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CLC chloride channels and transporters

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CLC proteins are found in cells from prokaryotes to mammals and perform functions in plasma membranes and intracellular vesicles. Several genetic human diseases and mouse models underscore their broad physiological functions in mammals. These functions range from the control of excitability to transepithelial transport, endocytotic trafficking and acidification of synaptic vesicles. The recent crystallization of bacterial CLC proteins gave surprising insights into CLC Cl^--channel permeation and gating and provides an excellent basis for structure–function studies. Surprisingly, the CLC from *Escherichia coli* functions as a Cl^-/H^+ exchanger, thus demonstrating the thin line separating transporters and channels.

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Introduction

The CLC gene family (see glossary) was identified almost 15 years ago [1] by the cloning of ClC-0, a chloride channel enriched in the electric organ of the marine ray *Torpedo* [2]. Since then, nine mammalian CLC genes have been identified, their products characterized, and prokaryotic CLCs crystallized. Mammalian CLC proteins can be grouped into three branches by homology (see Table 1). Members of the first branch, which includes ClC-1, the ortholog of ClC-0, function as plasma membrane ion channels. Members of the two other branches reside mainly in intracellular membranes. This prevented a biophysical characterization of ClC-6 and ClC-7, but upon overexpression, ClC-3 to -5 yielded plasma membrane currents. However, in the absence of single-channel recordings, and because their extreme rectification precludes determinations of reversal potentials, it is uncertain whether they are Cl^--channels or electrogenic Cl^-/H^+ exchangers, as was recently shown for the prokaryotic protein ClC-e1 [3••]. The crystal structures of prokaryotic CLC proteins [4,5••] provided detailed insights into their structure and function. The broad physiological roles of CLC channels are apparent from mouse models and human genetic diseases, which range from myotonia to renal salt loss, kidney stones, deafness, and osteopetrosis. A recent in-depth review of the physiological functions of CLC proteins is available [6].

The structure and function of CLC proteins

CLC Cl^--channels are (homo)dimers in which each of the two subunits has its own pore. This 'double-barrel' model was postulated based on the biophysical analysis of reconstituted *Torpedo* ClC-0 channels [2]. After the cloning of ClC-0 [1], patch-clamp analysis of ClC-0 wild type/mutant [7,8] and ClC-0/ClC-2 heteromers [9] strongly supported this concept and revealed that a pore is formed entirely within each subunit [9]. The crystal structures of bacterial CLC proteins [4,5••] confirmed these conclusions and marked a breakthrough in understanding CLC ion permeation and gating. Each CLC subunit has 17 intramembrane α helices [4]. Three chloride binding sites were identified on each subunit [4,5••]. In the central site, which might represent the selectivity filter, the anion is coordinated by the positive dipoles at the ends of four α helices [4]. Even before crystallization, several of these Cl^--coordinating residues had been mutated in vertebrate CLC channels, and this process moderately changed their ion selectivity. In the crystal, the extracellular access of chloride to this central binding site is blocked by a glutamate side chain [4]. This suggested that this side chain was responsible for the previously described gating by the permeant anion [10]. The glutamate is highly conserved, with the notable exception of ClC-K channels, which have valine at this position. ClC-K channels indeed show little gating [11,12]. Gating was introduced, however, by mutating this valine to glutamate [11]. Conversely, voltage- and Cl^--dependent gating of ClC-0 was abolished when its 'gating glutamate' was mutated [5••,13•]. The crystal structure of an *Escherichia coli* ClC-e1 mutant in which an alanine replaced the 'gating glutamate' revealed that the space normally occupied by the side chain now contained a Cl^- ion [5••]. This mutant might mimic a conducting configuration, and suggests that chloride opens the channel by replacing the negative glutamate side chain.

Surprisingly, the prokaryotic ClC-e1 is an ion exchanger and not a Cl^- channel [3••]. It is highly electrogenic and probably exchanges 2 Cl^- ions for 1 H^+. Mutating the 'gating glutamate' to alanine eliminated the H^+ coupling to Cl^- flux [3••]. The mutant mediated electrogenic Cl^-

Glossary

Bartter syndrome: Hereditary kidney diseases that are associated by a massive renal salt loss. The syndrome is genetically heterogeneous, with Bartter syndrome III caused by mutations in *CLCNKB*, the gene encoding the ClC-Kb α-subunit, and Bartter syndrome IV caused by mutations in *BSND*, the gene encoding the barttin β-subunit. Bartter IV is additionally associated with congenital deafness.

CBS domains: Protein domains that are named after cystathionine β-synthase, a protein in which they are found; in most proteins (including CLCs) there are two copies present and they were recently shown to bind to ATP and AMP.

CFTR: The 'cystic fibrosis transmembrane conductance regulator' is a cAMP-regulated Cl^- channel that belongs to the gene family of ABC transporters, which generally function as active pumps.

CLC gene family: Family of genes encoding Cl^- channels and transporters as originally identified by the expression cloning of ClC-0 from the electric organ of the marine ray *Torpedo marmorata*. The gene family is conserved from bacteria to mammals. Some of its members function as Cl channels (such as ClC-0, -1, and -2), whereas others (such as the bacterial ClC-e1 from *E. coli*) function as Cl^-/H^+ exchangers. The mammalian CLC proteins are called ClC-1, ClC-2, and so on, whereas the nomenclature for the human genes is *CLCN1*, *CLCN2* and so on.

Dent's disease: Inherited, X-chromosomal kidney disease caused by mutations in ClC-5. It is associated with the loss of low molecular weight proteins into the urine (LMW proteinuria) and — more variable but clinically more important — by hyperphosphaturia, hypercalciuria, and kidney stones.

Gating of 'double-barreled' CLC channels: CLC proteins assemble to form dimers, in which each monomer has its own ion-translocation pathway. This applies both for the bacterial proteins — which are now known to be Cl^-/H^+ exchangers — and for the Cl^- channels such as the prototype ClC-0 channel from *Torpedo*. In the latter 'double-barreled' channel, each of the pores can open and close individually (controlled by a fast 'protopore gate'), or by way of a 'common' gate that closes both pores simultaneously. The gating of the protopores is best understood: it is gated by the permeant anion and depends on a glutamate side-chain that blocks access to the narrowest part of the permeation pathway in the crystallized *E. coli* protein.

Hypercalciuria: Excessive calcium loss into the urine.

Hyperphosphaturia: Excessive phosphate loss into the urine.

Osteopetrosis: An inherited bone disease that results in fragile bone that is excessively calcified. It is most often caused by impaired bone resorption due to a defect in osteoclasts, the specialized cells that degrade bone.

Resorption lacuna: Acidic compartment between an osteoclast and the bone substrate. It is faced by the 'ruffled border' of the osteoclast, an highly infolded, specialized plasma membrane that secretes acid through an H^+-ATPase.

WW-domain: Protein interaction domains containing two conserved tryptophans (W). They are found many proteins, for example in the ubiquitin ligases Nedd4-2 and WWPII, and can interact with several recognition sequences, for example proline and tyrosine- containing 'PY' motifs.

movement, but did not exhibit significantly higher fluxes as might be expected when converting a transporter into a channel. The mechanism of the exchange activity and its relation to CLC channel gating remains unclear.

Although the bacterial protein is an ion exchanger, its crystal structure proved highly useful to explain results from previous mutagenesis studies and to guide new experiments [5••,13•–15•,16]. It also served as the basis for model calculations [17–19]. An inhibitor binding site in ClC-1 has been mapped with considerable detail [14•]. It lies on the cytoplasmic side of the 'selectivity filter' and overlaps with the more cytoplasmic Cl^- binding site [5••]. An extracellular binding site for inhibitors of ClC-Ka/barttin heteromeric channels was also characterized [15•]. The voltage-dependent block of ClC-0 and several mutants indicated that gating involves a larger conformational change than just a relocation of the glutamate side chain [13•,16]. This concurs with the observation that the E166A mutant retained some gating transitions in patch-clamp recordings [5••,13•].

In contrast to the bacterial CLC proteins that were crystallized [4], mammalian CLC proteins have large cytoplasmic tails that contain two CBS domains (CBS domains are protein domains named after cystathionine β-synthase, one of the proteins in which they are found; see glossary). Two recent mutagenesis studies investigated the roles of the CBS domains in ClC-1 [20,21]. Mutating crucial amino acids in the CBS domains of ClC-1 and ClC-0 affected the common gate, which simultaneously affects both pores of the dimeric channel [20]. The structural basis for this effect might involve the binding of CBS domains to the transmembrane part of the channel. Alternatively, these domains might affect gating through the last intramembrane helix R, which participates in the selectivity filter [4]. Future insights will come from the crystallization of other bacterial CLC proteins that have CBS domains. Interestingly, CBS domains bind ATP and AMP [22••] and were, therefore, suggested as energy-sensing modules. There is only limited information on a possible role of ATP and/or AMP in regulating CLC channels. The total replacement of cytoplasmic ATP by AMP accelerated the gating of ClC-2 [23•]. This effect was diminished with a point mutation that changed a residue between the two CBS domains [23•] and that was found in an epileptic family [24•].

ClC-2, a broadly expressed plasma membrane channel

ClC-2 is a plasma membrane channel that is activated by hyperpolarization, cell swelling and mild extracellular acidification. The disruption of this nearly ubiquitous channel in mice caused retinal and testicular degeneration [25]. It was suggested that photoreceptors and germ cells die because the pH in the narrow cleft between these cells and retinal pigment epithelial cells and Sertoli cells, respectively, cannot be regulated properly in the absence of ClC-2. The transepithelial transport of lactate that occurs in both tissues imposes an acid load on these clefts. Its pH might be regulated by Cl-/HCO3- exchangers that need Cl- recycling through ClC-2 for their operation [25].

ClC-2 has been discussed as an alternative pathway for cystic fibrosis transmembrane conductance regulator

Table 1

Properties of CLC proteins

Name	Species	Expression		Biophysics	Function	Mouse model	Human disease
		Tissue	Membrane				
ClC-0	*Torpedo* (fish)	Electric organ	PM(non-innervated membrane)	Cl$^-$channel common gate: activated by hyperpol. Individual gates: activated by depol.	Stabilization of V	n.a.	n.a.
ClC-1	Mammals	Skeletal muscle	PM	Cl$^-$ channel common and individual gates: activated by depol.	Stabilization of V	Myotonia (spontaneous mutant *adr)*	Myotonia (recessive and dominant)
ClC-2	Mammals	Broad	PM (basolateral in intestine)	Cl$^-$ channel activated by: hyperpol, swelling, acidic pH$_o$	Epithelial transport, regulation of pH, cell volume, [Cl]$_i$?	Degeneration of testes and retina	Epilepsy (?)
ClC-Ka + barttin	Mammals	Kidney and ear	PM (basolateral)	Cl$^-$ channel V-independent activated by: alkaline pH$_o$, [Ca]$_o$	Epithelial transport	Diabetes insipidus	Together: Bartter IV (renal salt loss + deafness)
ClC-Kb + barttin	Mammals	Kidney and ear	PM (basolateral)	Cl$^-$ channel V-independent activated by: alkaline pH$_o$, [Ca]$_o$	Epithelial transport		Bartter III renal salt loss
ClC-3	Mammals	Broad	Endosomes, synaptic vesicles	Cl$^-$ channel or Cl$^-$/H$^+$ exchanger? Depol.-activated, pH- sensitive currents	Endosomal and/or synaptic vesicle acidification	CNS degeneration (loss of hippocampus, blindness)	
ClC-4	Mammals	Broad	Endosomes	Cl$^-$ channel or Cl$^-$/H$^+$ exchanger? Depol.-activated, pH- sensitive currents	Endosomal acidification (?)		
ClC-5	Mammals	Kidney intestine	Endosomes	Cl$^-$ channel or Cl$^-$/H$^+$ exchanger? Depol.-activated, pH- sensitive currents	Endosomal acidification, endocytosis	Proteinuria, change in calciotropic hormones	Dent's disease, proteinuria, kidney stones
ClC-6	Mammals	Broad	Endosomes	Channel or exchanger? (no currents available)	Endosomal acidification (?)		
ClC-7	Mammals	Broad	Late endosomes, lysosomes	Channel or exchanger? (no currents available)	Lysosomal acidification (?) (no change in pH detected)	Osteopetrosis, blindness, lysosomal storage disease	Osteopetrosis (recessive and dominant)
ClC-e1	Bacteria (*E. coli*)	n.a.		2 Cl$^-$/H$^+$ exchangers	Acid resistance	n.a.	n.a.

The nine mammalian CLC proteins are shown within the lines, with the three branches indicated by different color shading. Members of the ClC-3,-4,-5 branch, in addition to ClC-6 and ClC-7 of the third branch, reside predominantly in intracellular membranes. The 'founding member', ClC-0 from the *Torpedo* electric organ, is shown at the top. Its closest relation is the mammalian skeletal muscle Cl$^-$ channel ClC-1 of the first mammalian branch. These proteins function as Cl$^-$ channels and perform their function in the plasma membrane (PM). The bacterial ClC-e1, shown at the bottom, has been crystallized and surprisingly functions as a highly electrogenic 2Cl$^-$/H$^+$ exchanger. It is not yet clear whether the mammalian vesicular CLCs (indicated by blue shading) function as Cl$^-$ channels (as previously assumed) or, similar to the bacterial protein, as ion exchangers. Currents could only be measured for ClC-3, -4, and -5, because they can reach the plasma membrane upon overexpression. However, their extreme outward rectification precluded precise determinations of reversal potentials and no single channel recordings are available. The voltage-dependence of currents, when available, is indicated. ClC-0 and ClC-1 clearly have two gates, a 'common gate' that closes the two pores of the double-barreled channel simultaneously, and individual gates for each pore. Abbreviations: depol, depolarized; hyperpol, hyperpolarized.

(CFTR; see glossary) in Cl$^-$-transporting epithelia. However, mice with both CFTR and ClC-2 disrupted survived longer than mice lacking only CFTR, indicating a mitigation of their intestinal problems [26•]. Indeed, ClC-2 knockout (KO) colon showed more Cl$^-$-secretion than wild type colon [26•]. This suggested that ClC-2 is not co-localized with apical CFTR, but, rather, is present in the basolateral membrane. This is supported by functional data from permeabilized colonic epithelia [27] and by recent knockout-controlled immunocytochemistry (AA Zdebik, TJ Jentsch, unpublished).

ClC-2 might influence the concentration of cytoplasmic Cl$^-$ in neurons, prompting speculation that its disruption might cause epilepsy by interfering with γ-amino-butyric acid (GABA)-mediated inhibition [28]. Although ClC-2 KO mice do not have convulsions [25], ClC-2 mutations co-segregated with epilepsy in three families [24•]. The most convincing mutation abolished channel function by truncating the protein. The reported dominant negative effect of this mutant [24•], however, could not be reproduced [23•]. This implies that its heterozygous presence in the reported family [24•] reduces ClC-2 currents by

only 50%. As searches for ClC-2 mutations in other cohorts with epilepsy revealed sequence changes that were probably only polymorphisms [29], a final conclusion about ClC-2 and epilepsy awaits further findings.

ClC-K/barttin heteromeric chloride channels: transepithelial transport in the kidney and the inner ear

ClC-Ka and ClC-Kb are homologous channels with a high degree of amino acid identity that need the accessory β-subunit barttin for functional expression [12]. Barttin is a ~ 40 kDa protein with two predicted transmembrane domains that is crucial for the transport of ClC-Ka and ClC-Kb to the plasma membrane.

ClC-K/barttin heteromeric channels perform transepithelial transport in the kidney and in the inner ear. ClC-Kb mutations underlie the severe renal salt loss in Bartter syndrome III (see glossary) [30], showing that basolateral chloride exit through ClC-Kb/barttin is crucial for salt reabsorption in the thick ascending limb of Henle's loop. The disruption of the mouse ortholog of ClC-Ka causes diabetes insipidus [31], whereas the inactivation of barttin entails severe salt loss and deafness in human Bartter syndrome IV [32]. Congenital deafness is ascribed to defective K^+ secretion by the stria vascularis, which controls the composition of the fluid contacting apical membranes of sensory hair cells. ClC-Ka/barttin and ClC-Kb/barttin, which are both present in the stria, are necessary for basolateral Cl^--recycling [12]. Only the elimination of both ClC-Ka/barttin and ClC-Kb/barttin, as observed with a loss of the common β-subunit, reduces Cl^--recycling enough to cause deafness [12]. This model has now been confirmed by an exceptional family with Bartter syndrome IV displaying mutations in both ClC-Ka and ClC-Kb [33].

In its carboxy-terminus, barttin contains a sequence motif that might serve as a so-called 'PY motif' that interacts with WW-domains of other proteins (see glossary). Mutating this motif increased surface expression and currents of CIC-K channels [12]. Although strong overexpression of the WW-domain containing ubiquitin ligase Nedd4-2 was reported to reduce ClC-K/barttin currents [34], the missing stimulatory effect of Nedd4-2 mutants on ClC-K/barttin currents in oocytes contrasts with experiments described for ClC-5 [35,36] or the epithelial channel EnaC, which gave more currents after this procedure. These observations, taken together with the fact that the sequence motif in barttin (PQPPYYVRL) is also compatible with a tyrosine-based internalization signal (YxxL), should add a note of caution.

Because ClC-K/barttin is important for salt reabsorption, it might have a role in blood pressure regulation. Interestingly, a common polymorphism in the human gene encoding ClC-Kb that strongly increased Cl^--currents [37] was associated with high blood pressure [38]. However, a replication of this result in additional cohorts is warranted before drawing firm conclusions [39]. To have an effect on blood pressure, CIC-Kb/barttin would need to be rate-limiting for NaCl-reabsorption in distal segments of the nephron.

ClC-3, -4 and -5: endosomal CLCs with diverse functions

ClC-3 to ClC-5 are expressed in endosomes. Upon heterologous expression, they can reach the plasma membrane where they mediate strongly outwardly rectifying, pH-dependent currents [40].

The best known of these proteins is ClC-5. It is expressed in early and recycling endosomes, with particularly increased levels in subapical compartments of the renal proximal tubule (PT). The role of ClC-5 in endocytosis is evident from the proteinuria in Dent's disease (an inherited kidney stone disorder caused by mutations in CIC-5; see glossary) and in ClC-5 KO mice [41,42]. The disruption of ClC-5 impairs receptor-mediated endocytosis, fluid-phase endocytosis, and the endocytotic retrieval of plasma membrane proteins in a cell-autonomous manner [41]. The amount of megalin, an endocytotic recycling receptor of PT cells, was also reduced in a cell-autonomous manner, suggesting a role of ClC-5 in megalin recycling [41]. Electron microscopy and density centrifugation confirmed that both megalin and its co-receptor cubulin were reduced in apical plasma membranes of PTs from ClC-5 KO mice [42]. Hence, receptor-mediated endocytosis should be decreased more than fluid-phase endocytosis [41]. The primary defect underlying impaired endocytosis is thought to be insufficient luminal acidification of endosomes. In the absence of Cl^--currents, the voltage created by the V-type H^+-ATPase would inhibit further proton pumping. As predicted, the ATP-driven acidification of endosomal fractions from ClC-5 KO kidneys was significantly reduced [41,43•].

The other phenotypes observed in Dent's disease, including hyperphosphaturia, hypercalciuria (see glossary) and kidney stones, might represent indirect consequences of impaired endocytosis [41,43•]. Several hormones involved in Ca^{2+}-homeostasis are endocytosed or processed in the PT. In the absence of ClC-5, the decreased apical endocytosis of parathyroid hormone (PTH) increases urinary PTH levels, thereby stimulating apical PTH receptors in the PT. This stimulates the endocytosis of apical phosphate transporters [41] and induces 1α-hydroxylase, the enzyme that generates the biologically active vitamin D metabolite in the PT [43•].

Between its two CBS domains, ClC-5 displays a PY motif that can interact with WW-domains of the ubiquitin ligase WWPII [35]. This interaction stimulates the endocytosis of ClC-5 [35]. ClC-5 can also interact with another

WW-domain containing ubiquitin ligase, Nedd4-2 [36]. Interestingly, ubiquitination of ClC-5 by Nedd4-2 requires the presence of albumin, but the underlying mechanism is unclear.

The disruption of ClC-3, which is expressed in endosomes and synaptic vesicles, leads to severe CNS degeneration [44]. The mechanism underlying this degeneration is unknown, but might relate to intracellular trafficking defects. ClC-3 is important for the acidification of synaptic vesicles [44] and endosomes [45•], probably by shunting proton pump currents. Endosomal ion concentrations were estimated at various time points after the endocytotic uptake of indicator dyes into wild type and ClC-3 KO hepatocytes [45•]. The parallel increase in [Cl^-] and [H^+] along the endosomal pathway was attenuated but not abolished in cells lacking ClC-3. Likewise, synaptic vesicle acidification *in vitro* was reduced but not abolished [44], suggesting that other ion-conductive pathways might contribute to their acidification. Upon overexpression, ClC-3 stimulated the uptake of Zn^{2+} into synaptic vesicles [46], possibly by increasing the driving force for the ZnT3 transporter.

The analysis of mice deficient for components of AP-3 adaptor complexes revealed that the targeting of ClC-3 to synaptic vesicles depends on the neuronal isoform of this complex [46,47]. Interestingly, a splice variant of ClC-3 introduces a PDZ-domain binding motif at its carboxy-terminus [48]. This splice variant, called ClC-3B, interacts with several PDZ-domain containing proteins, including EBP50 [48], PDZK1 [49] (both of which are present at the plasma membrane and bind CFTR), and the Golgi-associated GOPC [49]. In contrast to earlier speculations that ClC-3B might be linked to CFTR at the plasma membrane [48], ClC-3B was predominantly found in the Golgi [49]. The physiological function of ClC-3B, the abundance and tissue distribution of which remain poorly defined, is unclear.

The least is known about ClC-4, which is mainly found in brain, kidney and liver. Antisense experiments suggested that ClC-4 facilitates endosomal acidification and is important for endocytosis [50]. ClC-4 could be co-immunoprecipitated with ClC-5 [50]. Unlike ClC-5, however, ClC-4 is not crucial for renal endocytosis because ClC-4 KO mice do not display proteinuria (S Schaffer, S Stobrawa, TJ Jentsch, unpublished). ClC-4 was proposed to facilitate the incorporation of copper into ceruloplasmin by shunting currents of Cu^{2+}-ATPases in the secretory pathway [51]. This conclusion relies on strong ClC-4 overexpression and should be tested in mice lacking ClC-4.

Disruption of lysosomal ClC-7 leads to osteopetrosis and lysosomal storage disease

In contrast to the other vesicular CLC proteins, which are predominantly expressed on endosomes, ClC-7 is mainly found on lysosomes and late endosomes [52,53•]. ClC-7 is ubiquitously expressed [52,53•]. In osteoclasts, the cells that degrade bone, ClC-7 is also present in the 'ruffled border'. It can be inserted into this specialized plasma membrane domain together with a V-type ATPase by an exocytotic insertion from late endosomal–lysosomal membranes.

The disruption of ClC-7 resulted in osteopetrosis (see glossary) [52]. This was explained by a failure of the ruffled border to acidify the resorption lacuna (see glossary), a process that is essential for bone resorption [52]. The osteopetrosis of ClC-7 KO mice was indeed rescued by the osteoclast-specific, transgenic expression of ClC-7 [53•]. ClC-7 mutations were also identified in a subset of humans with recessive malignant infantile osteopetrosis [52], and later in autosomal dominant osteopetrosis [54,55]. Because heterozygous dominant negative mutations in ClC-7 are expected to only reduce but not abolish ClC-7 function, dominant osteopetrosis is clinically less severe.

ClC-7 KO mice also display retinal degeneration [52] and a neurodegeneration that resembles lysosomal storage disease [53•]. Intracellular storage material was found in neurons and in proximal tubular cells of the kidney, but also in PTs. Although the lysosomal storage was believed to result from impaired lysosomal acidification, based on the effects in endosomal acidification with the KO of ClC-5 [43•] or ClC-3 [44,45•], no change in steady state lysosomal pH was detected in ClC-7 KO neurons in culture [53•].

Conclusions

The combination of broad physiological functions and roles in disease, the availability of crystal structures providing an excellent basis for structure–function studies, and the unexpected finding that prokaryotic CLCs function as coupled transporters guarantee that the CLC field will continue to generate excitement. Future work will improve our understanding of permeation and gating, clarify the structural basis for transporter versus channel activity, and address the roles of CBS domains. It will be important to determine whether some mammalian CLCs display exchange activity and whether such an activity might be important for their physiological function. Finally, investigation of the targeting and regulation of CLC proteins, and their involvement in protein networks, remain challenging tasks for the future.

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In this landmark study, the *E.coli* CLC that has been used for crystallization is shown not to be a Cl^- channel, but to function as a highly electrogenic $2Cl^-/H^+$ exchanger. Neutralizing a glutamate residue implicated in the gating of CLC channels abolished the coupling to protons, resulting in an anion conductive pathway. Because the plasma membrane branch of CLC proteins encodes chloride channels, one wonders whether some vesicular CLC proteins also display exchange activity.

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