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CFTR and defective endocytosis: new insights in the renal phenotype of cystic fibrosis

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Abstract Inactivation of the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR) causes cystic fibrosis (CF). Although CFTR is expressed in the kidney, no overwhelming renal phenotype is associated with CF. Recent studies have shown that the level of CFTR mRNA in mouse kidney approaches that found in lung. CFTR is particularly abundant in the apical area of proximal tubule cells, where it co-distributes with the Cl^-/H^+ exchanger ClC-5 and Rab5a in endosomes. The biological relevance of CFTR in proximal tubule endocytosis has been tested in CF mouse models and CF patients. Mice lacking CFTR show a defective receptor-mediated endocytosis, as evidenced by impaired uptake of ^{125}I-β_2-microglobulin, a decreased expression of the cubilin receptor in the kidney, and a significant excretion of cubilin and its low-molecular-weight ligands into the urine. Low-molecular-weight proteinuria (and particularly transferrinuria) is similarly detected in CF patients in comparison with normal controls or patients with chronic lung inflammation. These studies suggest that the functional loss of CFTR impairs the handling of low-molecular-weight proteins by the kidney, supporting a role of CFTR in receptor-mediated endocytosis in proximal tubule cells. The selective proteinuria should be integrated in the pathophysiology of multi-systemic complications increasingly observed in CF patients.

Keywords Proximal tubule · Kidney · Chloride channel · Cystic fibrosis transmembrane conductance regulator · Protein metabolism

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Cystic fibrosis: pathology and genetics

Cystic fibrosis (CF, OMIM #219700) is the most common lethal autosomal recessive disease in Caucasians, affecting as many as one in 2,500 live births. Cystic fibrosis is a multisystemic disease that essentially results from the obstruction of exocrine glands by an excessive mucus production. The accumulation of thick mucus in the human airways causes obstructive lung disease promoting chronic bacterial infection and inflammation, the hallmark of CF. A similar mucus accumulation in pancreatic ducts, bile ducts, and the intestine may lead to pancreatic insufficiency, liver damage, and meconium ileus (intestinal obstruction), respectively, in some patients with CF [73]. In addition, the absence or obstruction of the vas deferens causes male infertility in most CF patients, and altered cervical mucus production reduces female fertility [55]. The airway manifestations represent the main cause of morbidity and mortality in CF patients. Nowadays, improvement in medical care often preserves life expectancy beyond the third decade [73].

The fact that patients with CF have a typically high concentration of NaCl in the sweat, coupled with an increased lumen-negative transepithelial voltage, pointed early on to a defective chloride transport and salt homeostasis as the cause of organ damage in CF [73, 79]. In 1989, Collins, Riordan and colleagues demonstrated that CF was due to loss-of-function mutations in the *CFTR* gene that encodes a Cl^- channel named CFTR—for cystic fibrosis transmembrane conductance regulator [48, 71]. The *CFTR* gene, also named as *ABCC7*, is located on chromosome 7q31.2 and spans approximately 290 kb of genomic DNA (27 exons) encoding a 1,480-amino acid protein [20]. Over 1,000 CF-associated mutations have been reported thus far in *CFTR* (CF Genetic Analysis Consortium, http://www.genet.sickkids.on.ca/cftr/) and

have been classified into five groups according to their structural or functional consequences on Cl^- conduction [73]. The in-frame deletion of three bases encoding a phenylalanine residue at position 508 (ΔF508) represents the most common mutation in CF population, affecting the correct processing and maturation of CFTR [5]. About 5% to 10% of *CFTR* mutations are due to premature truncation or non-sense alleles and are associated with the most severe CF phenotypes [74]. Most other *CFTR* defects are unique to a particular family or to only a handful of cases across the world. The type of *CFTR* mutations seems directly linked to the pancreatic phenotype, whereas the high variability in pulmonary complications among siblings carrying identical mutations strongly supports the influence of the environment and modifier genes in the disease severity [74]. Moreover, patients with the CF phenotype have been reported with no or only one mutation in the *CFTR* gene [29]. These cases exemplify the broad clinical spectrum of *CFTR*-linked disease and support the existence of additional genes involved in the CF phenotype.

Structure and function of CFTR

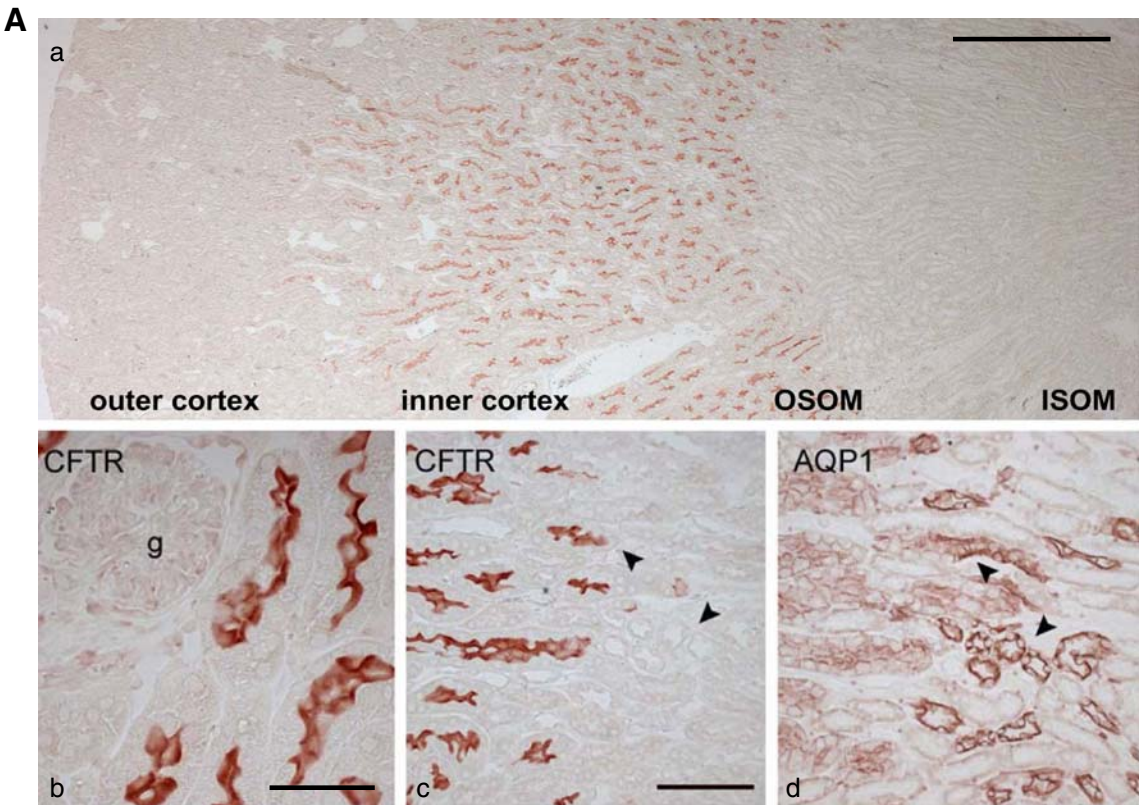
The CFTR protein is a member of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of integral membrane transporters [25]. The ABC transporters function as mediators of unidirectional organic solute transport and include multidrug resistance proteins, such as MDR and P-glycoprotein, and a number of prokaryotic and eukaryotic small nutrient and molecule transporters [38]. CFTR is organized symmetrically in two transmembrane domains (TMD1 and TMD2) and two nucleotide binding domains (NBD1 and NBD2), separated by a large, polar, regulatory (R) domain unique within the ABC family [71]. Each membrane-spanning domain contains six α helices, portions of which form the Cl^- pore. CFTR is regulated by cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of the R domain via protein kinase A (PKA), followed by ATP-dependent gating events initiated by ATP binding to the cytoplasmic nucleotide-binding domains (NBD1 and NBD2) and resulting in transepithelial Cl^- transport [25, 79].

The N and C termini of CFTR are both intracellularly oriented. The C terminus harbors a conserved type I PDZ domain-binding motif, which interacts with several PDZ-domain proteins including the Na^+/H^+ exchange regulatory factors (NHE-RF1 and NHE-RF2) and the actin-binding protein ezrin [31]. The dynamic regulation of CFTR binding to such scaffolding proteins may determine its dimeric organization into macromolecular functional units containing regulatory partners and other channels. Furthermore, the phosphorylation of the R domain is also regulated by NHERF1, NHERF2, and ezrin [32]. The insertion of CFTR channels in the plasma membrane also involves a complex of proteins including the PDZ-domain proteins NHERF1 and CAL as well as the SNARE SYN6 and the Rho GTPase TC10 [6]. Consequently, molecular switches regulate CFTR-mediated Cl^- secretion by modulating both its channel activity and its intracellular trafficking [52]. On the other hand, PKA is involved in the exocytic insertion and endocytic retrieval of CFTR from the plasma membrane and distinct Rab GTPases, which are small catalysts responsible for cargo selection, vesicle motility, and endosome docking and fusion to the appropriate membranes, participate in the intracellular transport and the plasma membrane localization of CFTR [32]. CFTR interacts functionally with other channels, including the outwardly rectifying Cl^- channels and the Na^+ channel ENaC, and it participates in exocytosis and the formation of macromolecular complexes at the plasma membrane, in close contact with receptors, signaling proteins, and the cytoskeleton [32]. Therefore, the role of CFTR extends well beyond Cl^- permeability, as supported by its unique structure and its subcellular distribution in epithelial cells.

Segmental and subcellular distribution of CFTR in the kidney

CFTR is located in the apical membrane of numerous secretory epithelia, including airways, colonic crypts, pancreatic and sweat ducts, and male genital tract [79]. However, several studies have demonstrated the expression of CFTR in the developing and mature mammalian kidney [12, 15, 43, 58]. CFTR mRNA is detected in all nephron segments of the rat and human kidney, but is particularly abundant in the cortex and outer medulla [58]. By immunostaining, CFTR was detected at the apical surface of both proximal and distal tubules of rat kidney but not in the outer medullary collecting ducts [12]. Recent studies in mouse kidney [43] revealed that CFTR is mainly expressed in the apical area of proximal tubular (PT) cells (pars recta, S3 segment; Fig. 1a), with a subcellular distribution compatible with endosomes as shown by co-distribution with ClC-5 and vacuolar H^+-ATPase (V-ATPase) in Rab5a-enriched fractions (Fig. 1b). In the human kidney, CFTR protein expression was detected in the PT, in addition to the thin limbs of Henle's loop, distal tubules, and collecting ducts [12, 15, 58]. CFTR is also expressed in the branching ureteric bud during early nephrogenesis [15]. Of note, a functional truncated isoform (TNR-CFTR) made of the TMD1, NBD1 and R domains has also been detected in rat and human kidney with a distinct ontogeny pattern and a minor plasma membrane expression [15, 39, 58].

Besides its location in the plasma membrane, CFTR is located in intracellular organelles along the endocytic and



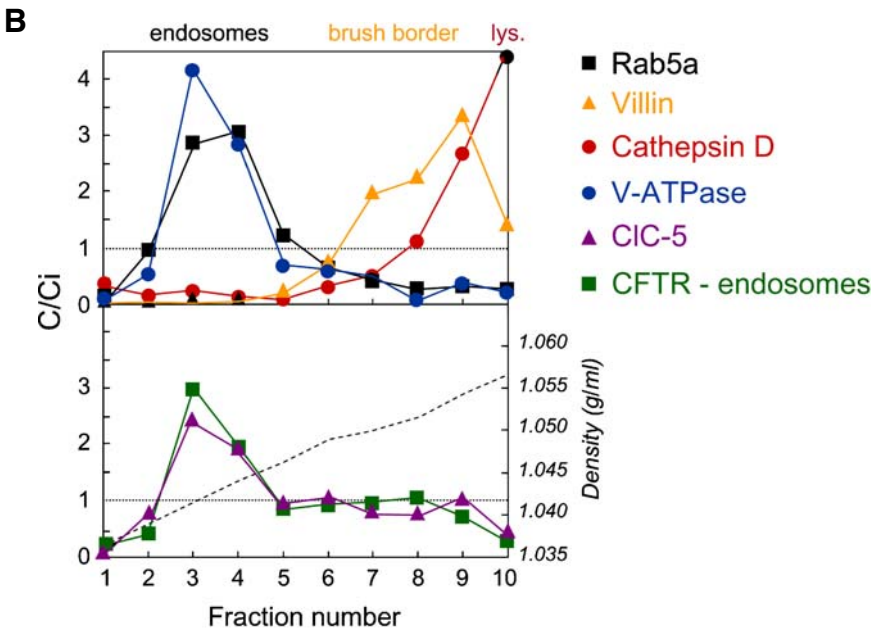


Fig. 1 Localization of CFTR in mouse kidney. **A** Distribution of CFTR in mouse kidney. (a) CFTR is detected preferentially at the junction between the inner cortex and the outer stripe of the outer medulla (OSOM). (b) In the cortex, CFTR is located in the apical area of proximal tubules. (c, d) The segmental co-localization of CFTR and AQP1 indicates that CFTR is particularly abundant in the apical area of the distal S3 segments of the proximal tubule, just before the transition with the descending thin limb (arrowheads). No specific staining is observed in *Cftr*$^{-/-}$ kidney (not shown). Bars 500 μm in a; 50 μm in b; and 100 μm in c and d. g glomerulus. **B** Subcellular distribution of CFTR in mouse kidney: Percoll gradient analyses. Percoll gradients of total mouse *Cftr*$^{+/+}$ kidney resolve a low-density peak (fractions 2–4) including the early endosomal maker Rab5a, an intermediate density peak (fractions 7–9) including the brush border component villin, and a bottom peak enriched in lysosomes (cathepsin D). Distributions after centrifugation are presented by comparison with the initial concentration (C/Ci values >1 reflect organelle enrichment, and values <1 reflect organelle depletion). Typical densities are indicated by a broken line in the lower panel. CFTR co-distributes with ClC-5 and the vacuolar H-ATPase (E1 subunit) in the endosomal fractions. Modified from [43]

secretory pathways, in which it might act as a pH regulator by importing Cl^- in parallel to H^+ accumulation [3]. Mutant epithelial cells derived from CF patients exhibit no cAMP-dependent regulation of endocytosis and exocytosis, unless transfected with cDNA encoding wild-type CFTR [4]. Incubation of freshly isolated nasal polyps from CF patients harboring the ΔF508 mutation with 3-(2,4-dinitro-anilino)-3′-amino-*N*-methyldipropylamine (DAMP), used as a semi-quantitative marker of vesicular acidification, showed that DAMP accumulation was significantly lowered in specific biosynthetic compartments, i.e. *trans*-Golgi and pre-lysosomal organelles [1]. Moreover, the monitoring of membrane potential in a light microsomal fraction from CF and non-CF epithelial cells showed that acidification is limited in CF cells by a high $\Delta\Psi$ resulting from insufficient Cl^- counterion conductance. In turn, defective acidification may induce lysosomal enzyme deficiencies and abnormal trafficking and processing of newly synthesized polypeptides in cells lacking CFTR [3]. However, the role of CFTR in regulating organelle pH remains controversial, with hyper- rather than hypo-acidification suggested to occur in CF respiratory epithelial cells. Indeed, cell ratiometric imaging with luminally exposed pH-sensitive green-fluorescent protein have demonstrated that CFTR decreases the pH of endosomal organelles because of a loss of CFTR inhibitory effects on Na^+ transport and a defect in cyclic guanosine 3,5 monophosphate signaling cascade [69]. In addition, recycling of transferrin receptor is impaired in CFTR mutant lung epithelial cells, with possible functional consequences at both the plasma membrane and within endosomal compartments [70].

The renal phenotype of cystic fibrosis

Despite the expression of CFTR in the mammalian kidney [12, 15], no overwhelming renal phenotype is associated with CF. Patients with CF are prone to develop episodes of hyponatraemic, hypochloraemic dehydration with metabolic alkalosis, resembling Bartter syndrome [47, 81]. A reduced renal NaCl excretion and decreased capacity to dilute and concentrate urine has also been reported [59]. However, some of these modifications could result from a primary defect in kidney function or simply reflect changes in the extracellular fluid volume caused by excessive losses of NaCl in sweat and feces. Microscopic nephrocalcinosis has been detected in an autopsy series of CF patients ranging in age from birth to 36 years [46], and the incidence of kidney stones in CF patients may also be increased [27]. However, the relative contribution of lithogenic factors, such as hypocitraturia, hyper-oxaluria and -uricosuria, or impaired hydration remains elusive.

In CF mouse models, renal Na^+ clearance studies showed no significant difference between controls and *Cftr*^{tm2Cam} mice harboring the ΔF508 mutation under basal conditions or after acute extracellular volume expansion [49]. In addition, *Cftr*^{tm2Cam} ΔF508 mice were equally able to reduce Na^+ excretion under chronic dietary salt restriction but displayed an increased amiloride sensitivity, compatible with a functional interaction between CFTR and the Na^+ channel ENaC in collecting duct principal cells [50, 51]. Recently, Lu et al. used CFTR-deficient mice to demonstrate that CFTR regulates the ATP sensitivity of the K^+ channel renal outer medullary potassium channel (ROMK) in the thick ascending limb (TAL) of the loop of Henle, which could explain why CF patients are prone to develop the pseudo-Bartter features of hypokalemic metabolic alkalosis [54].

Interestingly, CF patients show an enhanced renal clearance of many drugs including aminoglycosides [75], which pointed to a putative defect in receptor-mediated endocytosis in proximal tubule cells [78]. Indeed, by analogy to other intracellular Cl^- transporters such as the Cl^-/H^+ exchanger, ClC-5, CFTR may play a role in membrane recycling and/or vesicular pH regulation in kidney cells [17]. However, until recently, the possible role of CFTR in regulating endocytosis in kidney PT cells had not been substantiated in mouse models or CF patients.

Receptor-mediated endocytosis in the proximal tubule

A significant amount of albumin and low-molecular-weight (LMW) plasma proteins is continuously filtered through the glomerular basement membrane, to be reabsorbed by PT cells [2]. As an example, albumin concentration in the ultrafiltrate is in the range of 20 to 30 mg/l, which corresponds to a daily filtered load of albumin of 3.5 to 5.5 g, of which less than 1% is excreted in the final urine [26]. By definition, LMW proteins are characterized by a molecular mass lower than that of albumin (~69 kDa). They include hormones (parathormone (PTH), insulin, growth hormone), carrier or storage proteins (retinol-, vitamin D- and folate-binding proteins), enzymes (cytochrome C, lysozyme), cell-surface antigen components (β_2-microglobulin), immunoglobulin light chains, and other proteins (cystatin C, Clara cell CC16 protein, and α_1-microglobulin). Most of these filtered LMW proteins are reabsorbed and metabolized by PT cells, and the human urine is virtually devoid of plasma proteins under physiological conditions (Fig. 2). Such massive uptake of proteins accounts for as much as 80% of the total metabolic clearance of small proteins and peptides and plays a key role in hormone and vitamin homeostasis [7].

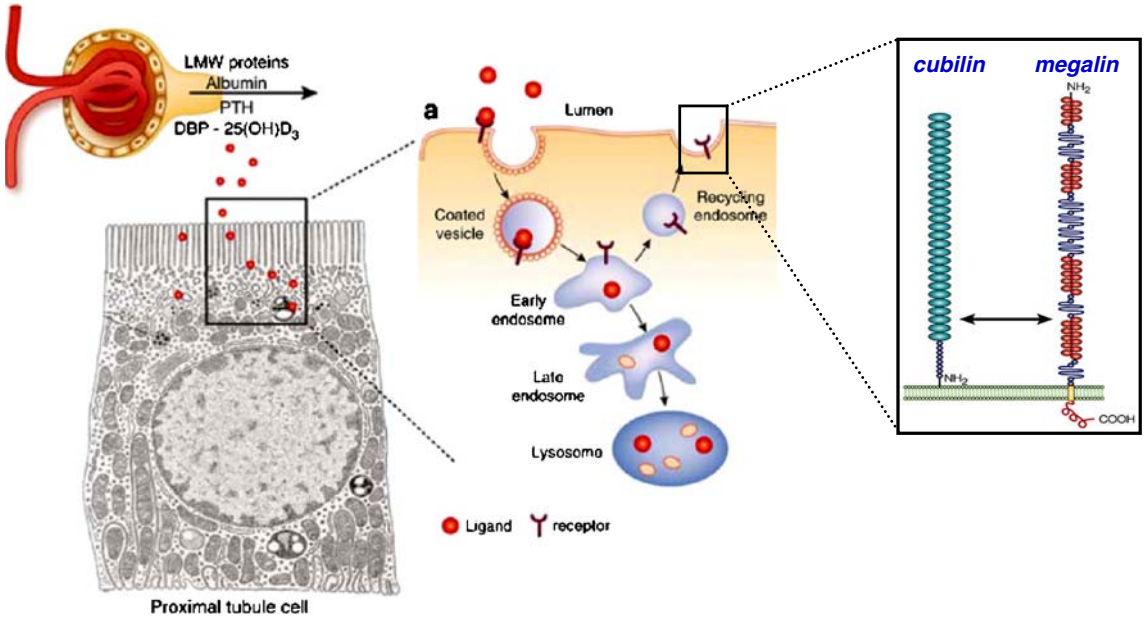


Fig. 2 Reabsorption of low-molecular-weight proteins by proximal tubule cells. Albumin and low-molecular-weight (*LMW*) proteins (including PTH, vitamin-D binding protein (*DBP*), and 25(OH) vitamin D) that are naturally filtered by the glomerulus into the primary urine are endocytosed by PT cells via the megalin–cubilin receptor pathway. Following internalization in coated vesicles, the receptor–ligand complexes progress along the endocytic pathway. The endosomes undergo a progressive acidification that results in the dissociation of the receptor–ligand complexes, with megalin and cubilin (*inset*) being recycled in the apical membrane, whereas the ligand is directed to lysosomes for degradation. Modified from [2, 18]

The uptake of LMW proteins by PT cells essentially involves receptor-mediated endocytosis, while fluid-phase capture can be considered as quantitatively negligible [9]. During receptor-mediated endocytosis, filtered proteins are concentrated at the apical cell surface, and their concentration in the endocytic invagination exceeds that in the extracellular space several fold. Clathrin-mediated endocytosis represents the predominant pathway for protein uptake across the apical membrane of PT cells, with an endocytic pathway consisting of five main interrelated compartments: (1) microvilli and clathrin-coated pits, (2) early endosomes, (3) dense apical tubules responsible for apical recycling, (4) late endosomes, and (5) lysosomes [10]. The process requires two multiligand receptors, megalin and cubilin, that are abundantly expressed at the brush border of PT cells [7]. Ligand binding and interactions between both receptors induce their internalization into coated vesicles and their subsequent delivery to endosomes and lysosomes for ligand processing and receptor degradation or recycling (Fig. 2). Receptor-mediated endocytosis of albumin depends on the integrity of the actin cytoskeleton and the microtubules [26], whereas progression along the endocytic apparatus requires a sustained vesicular acidification from early to late endosomes and finally to lysosomes [22, 80] (Fig. 3). Indeed, the drop in pH in the successive endocytic compartments triggers receptor–ligand dissociation and modulates vesicle trafficking, endosomal fusion events, and coat formation [40]. In PT cells, the endosomal acidification is driven by the electrogenic vacuolar H^+-ATPase (Fig. 4), whose inhibition by pharmacological agents like bafilomycin A-1 or toxic agents like the heavy metal cadmium severely impairs the uptake of albumin and LMW proteins in vitro and in vivo

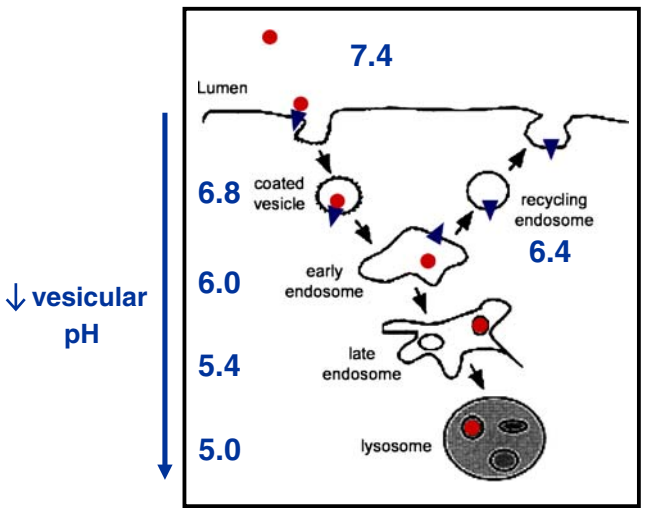


Fig. 3 Vesicular acidification and receptor-mediated endocytosis. The endocytic pathway in proximal tubule cells involves coated pits and coated vesicles, followed by early endosomes that form recycling endosomes or mature to late endosomes and lysosomes. There is a progressive, ATP-dependent acidification of the endosomes (pH 5 to 6) and lysosomes (pH 4.6–5.0) that is necessary for dissociation of the ligand–receptor complex, recycling of receptors to the apical membrane, and progression of ligands into lysosomes. The approximate pH values of the different compartments are indicated. Modified from [17]

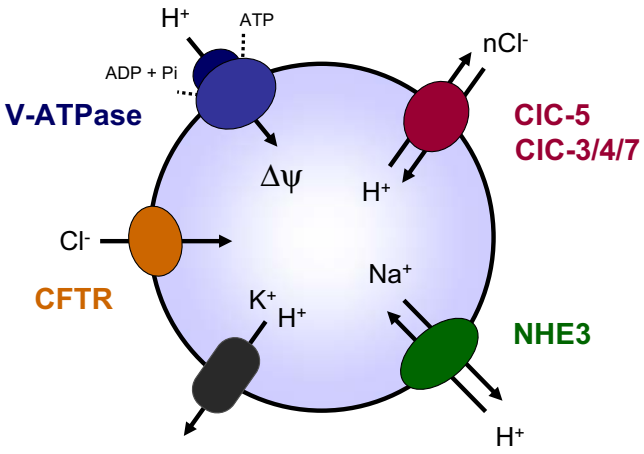


Fig. 4 Ion transport processes involved in endosomal acidification. The endosomal acidification is achieved by ATP-driven transport of cytosolic H^+ through the V-ATPase. The positive electrical gradient ($\Delta\Psi$) is dissipated by cation (H^+, K^+, Na^+) leakage, as well as by parallel Cl^- permeability through ClC-5 and most likely other anion transporters. Recent studies have demonstrated that vesicular CLC isoforms (ClC-3 to ClC-7) act as voltage-dependent Cl^-/H^+ exchangers. ClC-6, restricted to the nervous system, is not depicted. Adapted from [22]

[37, 84, 86]. The translocation of H^+ from the cytoplasm into the endosomes generates a transmembrane electrical potential ($\Delta\Psi$) resulting in a rapid inhibition of V-ATPase activity. Thus, in order to limit the formation of an endosomal-positive membrane potential, either anions have to concurrently enter vesicles or cations have to leave (Fig. 4). In most cases, acidification of intracellular vesicles seems dependent on a parallel Cl^- conductance that provides the electrical shunt necessary to neutralize the H^+ electrical gradient [42]. Furthermore, the intravesicular Cl^- concentration itself could directly affect the V-ATPase activity [60] as well as the vesicle recycling independently of its effect on pH [22].

The paradigm of Dent's disease

Recent investigations on the pathophysiology of Dent's disease and the Cl^-/H^+ exchanger, ClC-5, have provided exciting information on the role of chloride transporters along the endocytic pathway [42]. Dent's disease (OMIM #300009) is a rare X-linked renal tubulopathy characterized by LMW proteinuria associated with hypercalciuria, which may provoke nephrolithiasis, nephrocalcinosis, and renal failure [18, 77]. Low-molecular-weight proteinuria is the most consistent manifestation of Dent's disease, detected in all affected males and obligate carrier females. In contrast, there is considerable inter- and intra-familial variability in the other manifestations of the disease, including progression to renal failure [77]. Dent's disease is caused by mutations in the *CLCN5* gene that encodes ClC-5, an electrogenic Cl^-/H^+ exchanger [53, 66, 76]. ClC-5 belongs to the CLC family of Cl^- channels/transporters that have been discovered and characterized by Jentsch and colleagues ([41] for review). ClC-5 consists of 746 amino acids and forms diamond-shaped homodimers composed of two repeated halves that span the membrane in opposite orientation [19]. Each subunit has its own pore responsible for the selective coupling of the Cl^- flux to H^+ counter-transport [66, 76]. In vitro studies have demonstrated that natural mutations in ClC-5 lead to a loss of function [53]. Furthermore, genetic inactivation of the *Clcn5* gene in mouse mimics the severe PT dysfunction observed in Dent's disease [67, 85].

The complex phenotype of Dent's disease is probably explained by the expression of ClC-5 in multiple nephron segments, including the PT, the TAL, and the α-type intercalated cells [16, 18, 33]. Particularly, ClC-5 co-distributes with the V-ATPase in early endosomes along the endocytic apparatus in PT cells [16, 33], where it is thought to ensure the Cl^- permeability in parallel with H^+ transport (Fig. 4), as suggested by a decreased ATP-dependent fluorescence quenching of low-density vesicles isolated from ClC-5-deficient mice and loaded in vitro by acridine orange [34]. Further measurements of endosomal pH and Cl^- concentration in PT cells cultured from ClC-5-deficient mice provided additional evidence for ClC-5 involvement in acidification of early endosomes but not of late endosomes and Golgi [36]. Other members of the CLC family of vesicular channels/transporters have been shown to play a role in vesicular acidification, including ClC-3 and ClC-7 [28, 36]. Furthermore, recent studies have demonstrated that ClC-5 inactivation induces a generalized trafficking defect in PT cells, with loss of megalin and cubilin at the brush border and impaired lysosome biogenesis, which also contributes to defective endocytosis and urinary loss of LMW ligands and lysosomal enzymes [8, 61].

Receptor-mediated endocytosis in CFTR-deficient mouse kidney

As mentioned above, the distribution of CFTR in the apical endosomes of mouse PT cells [43] pointed to its possible involvement in renal endocytosis. This hypothesis was recently substantiated by using CF mouse models to characterize the role of CFTR in the kidney. Plasma and urine analyses revealed that baseline renal function was normal in $Cftr^{-/-}$ ($Cftr^{tm1Cam}$) mice. However, the urinary excretion of the LMW Clara Cell protein (CC16, 16 kD) was significantly increased in $Cftr^{-/-}$ mice in comparison to controls, reflecting a defect in PT cell apical endocytosis. This was supported by the demonstration of a significant decrease in the renal uptake of radiolabelled ^{125}I-β_2-microglobulin in $Cftr^{-/-}$ mice and a lower renal uptake of aminoglycosides in comparison to wild-type littermates [43].

The endocytic uptake of aminoglycosides and LMW proteins, like CC16 and β_2-microglobulin, is mediated by the multiligand receptors, megalin and cubilin [7]. In contrast to megalin, which is a member of the low-density lipoprotein receptor family, cubilin, also known as the intestinal intrinsic factor (IF)-B12 receptor, is a highly conserved membrane glycoprotein with little structural homology to known endocytic receptors and is characterized by the absence of a transmembrane domain [2]. High-affinity binding of purified megalin to cubilin N-terminal region has been shown in vitro, suggesting that megalin participates in the endocytosis and intracellular trafficking of cubilin [57]. The apical sorting of cubilin and its participation in receptor-mediated endocytosis critically depend on its reciprocal interaction with the transmembrane protein amnionless (AMN) identified as a key factor for mouse gastrulation [11, 44]. Cubilin contributes ligand-binding regions of the receptor complex, whereas AMN ensures the membrane anchorage, biosynthetic processing, and recycling of the complexes at the plasma membrane [24]. Interestingly, there was a selective decrease of cubilin expression in the straight (S3) segment of the PT of *Cftr*$^{-/-}$ mice mirrored by an increased urinary excretion of cubilin ligands such as transferrin and CC16 [43]. Further investigations demonstrated that the lack of CFTR in the kidney was not associated with changes in the biosynthesis of cubilin but, rather, with a significant increase in the excretion of cubilin in the urine of *Cftr*$^{-/-}$ mice. No significant changes in kidney and urine abundance of megalin were observed. Taken together, these data suggest that the lack of CFTR in renal PT cells induces instability of cubilin at the brush border, leading to its accelerated shedding into urine. Although the lack of suitable reagents prevented to investigate the specific role of AMN in the process, it is tempting to hypothesize that such cubilin instability could be due to the improper processing or trafficking of one of its partners in relation with the lack of CFTR in that nephron segment [43].

Renal phenotype in mice and humans harboring the ΔF508 mutation

The ΔF508 mutation is the most common mutation in the CF population, with almost 90% of Caucasian CF patients having at least one ΔF508 allele [73]. This mutation affects the processing and maturation of CFTR to its fully glycosylated form [5], with retention of ΔF508-CFTR in the endoplasmic reticulum (ER) by molecular chaperones [20] and subsequent degradation via the ubiquitin-proteasome pathway [87]. However, the ΔF508-CFTR can essentially function as a cAMP-regulated Cl^- channel both in the ER and at the plasma membrane under distinct permissive conditions [23, 64]. The processing of ΔF508-CFTR has been shown to revert to that of wild-type CFTR as the incubation temperature is reduced [13]. Airway and gallbladder cells from *Cftr*^{tm1Eur} mice homozygote for the ΔF508 mutation show increased cAMP-induced Cl^- conductance when cultured at 27°C [23].

The *Cftr*^{tm1Eur} mouse model expressing the ΔF508 mutant CFTR was generated by double homologous recombination [21, 82]. Six-week-old mice homozygous for the ΔF508 mutation (*Cftr*$^{\Delta F/\Delta F}$) have abnormalities typical of CF such as growth retardation, focal hypertrophy of goblet cells in the intestinal crypts, and higher basal nasal potential difference with reduced response to forskolin in trachea and intestine. However, the *Cftr*$^{\Delta F/\Delta F}$ tissues show a residual Cl^- conductance, suggesting that the mutant ΔF508-CFTR is partially processed and reaches the plasma membrane. This may explain the minor phenotype of the *Cftr*$^{\Delta F/\Delta F}$ versus *Cftr*$^{-/-}$ mice (e.g., absence of lethal intestinal obstruction). Although such rescue phenomenon has not been clearly demonstrated thus far in man, residual Cl^- transport activity has also been observed in rectal biopsies of ΔF/ΔF patients with milder CF phenotype [83]. Moreover, the expression of ΔF508-CFTR in man is tissue-specific, suggesting that the variable severity of CF in different organs may reflect heterogeneity of residual expression [45, 65].

In mouse *Cftr*$^{\Delta F/\Delta F}$ kidney, the mRNA abundance of ΔF508-CFTR was ~2-fold reduced in comparison to controls [43]. The mutant ΔF508-CFTR showed defective glycosylation, with a large individual variability in its residual expression in the apical area of PT cells. Consequently, the renal uptake of radiolabelled ^{125}I-β_2-microglobulin was either unchanged or decreased (~30%) in comparison to wild-type mice. In addition, the electrolyte and water handling has previously been demonstrated as largely preserved in the PT of *Cftr*^{tm2cam} ΔF508 mice [49]. Taken together, these data suggest that, in mouse kidney, the ΔF508-CFTR is variably processed into its mature form, reaching the plasma membrane and ensuring correct function in some *Cftr*$^{\Delta F/\Delta F}$ mice.

Clinical and biological investigations of a large cohort of CF patients, all harboring at least one ΔF508 mutation, showed a mild but significant albuminuria and LMW proteinuria versus healthy controls. The specificity of the LMW proteinuria in CF was supported by the lack of significant changes in patients with chronic lung inflammation due to active asthma [43]. These findings may impact on the long-term renal function since LMW proteinuria can trigger tubulo-interstitial injury progressively leading to renal failure [2]. Moreover, the increased urinary loss of the cubilin-ligand, transferrin, could participate in the iron deficiency and lower circulating transferrin levels that are commonly reported in CF patients [62, 68].

Conclusions and perspectives

The spectrum of CF, which was previously considered as a respiratory and digestive disease associated with a rapidly fatal outcome, has broadened considerably over the last decade. Recent studies performed in CF mouse models and patients with CF have demonstrated that the functional loss of CFTR is associated with a moderate but significant defect in LMW protein handling by the kidney. These data support a role of CFTR in cubilin-mediated endocytic pathway in PT cells. Data obtained in mice and patients harboring the ΔF508 mutation also give insights into the tissue-specific and species-dependent processing of wild-type and mutant CFTR [63]. Further investigations in different CF mouse models [30] and in other animal models that may be more relevant for the disease [72] should help to clarify the role of CFTR in other specialized kidney functions, such as Ca^{2+} handling and NaCl homeostasis [17]. One should also consider that subtle abnormalities in kidney development, function, and/or morphology may appear in CF patients at a later stage—as longer survival may be associated with a potential for developing multi-organ complications [73].

Finally, in addition to the functional loss of CFTR, specific kidney diseases may involve an abnormal activity or expression of CFTR. For example, several lines of evidence have shown that CFTR is upregulated and activated in the cells lining the cysts, playing a role in cyst fluid accumulation in autosomal dominant polycystic kidney disease, the most common inherited nephropathy [14, 35, 56, 65, 88]. Investigation of these situations may also prove useful to gain knowledge in the role of CFTR during nephrogenesis and in the mature kidney.

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