent possible strong evidence to the contrary.

LOCALIZATION

One of the most controversial issues regarding Cl^--stimulated ATPase activity is its site or anatomical localization within the microarchitecture of cells. Without question, the primary location of anion (specifically Cl^-)-stimulated ATPase activity within animal cells appears to be in the mitochondria; that is, a property of the mitochondrial H^+-ATPase (Racker, 1962). However, Gerencser and Lee (1985) presented strong evidence for the existence of Cl^--ATPase activity in a plasma membrane system free from any possible mitochondrial-contaminant ATPase. They presented evidence which indicated that purified basolateral membranes (BLM) of *Aplysia* foregut absorptive cells contained Cl^--ATPase activity. Their finding that the BLM subcellular membrane fraction had a high specific activity in (Na^++K^+)-ATPase, but had no perceptible cytochrome c oxidase activity nor succinic dehydrogenase activity, supported this conclusion. The failure of oligomycin to inhibit Cl^--ATPase activity in the BLM fraction was also consistent with the nonmitochondrial origin of the Cl^--ATPase. Supporting this contention was the corollary finding that oligomycin inhibited *Aplysia* mitochondrial Cl^--stimulated ATPase activity. The finding that efrapeptin, a direct inhibitor of mitochondrial F_1-ATPase activity (Bullough *et al*., 1982), significantly inhibited Mg^{2+}-ATPase activity in the mitochondrial and not in the BLM fraction (Gerencser and Lee, 1985) unequivocally supported the notion that the plasma membrane fraction was pure and was free from mitochondrial contamination. Additionally, Gerencser and Lee (1985) showed that vana-

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Table 1. Some zoological tissues in which anion$^-$-stimulated ATPase activity has been localized to cellular plasma membranes or microsomal fractions.

TISSUE	SPECIES	REFERENCE
Brain	Rat	Shiroya *et al.* (1989)
Embryo	Sea Urchin	Mitsunaga *et al.* (1987)
Gastric mucosa	Dog	Sachs *et al.* (1978)
	Frog	Durbin RP, Kasbekar DK (1965)
	Lizard	DePont JJHJM, Bonting SL (1981)
	Necturus	Wiebelhaus *et al.* (1971)
	Rabbit	Van Amelsvoort *et al.* (1977)
	Rat	Soumaron *et al.* (1974)
Gills	Goldfish	DeRenzis G, Bornancin M (1977)
	Eel	Bornancin *et al.* (1977)
	Trout	Bornancin *et al.* (1980)
		(Battram (1989)
	Fiddler Crab	Depew EF, Towle DW (1979)
	Blue Crab	Lee (1982)
Intestine	Eel	Morisawa M, Utida S (1976)
	Rat	Humphreys MH, Chou LYN (1979)
	Aplysia	Gerencser (1985)
Kidney	Dog	Sachs *et al.* (1978)
	Frog	Gerencser GA, Lee SH (1983)
	Mouse	Gerencser GA, Lee SH (1983)
	Rabbit	Liang CT, Sacktor B (1976)
	Rat	Kinne-Saffran E, Kinne R (1979)
Lens	Cow	Jose JG, Gassner D (1983)
Liver	Rat	Izutsu JT, Siegel IA (1972)
Mantle	Oyster	Wheeler AP, Harrison EW (1982)
Midgut	Moth	Turnbeck *et al.* (1968)
Pancreas	Cat	Simon B, Thomas L (1972)
	Dog	Simon *et al.* (1972)
	Rat	Van Amelsvoort *et al.* (1978)
Placenta	Human	Boyd CAR, Chipperfeld AR (1980)
Rectum	Larval Dragonfly	Gerencser GA, Lee SH (1983)
	Locust	Lechleitner RA, Phillips JE (1988)
Salivary gland	Dog	Izutsu JT, Siegel IA (1972)
	Rabbit	Simon *et al.* (1972)
	Rat	Gerencser GA, Lee SH (1983)
Seminiferous tubules	Rat	Gerencser GA, Lee SH (1983)
Spinal Motoneurons	Rat	Inagaki *et al.* (1985)
Uterus	Rat	Boyd CAR, Chipperfeld AR (1980)

date, an inhibitor of only “P-type ATPases” (Pedersen and Carafoli, 1987), inhibited Cl^--ATPase activity in the purified BLM fraction and not in the mitochondrial fraction. This result is consistent with the others since mitochondrial H^+-ATPase is an F-type ATPase (Pedersen and Carafoli, 1987). Taken together, all of these observations strongly supported the hypothesis that Cl^--stimulated ATPase activity exists in, at least, one subcellular locus other than mitochondria. It appears that in numerous biological cells, which transport Cl^-, Cl^--stimulated ATPase activity forms an integral part of the plasma membrane as a separate system (Gerencser, 1986; Gerencser, 1996; Gerencser and Zelezna, 1994).

FUNCTION OF Cl^--ATPases

To assign a direct role of Cl^- transcellular transport to an ATPase, the enzyme should be shown to be an integral component of the plasma membrane. The energy for active transport of Cl^- can, in principle, thus be obtained from the hydrolysis of ATP. Therefore, the following question can be asked. Is the anion-stimulated ATPase identical with a primary active transport mechanism (pump) for anions? The following discussion deals with this controversial question (Depont and Bonting, 1981; Gerencser, 1983).

The hindgut of the desert locust possesses an unusual Cl^- transport system (Hanrahan and Phillips, 1983). The isolated locust rectum absorbs Cl^- from the mucosal to the serosal side in the absence of an electrochemical potential gradient. Net Cl^- transport persists in nominally Na-free or $HCO_3(CO_2)$-free saline, is insensitive to normal inhibitors of NaCl co-transport and anion exchange, and is independent of the net electrochemical gradient for Na^+ across the apical membrane. Cl^- entry across the apical membrane is active, whereas the net electrochemical gradient across the basal membrane favors passive Cl^- exit from the cell. To determine if active Cl^- transport across rectal epithelia might be due to

an anion-stimulated ATPase, a microsomal fraction was obtained by differential centrifugation (Lechleitner and Phillips, 1988). Microsomal ATPase activity was stimulated in the following sequence: sulphite > bicarbonate > chloride. The microsomal fraction was enriched in plasma membrane markers and had little contamination from the mitochondrial enzymes. On the surface, these results indicate the presence of an anion-stimulated ATPase activity that could be responsible for active Cl^- transport across locust rectum. Recently, however, Phillips and his colleagues (Phillips *et al*., 1996) have discovered a bafilomycin-sensitive H^+ pump in the apical membrane of locust rectum. Since proton pumping could account for almost one-half of the Cl^- transported, the authors could not rule out a "proton recycling process" (Wieczorek, 1992) as responsible for at least partial net Cl^- absorption. Phillips *et al.* (1996) further states that Cl^--ATPase could be responsible for the remainder of net Cl^- absorption.

The following studies on rat brain motoneurons provided the strongest evidence in vertebrates for the existence and function of a Cl^- pump. Inagaki and her colleagues (Inagaki *et al*., 1985; Shiroya et al, 1989) demonstrated that EDTA-treated microsomes prepared from rat brain consisted mainly of sealed membrane vesicles 200–500 nm in diameter and were rich in both Cl^--ATPase and Na^+/K^+-ATPase activities. Such Cl^--ATPase-rich membrane vesicles accumulated Cl^- in an ATP-dependent and osmotically reactive manner in the presence of ouabain. The Cl^- uptake was maximally stimulated by ATP; GTP, ITP, and UTP partially stimulated Cl^- uptake, but CTP, beta, gamma-methylene ATP, ADP, and AMP did not. The ATP-dependent Cl^- uptake was accelerated by an increase in the medium Cl^- concentration. Such stimulation of Cl^- uptake by ATP was dependent on the pH of the medium, with an optimal pH of 7.4. Ethacrynic acid dose-dependently inhibited the ATP-dependent Cl^- uptake. N-ethylmaleimide completely inhibited and vanadate partially inhibited the ATP-dependent Cl^- uptake. The membrane vesicles did not accumulate H^+ in the Cl^- uptake assay medium. The ATP-dependent Cl^- uptake profile agreed with that of Cl^--ATPase activity reported previously (Inagaki *et al*., 1985; Inagaki *et al*., 1994). More recently, the Cl^--ATPase from rat brain has been reconstituted into liposomes and has been shown to support an ATP-dependent Cl^- uptake (Zeng *et al*., 1994). These data, collectively, strongly supported the idea that Cl^--ATPase in the brain actively transports Cl^- and does so for the partial maintenance of the nerve cell membrane potential.

One of the most rigorous and definitive proofs for a Cl^- pump's existence and its mode of operation rests with the following group of experiments by Gerencser and his colleagues (Gerencser, 1996; Gerencser and Lee, 1983).

BIOCHEMISTRY OF THE Cl^--ATPase

ATPase activity

Gerencser and Lee (1983, 1985) presented evidence which indicated that the BLM, and only the BLM, of *Aplysia* foregut absorptive cells contains true Cl^--ATPase activity. Biochemical properties of the *Aplysia* foregut absorptive cells BLM-localized Cl^--stimulated ATPase include the following: 1) pH optimum=7.8; 2) ATP being the most effective nucleotide hydrolyzed; 3) also stimulated by HCO_3^-, SO_3^{-2}, and $S_2O_3^{-2}$, but inhibited by NO_2^-, and no effect elicited by NO_3^- and SO_4^{-2}, 4) apparent K_m for Cl^- is 10.3 mM while the apparent K_m for ATP is 2.6 mM; and 5) an absolute requirement for Mg^{2+} which has an optimal concentration of 3 mM (Gerencser and Lee, 1985). Coincidentally, Cl^- has an intracellular activity (Gerencser, 1983) in the *Aplysia* foregut epithelial cell approximating its apparent K_m for the Mg^{2+}-dependent Cl^--ATPase, which supports the interrelationship of its physiological and biochemical activities.

Transport Activity

To elucidate both the nature and electrogenicity of the ATP-dependent Cl^- transport process, several experimental maneuvers were performed by Gerencser (1988) as follows. First, an inwardly directed valinomycin-induced K^+ diffusion potential, making the BLM inside-out vesicle interior electrically positive, enhanced ATP-driven Cl^- uptake compared with vesicles lacking the ionophore. Second, an inwardly directed FCCP-induced H^+ electrodiffusion potential, making the BLM inside-out vesicle interior less negative, increased ATP-dependent Cl^- uptake compared to control. Third, ATP increased intravesicular negativity measured by lipophilic cation distribution across the vesicular membrane. Both ATP and Cl^- appeared to be necessary for generating the negative intravesicular membrane potential, because substituting a nonhydrolyzable ATP analog for ATP, in the presence of Cl^- in the extravesicular medium, did not generate a potential above that of control. Likewise, substituting NO_3^- for Cl^- in the extra- and intravesicular media, in the presence of extravesicular ATP, caused no change in potential difference above that of control. These results also suggested that hydrolysis of ATP is necessary for the accumulation of Cl- in the vesicles. Furthermore, vanadate and thiocyanate inhibited both the (ATP+Cl^-)-dependent intravesicular negativity and ATP-dependent Cl^- uptake (Gerencser *et al*., 1988); and in addition, it had been demonstrated that the pH optimum of the Cl^--stimulated ATPase (Gerencser and Lee, 1985) coincided exactly with the pH optimum of 7.8 of the ATP-dependent Cl^- transport in the *Aplysia* foregut absorptive cell BLM vesicles. Bafilomycin had no effect on either ATP-dependent potential change or ATP-dependent Cl^- transport (Gerencser and Purushotham, 1995), supporting the notion that this transporter was a P-ATPase and not a V-ATPase, since bafilomycin is an inhibitor of V-ATPase activity (Wieczorek, 1992). Further buttressing this observation was the observation that DCCD, an inhibitor of P, V or F-ATPase proton pumps (Pedersen and Carafoli, 1987), had no effect on the ATP-dependent transport parameters: Cl^- transport or vesicular membrane potential change. These results negated a proton-recycling mechanism (Wieczorek, 1992) as the means for net Cl^- uptake in BLM vesicles. Finally, all three aspects of the BLM-localized Cl^- pump (ATPase, ATP-dependent Cl^- transport and ATP-

dependent vesicular membrane potential change) have the same pH optimum and have the same Mg^{2+} and Cl^- kinetic parameters (Gerencser and Purushotham, 1995; Gerencser and Purushotham, 1996), which suggests that these properties are part of the same molecular mechanism.

Reconstitution of the Cl^- Pump

Reconstitution of a membrane protein into a liposome provides one of the few methods needed to rigorously demonstrate the existence of a separate and distinct biochemical and physiological molecular entity. This method also provides evidence that all components of the solubilized protein have been extracted intact. With this premise in mind, Gerencser (1990) reconstituted both aspects of the Cl^- pump; that is, the catalytic (ATPase) and transport components from the BLM of *Aplysia* gut absorptive cells. Cl^--stimulated ATPase activity existed significantly above Mg^{2+}-stimulated ATPase activity found in the proteoliposome population extracted and generated with digitonin. Vanadate abolished this Cl^--stimulated ATPase activity. From this digitonin-generated proteoliposome population, there is a significant ATP-dependent Cl^- uptake into these proteoliposomes above that of control, and that this ATP-dependent Cl^- uptake was also abolished by vanadate. These data suggested that these two major observations are manifestations of one molecular mechanism: the Cl^- pump. Support of this contention rested with the findings that vanadate (an inhibitor of P-type ATPases) inhibited both Cl^--stimulated ATPase activity and ATP-dependent Cl^- transport in the digitonin-based proteoliposomes. Even though Krogh (1937) first coined the term "Cl^- Pump" in 1937, it was not until the reconstitution of all of its components into an artificial liposomal system through the study mentioned above (Gerencser, 1990) that the existence of this mechanism (primary active transport mechanism) was proven. Similar reconstitutions of Cl^- pump activity have since been reported in bacteria (Zimanyi and Lanyi, 1989), alga (Ikeda and Oesterhelt, 1990) and rat brain (Zeng et al, 1994; Inagaki et al, 1994). However, the alga studies (Ikeda and Oesterhelt, 1990) are somewhat ambiguous since Cl^- inhibited the Mg^{2+}-ATPase activity despite there being an ATP-dependent Cl^- uptake into the proteoliposomes.

Molecular Weight

Utilizing electrophoretic techniques to digitonin-generated proteoliposomes containing the Cl^- pump protein from *Aplysia* gut absorptive cells as shown previously (Gerencser, 1990), the approximate molecular weight of the Cl^- pump was ascertained (Gerencser and Zelezna, 1993). Since both aspects of the Cl^- pump were inhibited by vanadate, it was surmised that the approximate molecular weight of the Cl^- pump of *Aplysia* should be around 100 kDa since vanadate only inhibited "P" type ATPases and not "F", or "V" type ATPases (Pedersen and Carafoli, 1987). The alpha-subunit of all "P" type ATPases approximates 100 kDa in molecular weight. One major protein band was eluted through PAGE and its molecular weight was found to be 110 kDa (Gerencser and Zelezna, 1993). Recently, similar molecular weights have been obtained for Cl^- pump catalytic subunits in alga (Ikeda and Oesterhelt, 1990; Moritani *et al*., 1994) and rat brain (Inagaki *et al*., 1994) confirming the possible E_1-E_2 nature of the ATPase, although the authors of these studies postulate these structural subunits to be part of a V-type ATPase assembly. These conclusions were reached despite the ATPases being partially inhibited by vanadate, a specific inhibitor of P-ATPases (Pedersen and Carafoli, 1987).

Reaction Mechanism

The semi-purified protein (Cl^- pump) had been subjected to phosphorylation within the proteoliposome and the reaction sequence and kinetics of the reaction sequence of the enzyme have been determined: Mg^{2+} causing phosphorylation, Cl^- causing dephosphorylation, and all in a time frame consistent with an aspartyl phosphate linkage (Gerencser and Zelezna, 1993; Gerencser and Zelezna (1994). Hydroxylamine and high pH destablized this phosphorylation confirming an acyl phosphate bond as an intermediate in the reaction sequence (Vara and Serrano, 1982). Vanadate almost completely inhibited the Mg^{2+}-driven phosphorylation reaction, which corroborates the protein catalytic subunit molecular weight of 110 kDa and it also defines the protein as a "P" type ATPase, because vanadate is a transition state inhibitor of phosphate (Pedersen and Carafoli, 1987). Fig. 1 is an operational model of the reaction sequence of the *Aplysia* Cl^- pump.

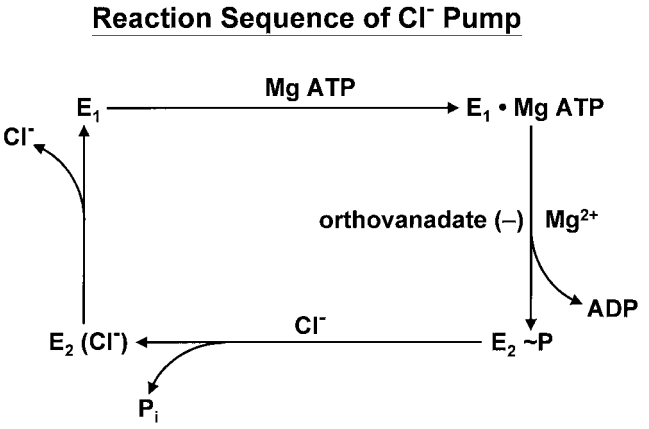

Fig. 1. Working model of reaction sequence for Cl^- pump. E_1 and E_2 are assumed to be different conformational states of the enzyme since it has been demonstrated that all P-type ATPases have at least two major conformational states (Schuurmans and Bonting, 1981). (–) represents inhibition by orthovanadate of the Mg^{2+}-driven phosphorylation reaction. Taken from reference (Gerencser and Zelezna, 1993) with permission.

Stoichiometry

The stoichiometry of ATP hydrolyzed to Cl^- transported during a single cycle of the reaction sequence was ascertained through thermodynamic means (Gerencser, 1993). Intracellular concentrations of ATP, ADP and inorganic phosphate were determined and, coupled with an estimate of the standard free energy of hydrolysis for ATP, the operant free

energy for ATP hydrolysis was calculated. Because the operating free energy of the Cl^- pump (electromotive force) was approximately one-half the energy (140 mV) obtained from the total free energy of ATP hydrolysis (270 mV), the only possible integral stoichiometries were one or, at the most, two Cl^- transported per cycle per ATP hydrolyzed. Physiologically, the electrogenic Cl^- pump (Gerencser, 1988), most likely, transports one Cl^- per ATP hydrolyzed per reaction cycle. This increased electrochemical driving force created by the electrogenic nature of the pump could fuel secondary, electrophoretic (or electroneutral) transport processes such as the nutritional uptake of sugars and/or amino acids (Gerencser *et al*., 1988).

Phosphorylation

When the relatively pure proteoliposomal preparation of Cl^--stimulated ATPase (Gerencser and Zelezna, 1993) was incubated with (γ-^{32}P)ATP, there was a fairly rapid formation of phosphoenzyme which remained stable for 4 min (Gerencser, 1993). This finding and the finding that there is a curvilinear relationship between labeled ATP concentrations and phosphoprotein levels suggests that at low ATP concentrations phosphoprotein formation is directly proportional to the ATP concentration; that is, the system follows Michaelis-Menten kinetics. The relatively high K_m of ATP for the protein (1.25 mM) suggests a relatively low affinity of the nucleotide for the enzyme. These findings are similar to what has been found for ATP affinity for fungal and plant P-type proton pumps (Vara and Serrano, 1982; Slayman and Zuckier, 1989). The finding of a millimolar K_m value was very surprising in view of well-established micromolar K_m values for ATP reactivity in P-type ATPases from various animal cells. During hydrolysis by brain or electroplax Na^+/K^+-ATPase, ATP reactivity to the enzyme exhibited a K_m below 1 μM (Plesner *et al*., 1981). Phosphorylation studies on the Ca^{2+}-ATPase of rabbit sarcoplasmic reticulum have yielded ATP-K_m values of approximately 10 μM (Fernandez-Belda *et al*., 1981). The H^+/K^+-ATPase from hog gastric mucosa displays two kinetic components for ATP hydrolysis; one with a K_m near 2 μM, and the other with a K_m near 50 μM (Wallmark *et al*., 1980).

Despite some exaggeration due to methodologic differences, it seems likely that the disparity of K_m values between the Aplysia Cl^--ATPase, fungal and plant proton ATPases and other cation P-type ATPases is real. One possible interpretation is that the high ATP-K_m ATPases might reflect obligatory activation by nucleotide binding at a nonhydrolyzing, low-affinity binding site, thereby masking the higher-affinity ATP at the catalytic site, such as has been proposed for the Ca^{2+}-ATPase of sarcoplasmic reticulum (Inesi and Kirtley, 1992). This suggestion is circumstantially supported by a slight sigmoidicity in plots of membrane potential, H^+-ATPase activity, and vesicular H+ transport, against ATP concentration in *Neurospora* (Perlin *et al*., 1984).

It was also found that adding large amounts of unlabeled ATP concentrations to the incubation medium increased the amount of enzyme dephophosphorylation which suggests that there is at least one binding site for phosphate on the protein (Gerencser, 1993). This premise was strengthened by the observation that the ATP molecule also needed to be hydrolyzed in order for this phenomenon to occur. These experiments also suggested that one of the rate-limiting factors for Cl^--ATPase phosphorylation-dephosphorylation is ambient ATP concentrations as also found in plant H^+-ATPase (Vara and Serrano, 1982).

In summary of these phosphorylation experiments, it appears that the Cl^--stimulated ATPase is a P-type ATPase similar in characteristics and reaction scheme to those described for various cation-pumping P-type ATPases present in plasma membranes from a diversity of biological organisms (Pedersen and Carafoli, 1987; Slayman and Zuckier, 1989). However, it appears that there are two types of this kind of P-type ATPase. One has a high (micromolar) affinity for ATP and is prevalent, for the most part, in plasma membranes of higher animals while the other group has a (millimolar) low affinity for ATP and is present in bacteria (Slayman and Zuckier, 1989), fungi (Slayman *et al*., 1973), plants (Vara and Serrano, 1982) and in molluscs (Gerencser and Zelezna, 1993; Gerencser, 1993).

Kinetics

Utilizing a purified BLM vesicle preparation containing Cl^--ATPase from *Aplysia* gut, it was demonstrated that ATP, and its subsequent hydrolysis, stimulated both intravesicular Cl^- accumulation and intravesicular negativity with almost identical kinetics (Gerencser and Purushotham, 1995). Additionally, in the proteoliposomal preparation the apparent K_m's of Cl^- concentration for ATP-dependent Cl^- uptake, ATP-dependent membrane potential change and Cl^--stimulated ATPase activity were almost identical to each other (Gerencser and Purushotham, 1996). These values were similar to what had been reported for Cl^--ATPase activity in the *Aplysia* BLM preparation (Gerencser and Lee, 1985) and in rat brain motoneurons (Inagaki *et al*., 1994).

Similarly, the apparent K_m's of ATP for ATP-dependent Cl^- uptake, ATP-dependent membrane potential change and Cl^--stimulated ATPase in the proteoliposomal preparation were similar to each other (Gerencser and Purushotham, 1996) and to the apparent K_m for ATP found for Cl^--ATPase in the BLM of *Aplysia* (Gerencser and Lee, 1985) and for ATP-induced phosphorylation of Cl^--ATPase in the same proteoliposomal preparation of *Aplysia* (Gerencser and Zelezna, 1994; Gerencser, 1993). These kinetic experiments demonstrate the correspondence between overall ATPase activity, Cl^--ATPase phosphorylation, ATP-dependent Cl^- transport, ATP-dependent membrane potential change and Cl^--ATPase activity which are similar to those characteristics detected in cation-activated and cation-motive ATPases (Pedersen and Carafoli, 1987; Slayman and Zuckier, 1989).

These kinetics are uniquely significant not only because they are the first and only results obtained with an isolated protein anion transporter ATPase but because they demonstrate the interrelationship, interchangability and universality

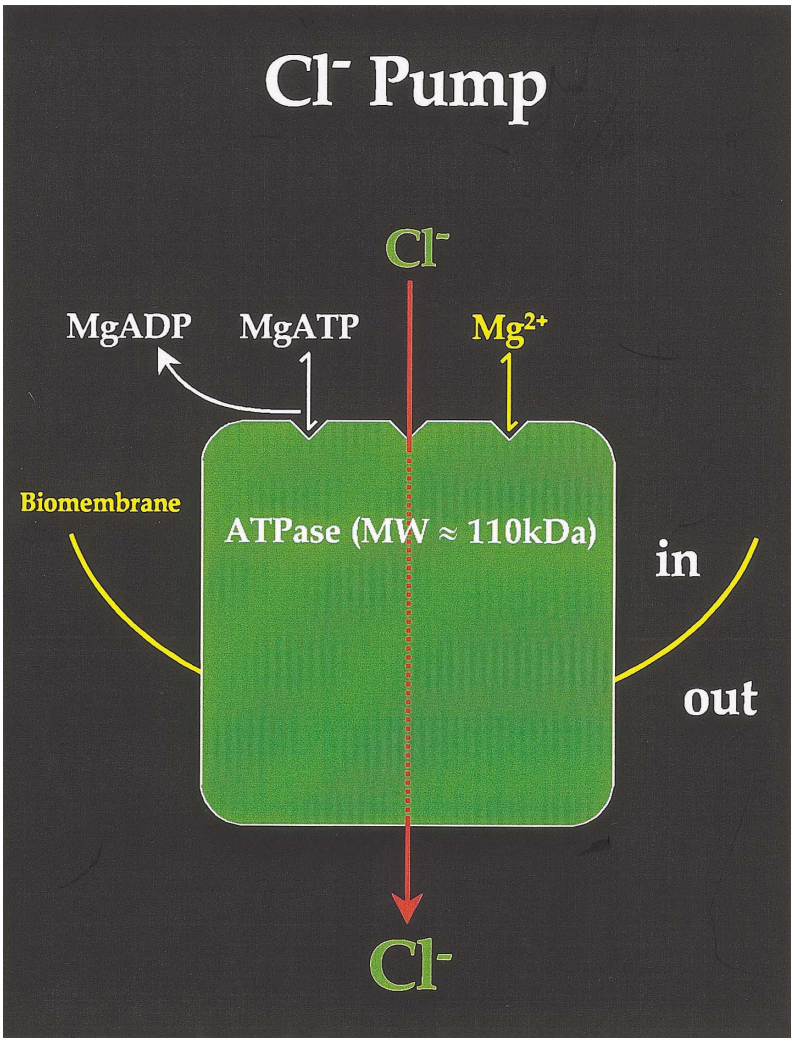

Fig. 2. Operational model of the Cl^--stimulated, Cl^--translocating ATPase, or Cl^- pump in *Aplysia* gut. Taken from reference (Gerencser and Purushotham, 1996) with permission.

between both transport and catalysis of the Cl^--ATPase ion pump. Figure 2 is an operational model of the Cl^- pump in *Aplysia* gut.

CONCLUSIONS

In summary, the demonstrations of reconstitution and phosphorylation of Cl^--ATPase provides the first direct evidence for the existence of a new P-type ATPase: the Cl^-- pump. Future studies should include constructing cDNA probes from a partially sequenced Cl^--ATPase protein that can then transcribe on RNAs of the Cl^- pump protein. The mRNAs should then be shown to translate into Cl^- pump proteins in a non-Cl^- pump containing plasma membrane system.

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