ClC-5 mutations associated with Dent's disease: a major role of the dimer interface

S. Lourdel, Teddy Grand, Johanna Burgos, Wendy Gonzales, Pv Sepulveda, Jacques Teulon

ClC-5 mutations associated with Dent's disease: a major role of the dimer interface

Journal:	*Pflugers Archiv-European Journal of Physiology*
Manuscript ID:	EJP-00246-2011.R1
Manuscript Type:	Invited Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Lourdel, Stéphane; UPMC Univ Paris 06, UMR_S 872; INSERM, UMR_S 872; CNRS, ERL 7226 Grand, Teddy; UPMC Univ Paris 06, UMR_S 872; INSERM, UMR_S 872; CNRS, ERL 7226 Burgos, Johanna; Centro de Estudios Científicos (CECs) González, Wendy; Universidad de Talca, Centro de Bioinformática y Simulación Molecular Sepúlveda, Francisco; Centro de Estudios Científicos (CECs) Teulon, Jacques; UPMC Univ Paris 06, UMR_S 872; INSERM, UMR_S 872; CNRS, ERL 7226
Keywords:	Cl- channels, Cloned ion channels, Kidney, Electrophysiology, Renal proximal convoluted tubule

ClC-5 mutations associated with Dent's disease: a major role of the dimer interface

Stéphane Lourdel[1,2,3], Teddy Grand[1,2,3], Johanna Burgos[4], Wendy González[5], Francisco V. Sepúlveda[4], Jacques Teulon[1,2,3]

[1]UPMC Univ Paris 06, UMR_S 872, Laboratoire de génomique, physiologie et physiopathologie rénales, F-75005, Paris, France

[2]INSERM, UMR_S 872, Laboratoire de génomique, physiologie et physiopathologie rénales, F-75005, Paris, France

[3]CNRS, ERL 7226, Laboratoire de génomique, physiologie et physiopathologie rénales, F-75005, Paris, France

[4]Centro de Estudios Científicos (CECs), Valdivia 5110466, Chile

[5]Centro de Bioinformática y Simulación Molecular, Universidad de Talca, Talca, Chile

Address for correspondence:

Stéphane Lourdel, UMR_S 872, ERL 7226, Laboratoire de génomique, physiologie et physiopathologie rénales, 15 rue de l'Ecole de Médecine, 75270 Paris cedex 06, France

phone: 33.1.55.42.78.55

fax: 33.1.46.33.41.72

e-mail: stephane.lourdel@upmc.fr

Abstract

Dent's disease is an X-linked recessive disorder affecting the proximal tubules. Mutations in the $2Cl^-/H^+$ exchanger ClC-5 gene *CLCN5* are frequently associated with Dent's disease. Functional characterization of mutations of *CLCN5* have helped to elucidate the physiopathology of Dent's disease, and provided evidence that several different mechanisms underlie the ClC-5 dysfunction in Dent's disease. Modeling studies indicate that many *CLCN5* mutations are located at the interface between the monomers of ClC-5, demonstrating that this protein region plays an important role in Dent's disease. On the basis of functional data, *CLCN5* mutations can be divided into three different classes. Class 1 mutations impair processing and folding, and as a result, the ClC-5 mutants are retained within the endoplasmic reticulum and targeted for degradation by quality control mechanisms. Class 2 mutations induce a delay in protein processing and reduce the stability of ClC-5. As a consequence, the cell surface expression and currents of the ClC-5 mutants are lower. Class 3 mutations do not alter the trafficking of ClC-5 to the cell surface and early endosomes, but induce altered electrical activity. Here, we discuss the functional consequences of the three classes of *CLCN5* mutations on ClC-5 structure and function.

Key words

Dent's disease; Chloride/proton exchanger; *CLCN5*; ClC-5; mutation.

Genetics of Dent's disease

Dent's disease is a heterogeneous group of X-linked inherited disorders characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and in many cases, renal failure [8,31,32,43,47,58]. Positional cloning analysis in families affected by Dent's disease has identified mutations of *CLCN5* (MIM #300009), which is located on chromosome Xp11.22 and encodes the electrogenic $2Cl^-/H^+$ exchanger ClC-5 [41,46]. Although approximately two-thirds of patients with Dent's disease exhibit mutations of *CLCN5*, mutations of the gene for the Lowe syndrome *OCRL1* located on chromosome Xq25, which encodes the phosphatidylinositol 4,5-bisphosphate 5-phosphatase OCRL1, have also been found in about 15% of patients with Dent's disease (MIM #300555) [24]. Of note, the other patients with Dent's disease do not harbour mutations in either of these genes, suggesting an involvement of other genes [59].

Pathophysiology of Dent's disease

ClC-5 belongs to the ClC family of membrane proteins originally thought to function as Cl^- channels and consequently this was presumed to be its function upon its first identification [49]. In the kidney, ClC-5 is predominantly present in the early endosomes of proximal tubule cells and, to a lesser extent, in the thick ascending limb of Henle's loop and in the intercalated cells of the collecting duct [9,11,21,45,50,55]. ClC-5 knock-out mouse models have provided important clues about the mechanisms underlying Dent's disease. These mice reproduce the most common features of Dent's disease, such as low molecular weight proteinuria [22,42,53]. Because of its co-distribution with v-type H^+-ATPase in early endosomes, it has long been suggested that ClC-5 may provide Cl^- shunt conductance that

permits efficient intraluminal acidification of these organelles by v-type H^+-ATPase. ClC-5 loss-of-function would therefore impair endosomal acidification [21-23,42]. This could explain the defective endocytosis observed in Dent's disease, because progression along the endocytic pathway depends on an acidic endosomal lumen. However, the direct measurement of pH in endocytic vesicles in ClC-5 knock-out mice showed that acidification was reduced, but not abolished [22,23]. In addition, ClC-5 disruption also led to a trafficking defect of megalin and cubilin, two multiligands receptors for endocytosis that are heavily expressed at the brush border of proximal tubule cells [6,42].

The discovery that ClC-5, together with other intracellular ClC proteins, functions as an obligatory $2Cl^-/H^+$ exchanger presented the problem that shunting the electrical current of v-type H^+-ATPase via Cl^- flux would entail wasteful H^+ recycling. To unravel the role of ClC-5 in proximal tubule endocytosis, Novarino *et al.* have more recently generated mice that carry a mutation of a critical glutamate residue (i.e, the "gating glutamate", see below) that converts ClC-5 into a pure Cl^- conductor [40]. Surprisingly, despite normal endosomal acidification, the mice exhibited impaired proximal tubule endocytosis like ClC-5 knock-out mice. As observed in patients with Dent's disease and ClC-5 knock-out mice, these mice also showed low molecular weight proteinuria, hypercalciuria and hyperphosphaturia. This work indicates that modulation of the endosomal Cl^- concentration in the endosomes of the proximal tubule cells during H^+ transport by ClC-5 may play a crucial role in proximal tubule endocytosis. In addition to its prominent endosomal expression, small amounts of ClC-5 are also found on the apical surface of proximal tubule cells, where it may play additional roles. This might arise from a role in the protein-protein interactions required for receptor-mediated endocytosis and in microtubular transport as a result of associations with other proteins such as cofilin, a protein involved in the depolymerization of actin in the vicinity of endosomes,

with the PDZ-domain protein NHERF2, with the ubiquitin-protein ligase Nedd-4, and with KIF3B, a member of the kinesin superfamily [25,26,28,44].

Structure-function relationship of ClC $2Cl^-/H^+$ exchangers

ClC-5 is a 746 amino-acid protein that belongs to the highly conserved ClC family of Cl^- channels and electrogenic $2Cl^-/H^+$ exchangers that are expressed in prokaryotic and eukaryotic organisms [30]. In mammals, ClC-1, ClC-2, and the ClC-K/barttin complex all function as plasma membrane Cl^- channels, whereas ClC-3 to -7 function as electrogenic $2Cl^-/H^+$ exchangers that reside mainly in the membranes of the endosomal/ lysosomal compartments and in synaptic vesicles.

X-ray crystal structures of three ClC $2Cl^-/H^+$ exchangers (*E. coli* EcClC-1, *S. typhimurium StClC*, and *C. merolae* CmClC) have provided significant insights into the structure of ClC proteins, by demonstrating that they function as homodimers with separate ion pathways within each subunit and suggesting molecular mechanisms for the ion permeation process [12-14,16]. In ClC $2Cl^-/H^+$ exchangers, a glutamate residue acts simultaneously as the outside Cl^- gate and the extracellular H^+ acceptor [13,14,16]. Interestingly, neutralizing this "gating glutamate" of EcClC-1 (E148) and CmClC (E210) by mutation to alanine abolished H^+ flux, and purely anionic conductance was observed [2,16]. Abolition of the rectification of the currents and similar loss of H^+ flux were obtained when equivalent mutations of the "gating glutamate" were introduced in human ClC-3 (E224A), ClC-4 (E224A), ClC-5 (E211A), and ClC-6 (E200A) [35,39,41,46]. Another glutamate facing the intracellular medium has been identified as being an additional H^+-binding site in EcClC-1 (E203), in ClC-4 (E281), in ClC-5 (E268), and in ClC-6 (E267) [3,39,62]. Neutralization of

this "proton glutamate", which is strictly conserved among ClC $2Cl^-/H^+$ exchangers, abolished H^+ flux in EcClC-1 [3], and Cl^- and H^+ flux in ClC-4, ClC-5 and ClC-6 [39,62].

All eukaryotic ClCs have a large cytoplasmic carboxy-terminus containing a pair of cystathionine beta-synthase (CBS) domains. Recent data have provided evidence that CBS domains bind nucleotides like ATP and are involved in regulating the activity of several ClC, including ClC-5 [36,56,64].

Spectrum of mutations of the *CLCN5* gene in Dent's disease

To date, 148 *CLCN5* mutations have been reported in patients with Dent's disease. They consist of about 36% nonsense mutations, 30% missense mutations, 18% deletional mutations, 7% splice site mutations and 6% insertional mutations [10]. An early modeling study of ClC-5 by Wu *et al.* postulated that a majority of ClC-5 missense mutations involve residues positioned at the interface of the homodimers [60]. Wu *et al.* speculated that mutated residues clustering at the dimer interface of ClC-5 would cause a loss of electrical activity by disrupting the assembly of the homodimers. This would lead to the formation of misfolded proteins in the endoplasmic reticulum, and their rapid degradation within the cell. However, at that time, the subcellular localization of the ClC-5 mutants had not been investigated, and so the model did not take into account the possibility that some of the mutants showed reduced currents as a result of a gating defect.

Despite the large number of *CLCN5* mutations identified, their functional impact at the cellular and molecular levels in Dent's disease remains unclear. The aim of this review is to describe the findings of a series of recent functional investigations using heterologous expression systems carried out by us and by others that have made it possible to divide the *CLCN5* mutations into different classes, and have helped to elucidate the pathophysiology of

Dent's disease. We have based the classification of *CLCN5* mutations on the well-established classification of mutations of the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) that encodes the CFTR Cl^- channel. *CFTR* mutations can be divided into five classes that reflect their molecular dysfunction [57,63]. *CFTR* class I mutations comprise nonsense, frameshift and aberrant mRNA splicing mutations that lead to impairment of protein synthesis. Class II mutations cause a defect of processing in the endoplasmic reticulum and the Golgi apparatus. As a consequence, the CFTR mutants are misfolded, achieve partial N-glycosylation, and undergo retention in the endoplasmic reticulum, which is followed by their rapid degradation within the cell. Class III and class IV mutations lead to a normal amount of CFTR protein at the plasma membrane, but alter channel regulation and conduction, respectively. Class V mutations result in a decreased amount of functional protein, in most cases resulting from aberrant mRNA splicing.

To date, mutations in the *CLCN5* gene can be classified into three different classes that are discussed below. However, additional classes of *CLCN5* mutations might exist, as only 20% of the identified mutations have been functionally investigated. In addition, some mutations have been only partially explored (Table 1). Therefore, many mutations remain to be functionally analyzed.

Class 1 mutations inducing defective protein processing

After their translation, ClC-5 proteins move through the endoplasmic reticulum and the Golgi apparatus, where proper folding of the proteins occurs with the help of chaperones and other modifications take place, such as glycosylation. These post-translational modifications allow the $2Cl^-/H^+$ exchanger to traffic to the early endosomes and to the plasma membrane.

A remarkable number of naturally-occurring *CLCN5* mutations (about 60%) that have been functionally investigated in *Xenopus laevis* oocytes, in HEK-MSR cells, and in HEK293 cells can be assigned to a first class of *CLCN5* mutations that induce defective protein processing (Table 1) [18,19,34,38,48,51]. Although more recent studies have demonstrated that some mutations do not cluster at the dimer interface [18,19], the conclusions from the modeling study by Wu *et al.* [60] remain partially unchallenged, because it appears that many mutations are indeed localized at the interface between the monomers of ClC-5 (Fig. 1 and 2, Table 1) [18,19,34,48]. Immunocytochemical and biochemical analyses have revealed that this class of mutations results in the retention of ClC-5 within the endoplasmic reticulum, where it is subjected to early degradation by quality control systems. As a consequence, the mutant proteins are improperly N-glycosylated, and are non-functional due to defective trafficking to the cell surface and to the early endosomes.

The position of these *CLCN5* mutations in the protein sequence may explain their deleterious impact on ClC-5. As outlined by their position in ClC-5 and in view of the modeling study by Wu *et al.* [60], it looks as though the first subgroup of mutations may impair monomer dimerization. Because ClC-5 normally functions as a homodimer, the misfolded proteins could be then subjected to early degradation by the cells. Concerning the second subgroup of mutations (away from the dimer interface), they may significantly affect the stability of the α-helices, thus enhancing the degradation of the abnormal ClC-5 proteins. These studies have provided evidence that the proper folding of several protein regions, such as the interface, is crucial for the normal function of ClC-5 at the plasma membrane and in the early endosomes.

Defects in protein folding and processing have been shown to be a common cellular mechanism involved in the pathogenesis of several inherited disorders [20]. One well-known example is that of the most common p.F508del *CFTR* class II mutation in cystic fibrosis,

which results in the misprocessing of CFTR, producing a lack of functional protein at the cell surface [4]. Thus, the mechanisms that lead to ClC-5 dysfunction in this category of *CLCN5* mutations are similar to those responsible for the *CFTR* class II mutations in cystic fibrosis.

Class 2 mutations inducing delayed protein processing and lower stability of the mature protein

About 20% of the *CLCN5* mutations that have been functionally analyzed in heterologous expression systems generate ClC-5 proteins displaying delayed processing and reduced biological stability of the mature glycosylated form (Fig. 3, Table 1). As a consequence, the cell surface expression and the currents mediated by ClC-5 at the plasma membrane are reduced. However, the distribution of the mutants in the early endosomes is normal [18,48].These functional defects of ClC-5 resemble those observed with *CFTR* class V mutations that lead to lower protein levels at the plasma membrane due to a reduced synthesis of the CFTR Cl^- channel [63].

Four mutations of this type (c.779G>T, p.G260V; c.1581A>T, p.E527D; c.1637A>G, p.K546E and c.1639T>G, p.W547G) are located at the transporter dimer interface, and one mutation (c.834G>C, p.L278F) at the periphery of the interface (Fig. 1 and 2, Table 1). As shown by their complex N-glycosylation, plasma membrane and early endosome expression in HEK-293 transfected cells, a significant fraction of these mutants is not retained in the endoplasmic reticulum [18]. Despite lower abundance at the cell surface, the residual electrical activity and early endosomal distribution resulting from the mutation located at the edge of the dimer interface (p.L278F) are in accordance with the conclusions from the modeling study by Wu *et al.* [60]. These authors hypothesized that mutations of amino acids located at the periphery of the subunit interface may induce a relatively minor disruption of

the protein structure. This could explain why the p.L278F mutation is associated with residual function in HEK-293 transfected cells. The residual plasma membrane expression induced by the other mutations (p.G260V, p.E527D, p.K546E and p.W547G) makes the functional consequences of amino acid substitutions at the dimer interface of ClC-5 very difficult to predict in the absence of functional studies.

Smith *et al.* have investigated the effect of another mutant (c.1581A>T, p.E527D) on endosomal pH in HEK-MSR cells by means of a vesicular acidification assay [48]. The p.E527D mutation results in current abolition and reduced plasma membrane expression, but normal expression in early endosomes (Table 1). Expression of this mutant in HEK-MSR cells impairs endosomal acidification. To explain the abnormal intraluminal pH, the authors hypothesize that this occurred by loss of currents of the mutant ClC-5.

Ion conduction mediated by ClC-5 at the plasma membrane is not thought to be physiologically relevant. However, as we have already mentioned, several studies have provided evidence that the ClC-5 population at the cell surface of proximal tubule cells is a rate-limiting step in receptor-mediated endocytosis of low molecular weight proteins by establishing protein-protein interactions with proteins that are essential for renal endocytosis [27]. According to this view, in contrast to class 3 mutants that are normally targeted to the cell surface, the lower abundance of these mutants at the plasma membrane could severely reduce the rate of receptor-mediated endocytosis by disrupting the protein-protein interactions required for the formation of the endocytic macromolecular complex. This type of reasoning could explain the low molecular weight proteinuria observed in patients, but this point remains to be investigated.

Class 3 mutations inducing altered conduction without any change in subcellular distribution

In this third class of *CLCN5* mutations, ClC-5 mutants can be produced, transformed into a complex glycosylated form in the endoplasmic reticulum and in the Golgi apparatus, and then finally transported to the plasma membrane and to the early endosomes, much as wild-type ClC-5 proteins (Table 1). However, two-electrode voltage-clamp recordings in *Xenopus laevis* oocytes have demonstrated that the mutant proteins display either reduced or no current.

This class of *CLCN5* mutations account for approximately 20% of the mutations that we and others have analyzed [18,19,32,37,38,53,54,61]. These mutations are analogous to the *CFTR* class III and class IV mutations that alter regulation and conduction, respectively [57]. Even though two missense mutations (c.815A>G, p.Y272C and c.1558T>C, p.S520P) are positioned at the transporter interface (Fig. 1 and 2, Table 1), the resulting mutant proteins that were expressed in HEK293 cells and in the pig renal proximal tubule cell line, LLC-PK_1, escaped from endoplasmic reticulum retention and the subsequent degradation [18,53]. This finding breaks the rule that mutation of amino acids located at the subunit interface of ClC-5 necessarily cause protein-folding defects. A set of data for other ClCs also support these latter findings. For instance myotonia congenita is underlain by various missense mutations of the *CLCN1* gene that encodes the skeletal muscle Cl^- channel, ClC-1. It appears that many of the mutations that occur at the dimer interface of ClC-1 alter the function of ClC-1 at the plasma membrane by inducing a dramatic shift in the voltage dependence of channel activation [33]. Similarly, several artificially designed mutations positioned at the subunit interface in the *Torpedo* electric organ Cl^- channel ClC-0 induce altered gating behavior by inverting the voltage sensitivity of channel activation [5]. Further experiments are needed to find out

whether the p.Y272C and p.S520P mutations also cause altered gating behavior, as suggested by similarly located mutations in ClC-0 or ClC-1.

The p.G212A mutation induces reduced electrical activity. The mutated glutamate at position 212 is strictly conserved in ClCs. It lies very close to the ion pathway of ClC-5, because it directly follows the "gating glutamate", a residue that is involved in the coupling of the Cl^- flux to the H^+ flux in ClC $2Cl^-/H^+$ exchangers. The exact mechanism responsible for the decreased currents of ClC-5 remains to be elucidated, but one can hypothesize that mutation of a residue very close to the ion pathway could severely interfere with the gating process. Mutagenesis analysis from ClC-1 supports this hypothesis. Heterologous expression of the ClC-1 p.G233A mutation, which is located at the equivalent G212 position in ClC-5, caused a dramatic alteration of anion-selectivity sequence and gating of ClC-1 [15].

As we have already mentioned, the first hypothesis advanced to explain the defect of receptor-mediated endocytosis in patients with Dent's disease was that it resulted in altered endosomal acidification due to a loss of proton neutralization by ClC-5 [30]. However, recent work by Novarino *et al.* contradicts this hypothesis by suggesting that the endosomal Cl^- concentration, raised by ClC-5 in exchange for H^+ accumulated by H^+-ATPase, could be an important parameter in the physiology of the endosomal and lysosomal pathway, rather than vesicle acidification [40]. Thus, the altered currents of these ClC-5 mutants may induce severe impairment of endocytosis due to impaired function of v-type H^+-ATPases in early endosomes, or a reduction in Cl^- accumulation in early endosomes. It is interesting to note that Wang *et al.* have demonstrated that the S520P (c.1558T>C, p.S520P) mutant, which still displays residual currents and normal cell surface expression, cannot be internalized from the plasma membrane into the early endosomes in LLC-PK1-transfected cells [54]. This suggests that disorders in endocytosis could be caused either by inefficient endosomal acidification or endosomal Cl^- accumulation due to lack of targeting of ClC-5 to the early endosomes.

Analysis of the effects of these ClC-5 mutants on pH or Cl^- concentration in the early endosomes of proximal tubule cells would provide very useful information about the cellular mechanisms that lead to Dent's disease in this class of *CLCN5* mutations, but such investigations remain unaddressed.

Several mutations affecting the CBS1 and CBS2 domains also belong to this category of mutations. The p.R648X was shown to severely reduce currents [34], and the p.Y617X and p.R704X mutations displayed impaired electrical activity [32,37,54,61]. Data from the p.R718X mutation which causes truncation of the intracellular C terminus and loss of a part of CBS2 domain are not in accordance with these latter mutants that are able to traffic to the plasma membrane, because the R718X mutant protein is not detected at the cell surface due to retention in the endoplasmic reticulum. These findings raise the possibility that the p.R718X mutation may interfere with the proper folding of the C terminus of ClC-5 that is required to pass the process of protein quality control in the endoplasmic reticulum prior to trafficking to the early endosomes and to the plasma membrane.

Finally, four missense mutations (c.64T>G, p.W22G; c.731C>T, p.S244L; c.815A>G, p.Y272C; c.1558T>C, p.S520P) are not located in close proximity to the Cl^- or H^+ transport pathways of the ClC $2Cl^-/H^+$ exchangers [2,13,14,16,41,46,62] and do not belong to any region with assigned functional association.

Mutations altering the expression at the plasma membrane and in the early endosomes

It is not clear whether two other mutations (c.170G>T, p.G57V and c.839G>C, p.R280P) should also be assigned to another class of mutations. These two mutations have been shown to lead to a reduction both in plasma membrane expression and electrical activity in HEK-MSR transfected cells [48]. However they also induce alterations in the endosomal distribution of the mutant proteins: the R280P mutant accumulates in the early endosomes, whereas the G57V mutant accumulates in the late endosomes. Surprisingly, wild-type ClC-5 was found to localize in both early and late endosomes in HEK-MSR transfected cells. The expression of wild-type ClC-5 in late endosomes is at variance with its distribution in kidney tissue. Indeed, when investigated by immunofluorescence with specific antibodies, late endosomes of proximal tubule cells have clearly been shown to be devoid of ClC-5 [9,21,45,55]. Further investigations are needed to unravel the correct classification of these two *CLCN5* mutations, and to exclude the possibility that late endosomal expression of wild-type ClC-5 is not the consequence of an intracellular trafficking defect in HEK-MSR transfected cells due to overexpression or some other mechanism.

Conclusion

Functional studies of *CLCN5* mutations have made it possible to distinguish between three different classes of mutations (Fig. 3). ClC-5 class 1 mutants are characterized by a failure to induce currents, because of defective processing during N-glycosylation, resulting in their retention at the endoplasmic reticulum. ClC-5 class 2 mutants display delayed protein processing and lower stability of their mature form. ClC-5 class 3 mutants are correctly targeted to the plasma membrane and early endosomes, but display reduced electrical activity.

Further investigation is needed to elucidate the mechanisms (altered gating or conduction) leading to dysfunction of ClC-5.

Furthermore, the localization of the mutations deduced from molecular modeling studies demonstrates that about half of them cluster in the helices that form the dimer interface of ClC-5 (Table 1). This highlights the fact that impaired integrity of this region of ClC-5 is a mechanism that can frequently underlie Dent's disease. This observation clearly contrasts with the conclusions of modeling and functional studies of the mutations of ClC-1 that underlie myotonia congenita, and those of ClC-7 that cause recessive and dominant osteopetrosis. Although some mutations are localized at the dimer interface of ClC-1 and ClC-7, most of them appear to be excluded from this region [7,17,33,52].

Numerous studies have demonstrated that pharmacological therapies can rescue dysfunctional channels and transporters, as is the case, for instance, for the CFTR protein-carrying mutations for cystic fibrosis [4]. For example, chemical (e.g., quinazoline), pharmacological (e.g., benzo[c]quinolizinium compounds) and molecular chaperones (e.g., sodium 4-phenylbutyrate) have been shown to stabilize and facilitate the folding of class II CFTR mutants, thereby allowing them to escape from degradation in the endoplasmic reticulum and to be targeted to the plasma membrane. Furthermore, several compounds (e.g., genistein) have also been found to directly activate class III and class IV CFTR mutants that display defective regulation and conduction at the plasma membrane, respectively. A major future challenge will be to search for specific therapeutic molecules that will be able to restore sufficient function of mutant ClC-5 in patients with Dent's disease.

Acknowledgements

Work in our laboratories was funded in part by grants from the French ANR program (ANR-05-MRAR-033-01), the Fondation du Rein, and ECOS Conicyt C10S03. CECs is funded by Conicyt PFB. The English text has been edited by M. Ghosh.

References

1. Abagyan R, Trotov M, Kuznetsov D (1994) ICM-A a new method for structure modeling and design: applications to docking and structure prediction from the distorted native conformation. J Comput Chem 15:488-506
2. Accardi A, Miller C (2004) Secondary active transport mediated by a prokaryotic homologue of ClC Cl^- channels. Nature 427:803-807
3. Accardi A, Walden M, Nguitragool W, Jayaram H, Williams C, Miller C (2005) Separate ion pathways in a Cl^-/H^+ exchanger. J Gen Physiol 126:563-570
4. Becq F, Mall MA, Sheppard DN, Conese M, Zegarra-Moran O (2011) Pharmacological therapy for cystic fibrosis: from bench to bedside. J Cyst Fibros 10 Suppl 2:S129-145
5. Chen TY, Hwang TC (2008) CLC-0 and CFTR: chloride channels evolved from transporters. Physiol Rev 88:351-387
6. Christensen EI, Devuyst O, Dom G, Nielsen R, Van der Smissen P, Verroust P, Leruth M, Guggino WB, Courtoy PJ (2003) Loss of chloride channel ClC-5 impairs endocytosis by defective trafficking of megalin and cubilin in kidney proximal tubules. Proc Natl Acad Sci U S A 100:8472-8477
7. Cleiren E, Benichou O, Van Hul E, Gram J, Bollerslev J, Singer FR, Beaverson K, Aledo A, Whyte MP, Yoneyama T, deVernejoul MC, Van Hul W (2001) Albers-Schonberg disease (autosomal dominant osteopetrosis, type II) results from mutations in the ClCN7 chloride channel gene. Hum Mol Genet 10:2861-2867
8. Dent CE, Friedman M (1964) Hypercalcuric Rickets Associated with Renal Tubular Damage. Arch Dis Child 39:240-249
9. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV (1999) Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. Hum Mol Genet 8:247-257
10. Devuyst O, Thakker RV (2010) Dent's disease. Orphanet J Rare Dis 5:28
11. Dowland LK, Luyckx VA, Enck AH, Leclercq B, Yu AS (2000) Molecular cloning and characterization of an intracellular chloride channel in the proximal tubule cell line, LLC-PK1. J Biol Chem 275:37765-37773
12. Dutzler R (2007) A structural perspective on ClC channel and transporter function. FEBS Lett 581:2839-2844
13. Dutzler R, Campbell EB, Cadene M, Chait BT, MacKinnon R (2002) X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity. Nature 415:287-294
14. Dutzler R, Campbell EB, MacKinnon R (2003) Gating the selectivity filter in ClC chloride channels. Science 300:108-112
15. Fahlke C, Yu HT, Beck CL, Rhodes TH, George AL, Jr. (1997) Pore-forming segments in voltage-gated chloride channels. Nature 390:529-532
16. Feng L, Campbell EB, Hsiung Y, MacKinnon R (2010) Structure of a eukaryotic CLC transporter defines an intermediate state in the transport cycle. Science 330:635-641
17. Frattini A, Pangrazio A, Susani L, Sobacchi C, Mirolo M, Abinun M, Andolina M, Flanagan A, Horwitz EM, Mihci E, Notarangelo LD, Ramenghi U, Teti A, Van Hove J, Vujic D, Young T, Albertini A, Orchard PJ, Vezzoni P, Villa A (2003) Chloride channel ClCN7 mutations are responsible for severe recessive, dominant, and intermediate osteopetrosis. J Bone Miner Res 18:1740-1747

18. Grand T, L'Hoste S, Mordasini D, Defontaine N, Keck M, Pennaforte T, Genete M, Laghmani K, Teulon J, Lourdel S (2011) Heterogeneity in the processing of CLCN5 mutants related to Dent disease. Hum Mutat 32:476-483
19. Grand T, Mordasini D, L'Hoste S, Pennaforte T, Genete M, Biyeyeme MJ, Vargas-Poussou R, Blanchard A, Teulon J, Lourdel S (2009) Novel CLCN5 mutations in patients with Dent's disease result in altered ion currents or impaired exchanger processing. Kidney Int 76:999-1005
20. Gregersen N, Bross P, Vang S, Christensen JH (2006) Protein misfolding and human disease. Annu Rev Genomics Hum Genet 7:103-124
21. Gunther W, Luchow A, Cluzeaud F, Vandewalle A, Jentsch TJ (1998) ClC-5, the chloride channel mutated in Dent's disease, colocalizes with the proton pump in endocytotically active kidney cells. Proc Natl Acad Sci U S A 95:8075-8080
22. Gunther W, Piwon N, Jentsch TJ (2003) The ClC-5 chloride channel knock-out mouse - an animal model for Dent's disease. Pflugers Arch 445:456-462
23. Hara-Chikuma M, Wang Y, Guggino SE, Guggino WB, Verkman AS (2005) Impaired acidification in early endosomes of ClC-5 deficient proximal tubule. Biochem Biophys Res Commun 329:941-946
24. Hoopes RR, Jr., Shrimpton AE, Knohl SJ, Hueber P, Hoppe B, Matyus J, Simckes A, Tasic V, Toenshoff B, Suchy SF, Nussbaum RL, Scheinman SJ (2005) Dent Disease with mutations in OCRL1. Am J Hum Genet 76:260-267
25. Hryciw DH, Ekberg J, Ferguson C, Lee A, Wang D, Parton RG, Pollock CA, Yun CC, Poronnik P (2006) Regulation of albumin endocytosis by PSD95/Dlg/ZO-1 (PDZ) scaffolds. Interaction of Na^+-H^+ exchange regulatory factor-2 with ClC-5. J Biol Chem 281:16068-16077
26. Hryciw DH, Ekberg J, Lee A, Lensink IL, Kumar S, Guggino WB, Cook DI, Pollock CA, Poronnik P (2004) Nedd4-2 functionally interacts with ClC-5: involvement in constitutive albumin endocytosis in proximal tubule cells. J Biol Chem 279:54996-55007
27. Hryciw DH, Ekberg J, Pollock CA, Poronnik P (2006) ClC-5: a chloride channel with multiple roles in renal tubular albumin uptake. Int J Biochem Cell Biol 38:1036-1042
28. Hryciw DH, Wang Y, Devuyst O, Pollock CA, Poronnik P, Guggino WB (2003) Cofilin interacts with ClC-5 and regulates albumin uptake in proximal tubule cell lines. J Biol Chem 278:40169-40176
29. Humphrey W, Dalke A, Schulten K (1996) VMD : visual molecular dynamics. J. Mol. Graph. J Mol Graph 14:33-38
30. Jentsch TJ (2008) CLC chloride channels and transporters: from genes to protein structure, pathology and physiology. Crit Rev Biochem Mol Biol 43:3-36
31. Lloyd SE, Gunther W, Pearce SH, Thomson A, Bianchi ML, Bosio M, Craig IW, Fisher SE, Scheinman SJ, Wrong O, Jentsch TJ, Thakker RV (1997) Characterisation of renal chloride channel, CLCN5, mutations in hypercalciuric nephrolithiasis (kidney stones) disorders. Hum Mol Genet 6:1233-1239
32. Lloyd SE, Pearce SH, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SP, Wrong O, Jentsch TJ, Craig IW, Thakker RV (1996) A common molecular basis for three inherited kidney stone diseases. Nature 379:445-449
33. Lossin C, George AL, Jr. (2008) Myotonia congenita. Adv Genet 63:25-55
34. Ludwig M, Doroszewicz J, Seyberth HW, Bokenkamp A, Balluch B, Nuutinen M, Utsch B, Waldegger S (2005) Functional evaluation of Dent's disease-causing mutations: implications for ClC-5 channel trafficking and internalization. Hum Genet 117:228-237

35. Matsuda JJ, Filali MS, Collins MM, Volk KA, Lamb FS (2010) The ClC-3 Cl-/H+ antiporter becomes uncoupled at low extracellular pH. J Biol Chem 285:2569-2579
36. Meyer S, Savaresi S, Forster IC, Dutzler R (2007) Nucleotide recognition by the cytoplasmic domain of the human chloride transporter ClC-5. Nat Struct Mol Biol 14:60-67
37. Mo L, Xiong W, Qian T, Sun H, Wills NK (2004) Coexpression of complementary fragments of ClC-5 and restoration of chloride channel function in a Dent's disease mutation. Am J Physiol Cell Physiol 286:C79-89
38. Morimoto T, Uchida S, Sakamoto H, Kondo Y, Hanamizu H, Fukui M, Tomino Y, Nagano N, Sasaki S, Marumo F (1998) Mutations in CLCN5 chloride channel in Japanese patients with low molecular weight proteinuria. J Am Soc Nephrol 9:811-818
39. Neagoe I, Stauber T, Fidzinski P, Bergsdorf EY, Jentsch TJ (2010) The late endosomal ClC-6 mediates proton/chloride countertransport in heterologous plasma membrane expression. J Biol Chem 285:21689-21697
40. Novarino G, Weinert S, Rickheit G, Jentsch TJ (2010) Endosomal chloride-proton exchange rather than chloride conductance is crucial for renal endocytosis. Science 328:1398-1401
41. Picollo A, Pusch M (2005) Chloride/proton antiporter activity of mammalian CLC proteins ClC-4 and ClC-5. Nature 436:420-423
42. Piwon N, Gunther W, Schwake M, Bosl MR, Jentsch TJ (2000) ClC-5 Cl^- channel disruption impairs endocytosis in a mouse model for Dent's disease. Nature 408:369-373
43. Pook MA, Wrong O, Wooding C, Norden AG, Feest TG, Thakker RV (1993) Dent's disease, a renal Fanconi syndrome with nephrocalcinosis and kidney stones, is associated with a microdeletion involving DXS255 and maps to Xp11.22. Hum Mol Genet 2:2129-2134
44. Reed AA, Loh NY, Terryn S, Lippiat JD, Partridge C, Galvanovskis J, Williams SE, Jouret F, Wu FT, Courtoy PJ, Nesbit MA, Rorsman P, Devuyst O, Ashcroft FM, Thakker RV (2010) CLC-5 and KIF3B interact to facilitate CLC-5 plasma membrane expression, endocytosis, and microtubular transport: relevance to pathophysiology of Dent's disease. Am J Physiol Renal Physiol 298:F365-380
45. Sakamoto H, Sado Y, Naito I, Kwon TH, Inoue S, Endo K, Kawasaki M, Uchida S, Nielsen S, Sasaki S, Marumo F (1999) Cellular and subcellular immunolocalization of ClC-5 channel in mouse kidney: colocalization with H^+-ATPase. Am J Physiol 277:F957-965
46. Scheel O, Zdebik AA, Lourdel S, Jentsch TJ (2005) Voltage-dependent electrogenic chloride/proton exchange by endosomal CLC proteins. Nature 436:424-427
47. Scheinman SJ (1998) X-linked hypercalciuric nephrolithiasis: clinical syndromes and chloride channel mutations. Kidney Int 53:3-17
48. Smith AJ, Reed AA, Loh NY, Thakker RV, Lippiat JD (2009) Characterization of Dent's disease mutations of CLC-5 reveals a correlation between functional and cell biological consequences and protein structure. Am J Physiol Renal Physiol 296:F390-397
49. Steinmeyer K, Schwappach B, Bens M, Vandewalle A, Jentsch TJ (1995) Cloning and functional expression of rat CLC-5, a chloride channel related to kidney disease. J Biol Chem 270:31172-31177
50. Suzuki T, Rai T, Hayama A, Sohara E, Suda S, Itoh T, Sasaki S, Uchida S (2006) Intracellular localization of ClC chloride channels and their ability to form hetero-oligomers. J Cell Physiol 206:792-798
51. Tanuma A, Sato H, Takeda T, Hosojima M, Obayashi H, Hama H, Iino N, Hosaka K, Kaseda R, Imai N, Ueno M, Yamazaki M, Sakimura K, Gejyo F, Saito A (2007)

Functional characterization of a novel missense CLCN5 mutation causing alterations in proximal tubular endocytic machinery in Dent's disease. Nephron Physiol 107:p87-97

52. Waguespack SG, Koller DL, White KE, Fishburn T, Carn G, Buckwalter KA, Johnson M, Kocisko M, Evans WE, Foroud T, Econs MJ (2003) Chloride channel 7 (ClCN7) gene mutations and autosomal dominant osteopetrosis, type II. J Bone Miner Res 18:1513-1518
53. Wang SS, Devuyst O, Courtoy PJ, Wang XT, Wang H, Wang Y, Thakker RV, Guggino S, Guggino WB (2000) Mice lacking renal chloride channel, CLC-5, are a model for Dent's disease, a nephrolithiasis disorder associated with defective receptor-mediated endocytosis. Hum Mol Genet 9:2937-2945
54. Wang Y, Cai H, Cebotaru L, Hryciw DH, Weinman EJ, Donowitz M, Guggino SE, Guggino WB (2005) ClC-5: role in endocytosis in the proximal tubule. Am J Physiol Renal Physiol 289:F850-862
55. Wartosch L, Fuhrmann JC, Schweizer M, Stauber T, Jentsch TJ (2009) Lysosomal degradation of endocytosed proteins depends on the chloride transport protein ClC-7. Faseb J 23:4056-4068
56. Wellhauser L, Luna-Chavez C, D'Antonio C, Tainer J, Bear CE (2011) ATP induces conformational changes in the carboxyl-terminal region of ClC-5. J Biol Chem 286:6733-6741
57. Welsh MJ, Smith AE (1993) Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 73:1251-1254
58. Wrong OM, Norden AG, Feest TG (1994) Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. Qjm 87:473-493
59. Wu F, Reed AA, Williams SE, Loh NY, Lippiat JD, Christie PT, Large O, Bettinelli A, Dillon MJ, Goldraich NP, Hoppe B, Lhotta K, Loirat C, Malik R, Morel D, Kotanko P, Roussel B, Rubinger D, Schrander-Stumpel C, Serdaroglu E, Nesbit MA, Ashcroft F, Thakker RV (2009) Mutational analysis of CLC-5, cofilin and CLC-4 in patients with Dent's disease. Nephron Physiol 112:p53-62
60. Wu F, Roche P, Christie PT, Loh NY, Reed AA, Esnouf RM, Thakker RV (2003) Modeling study of human renal chloride channel (hCLC-5) mutations suggests a structural-functional relationship. Kidney Int 63:1426-1432
61. Yamamoto K, Cox JP, Friedrich T, Christie PT, Bald M, Houtman PN, Lapsley MJ, Patzer L, Tsimaratos M, Van'T Hoff WG, Yamaoka K, Jentsch TJ, Thakker RV (2000) Characterization of renal chloride channel (CLCN5) mutations in Dent's disease. J Am Soc Nephrol 11:1460-1468
62. Zdebik AA, Zifarelli G, Bergsdorf EY, Soliani P, Scheel O, Jentsch TJ, Pusch M (2008) Determinants of anion-proton coupling in mammalian endosomal CLC proteins. J Biol Chem 283:4219-4227
63. Zielenski J, Tsui LC (1995) Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Genet 29:777-807
64. Zifarelli G, Pusch M (2009) Intracellular regulation of human ClC-5 by adenine nucleotides. EMBO Rep 10:1111-1116

Figure legends

Fig. 1 Location of the *CLCN5* mutations in a three-dimensional model of ClC-5 based on the structure of StClC [13] viewed from the side of the membrane with the extracellular solution at the top. **a** Class 1 mutations inducing defective protein processing. **b** Class 2 mutations inducing delayed protein processing and lower stability of the mature protein. **c** Class 3 mutations inducing altered conduction without any change in subcellular distribution. Mutated residues are shown in spheres. The mutation numbers refer to those of Table 1. The helices involved in the formation of the dimer interface and those located at the edge are shown in yellow and in pale yellow respectively. Yellow numbers indicate mutated residues positioned at the dimer interface. A monomeric structure of the model was built using as template the crystallographic data of StClC channel (PDB ID code 1KPL) using the ICM programme [1]. The dimeric structure and the figures shown here were produced using VMD software [29]

Fig. 2 Location of the *CLCN5* mutations in the three-dimensional model of ClC-5 viewed from the subunit interface [13]. **a** Class 1 mutations inducing defective protein processing. **b** Class 2 mutations inducing delayed protein processing and lower stability of the mature protein. **c** Class 3 mutations inducing altered conduction without any change in subcellular distribution. Mutated residues are shown in spheres. The mutation numbers refer to those of Table 1. The helices involved in the formation of the dimer interface and those located at the edge are shown in yellow and in pale yellow respectively. Yellow numbers indicate mutated residues positioned at the dimer interface

Fig. 3 Molecular mechanisms responsible for the impaired function of ClC-5 in Dent's disease. A full description of each of the molecular mechanisms observed in the different *CLCN5* mutation classes is provided in the text

Table 1 Summary of the functional effects of *CLCN5* mutations in patients with Dent's disease

Number	Nucleotide change[a]	Amino acid change	Position in protein structure[b]	Currents	Surface expression	Intracellular localization		N-glycosylation	Reference
						E.R.	E.E.		
	WT			+	+	+	+	Complex	[31] [18] [17]
1	c.64T>G	p.W22G[e,i]	Helix A	-	+	N.T.	N.T.	N.T.	[41] [57]
2	88insACC	30insH[f]	Helix A	Reduced	N.T.	N.T.	N.T.	N.T.	[33]
3	c.170G>T	p.G57V[f]	Helix B, edge of interface	Reduced	Reduced	+	-	N.T.	[51]
4	c.536G>A	p.G179D[c]	Loop D-E	-	-	+	-	Core	[17]
5	c.599T>G	p.L200R[c]	Helix E	-	-	+	-	Core	[18]
6	c.608C>T	p.S203L[c]	Helix E	-	-	+	-	Core	[18]
7	c.635G>A	p.G212A[e]	Helix F	Reduced	+	+	+	Complex	[18]
8	c.557C>T	p.C219R[c]	Helix F	-	-	+	-	Core	[18]
9	c.661T>C	p.C221R[c]	Helix F	-	-	+	-	Core	[18]
10	c.674T>C	p.L225P[c]	Helix F	-	-	+	-	Core	[17]
11	c.731C>T	p.S244L[e]	Helix G	Reduced	+	+	+	Complex	[17]
12	c.779G>T	p.G260V[d]	Helix H, interface	-	Reduced	+	+	Complex	[17]
13	c.810C>G	p.S270R[c]	Loop H-I, interface	-	-	+	-	N.T.	[51]
14	c.815A>G	p.Y272C[e]	Loop H-I, interface	-	+	+	+	Complex	[17]
15	c.834G>C	p.L278F[d]	Helix I, edge of interface	Reduced	Reduced	+	+	Complex	[17]
16	c.837G>A	p.W279X[f]	Helix I, edge of interface	-	N.T.	N.T.	N.T.	N.T.	[34]
17	c.839G>C	p.R280P[f]	Helix I, edge of interface	Reduced	Reduced	+	Increased	N.T.	[51]
18	c.971T>G	p.L324R[c]	Helix J	-	-	N.T.	N.T.	N.T.	[37]
19	c.985G>C	p.G333R[c]	Helix J	-	N.T.	N.T.	N.T.	Core	[54]
20	c.1020C>A	p.N340K[c,i]	Helix J	-	-	+	-	Core	[17]

Table 1 Continued

Number	Nucleotide change[a]	Amino acid change	Position in protein structure[b]	Currents	Surface expression	Intracellular localization		N-glycosylation	Reference
						E.R.	E.E.		
21	c.1033C>T	p.R347X[c,i]	Helix J	-	-	N.T.	N.T.	N.T.	[41] [37]
22	c.1385G>T	p.G462V[c]	Helix N	-	-	N.T.	N.T.	N.T.	[37]
23	c.1406T>C	p.L469P[c]	Helix N	-	-	+	-	Core	[18]
24	c.1517G>A	p.G506E[c]	Helix O, interface	-	-	N.T.	N.T.	N.T.	[37]
25	c.1534G>C	p.G512R[f]	Helix O, interface	-	N.T.	N.T.	N.T.	N.T.	[35]
26	c.1538G>A	p.G513E[c]	Helix O, interface	-	-	+	-	N.T.	[51]
26	c.1539G>A	p.G513R[c]	Helix O, interface	-	-	+	-	Core	[17]
27	c.1546C>T	p.R516W[c]	Loop O-P, interface	-	-	N.T.	N.T.	N.T.	[37]
28	1842insT	M517fsX528[c]	Helix P, interface	-	-	N.T.	N.T.	N.T.	[37]
29	c.1558T>C	p.S520P[e]	Helix P, interface	Reduced	+	N.T.	N.T.	N.T.	[57]
30	1853-54delTT	L521RfsX526[c,h]	Helix P, interface	-	-	N.T.	N.T.	N.T.	[37]
31	1567-1569delGTC	p.V523del[f]	Helix P, interface	Reduced	N.T.	N.T.	N.T.	N.T.	[61]
32	c.1571T>A	p.I524K[c]	Helix P, interface	-	-	+	-	N.T.	[51]
33	c.1581A>T	p.E527D[d,g]	Helix P, interface	-	Reduced	+	+	N.T.	[51]
34	c.1637A>G	p.K546E[d]	Helix Q, interface	-	Reduced	+	+	Complex	[17]
35	c.1639T>G	p.W547G[d]	Helix Q, interface	Reduced	Reduced	+	+	Complex	[17]
36	c.1851C>G	Y617X[e,i]	CBS 1	-	+	N.T.	N.T.	N.T.	[63] [40]
37	c.1962C>T	R648X[e,i]	Between CBS 1-2	-	+	N.T.	N.T.	N.T.	[40] [34]
38	c.2110C>T	R704X[e,i]	CBS 2	-	+	N.T.	N.T.	N.T.	[40] [34]
39	C.2152C>T	p.718X[c,i]	CBS 2	-	-	+	-	Core	[18]

[a] Nucleotide numbering refers to the cDNA numbering with +1 being the A of the ATG translation initiation codon in the reference sequence. Codon 1 is the initiation codon. The GenBank accession number of human wild-type ClC-5 is NG_007159.2
[b] Position refers to the predicted topology of ClC-5 determined by Wu *et al.* [62]
[c] The mutation belongs to class 1 mutations inducing defective protein processing
[d] The mutation belongs to class 2 mutations inducing delayed protein processing and lower stability of the mature protein
[e] The mutation belongs to class 3 mutations inducing altered conduction without any change in subcellular distribution
[f] The mutation needs further investigation to allow its classification
[g] The mutation leads to an absence of endosomal acidification
[h] This mutation was functionally tested as L521R
[i] This mutation is not displayed on the tridimentional model of ClC-5 due to lack of sequence homology between StClC and ClC-5
E.E., early endosomes; *E.R.*, endoplasmic reticulum; *N.T.*, not tested; *WT*, wild-type

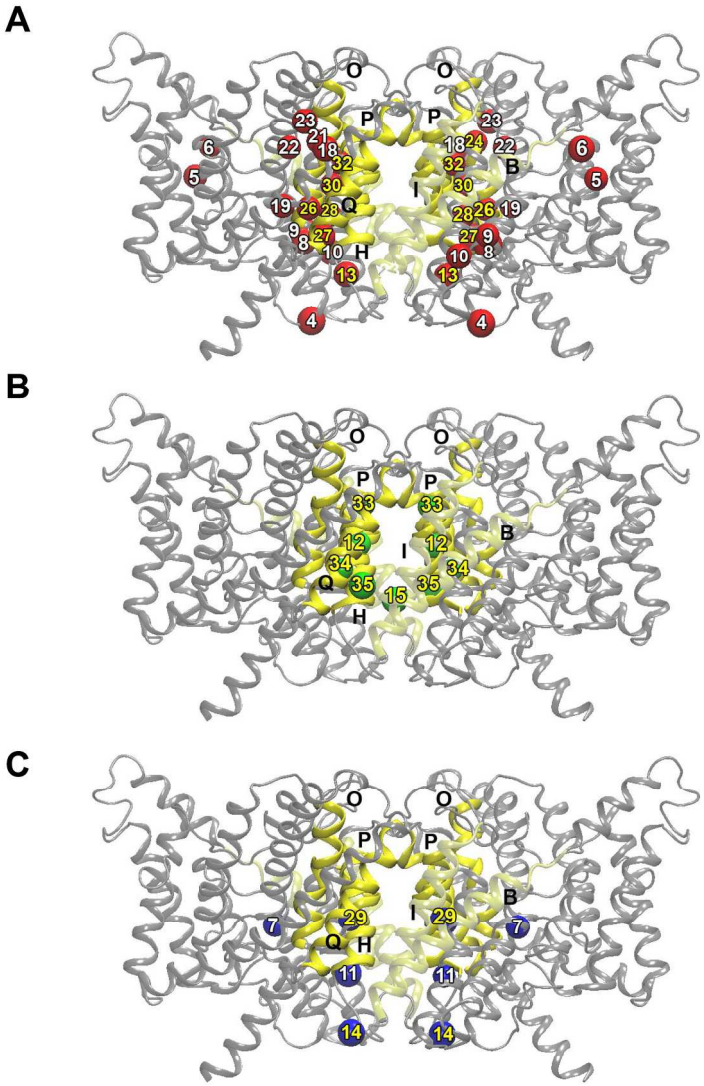

Figure 1

159x265mm (300 x 300 DPI)

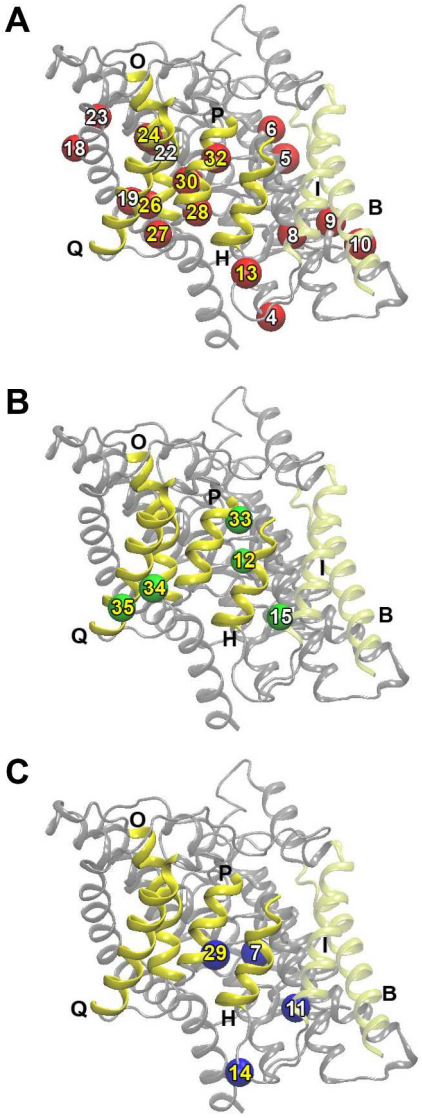

Figure 2

161x430mm (300 x 300 DPI)

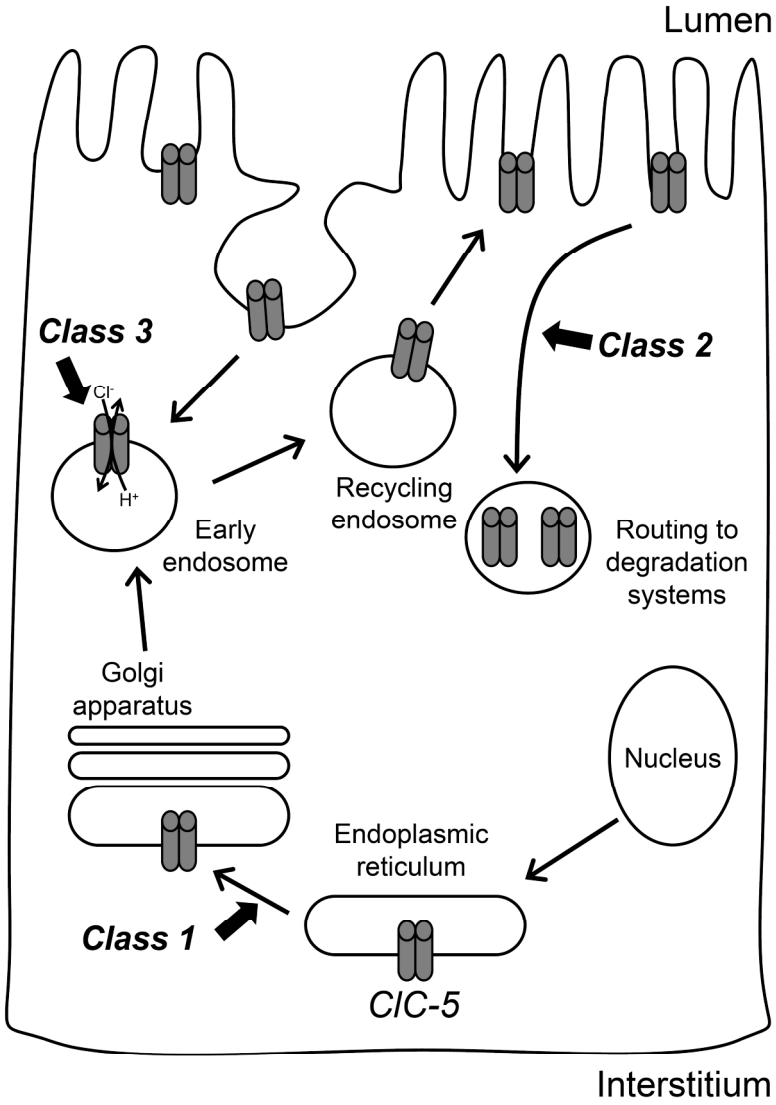

Figure 3

206x309mm (300 x 300 DPI)