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Review

Functional interplay between CFTR and pendrin: physiological and pathophysiological relevance

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Abstract

The transport of chloride and bicarbonate across epithelia controls the pH and volume of the intracellular and luminal fluids, as well as the systemic pH and vascular volume. The anion exchanger pendrin (SLC26A4) and the cystic fibrosis transmembrane conductance regulator (CFTR) channel are expressed in the apical membrane of epithelial cells of various organs and tissues, including the airways, kidney, thyroid, and inner ear. While pendrin drives chloride reabsorption and bicarbonate, thiocyanate or iodide secretion within the apical compartment, CFTR represents a pathway for the apical efflux of chloride, bicarbonate, and possibly iodide. In the airways, pendrin and CFTR seems to be involved in alkalinization of the apical fluid via bicarbonate secretion, especially during inflammation, while CFTR also controls the volume of the apical fluid via a cAMP-dependent chloride secretion, which is stimulated by pendrin. In the kidney, pendrin is expressed in the cortical collecting duct and connecting tubule and co-localizes with CFTR in the apical membrane of β intercalated cells. Bicarbonate secretion occurs via pendrin, which also drives chloride reabsorption. A functional CFTR is required for pendrin activity. Whether CFTR stimulates pendrin via a direct molecular interaction or other mechanisms, or simply provides a pathway for chloride recycling across the apical membrane remains to be established. In the thyroid, CFTR and pendrin might have overlapping functions in driving the apical flux of iodide within the follicular lumen. In other organs, including the inner ear, the possible functional interplay between pendrin and CFTR needs to be explored.

Keywords: CFTR; pendrin/SLC26A4; lung; kidney; thyroid; inner ear; review

1. Introduction

Transcellular Cl^- and HCO_3^- transport plays a crucial role in cell physiology. In several tissues, the anion transport through the epithelium is due to the coordinated function of the cystic fibrosis transmembrane conductance regulator (CFTR) channel (OMIM *602421) and members of the solute carrier SLC26 family of transporters, which share similar tissue expression patterns. In secretory epithelia, different SLC26 member proteins can have redundant functions when having the same tissue expression. Human SLC26A4 (OMIM *605646), also known as pendrin (PDS), is a 780 amino acid electroneutral anion exchanger displaying transport abilities for different substrates, including chloride, bicarbonate, iodide, formate, nitrate, and thiocyanate [1–4]. The local concentration of anions may partially explain the differentiated ion selectivity of pendrin. It cannot be excluded, however, the possibility that ion selectivity might be regulated by cell-specific pendrin protein interactomes. Pendrin was initially found expressed in the inner ear, kidney, and thyroid gland [5–7]. Other studies demonstrated that it is also expressed in the airways, liver, mammary glands, reproductive tissues, and placenta [5,8–13].

Sequence alterations in the *SLC26A4* gene, encoding pathogenic pendrin protein variants, cause Pendred syndrome [14] and non-syndromic sensorineural deafness DFNB4 [15]. Pendred syndrome (OMIM #274600) is an autosomal recessive disease that was first described, in terms of clinical manifestations, in 1896 by Vaughan Pendred [16]. This syndrome has an estimated prevalence ranging between 7.5 and 10 per 100,000 individuals [17,18]. Pendred syndrome is characterized by hearing loss and thyroid dysfunction possibly due to abnormal iodide organification. About 4–10% of the described inherited deafness cases are ascribed to Pendred syndrome [19]. DFNB4 (OMIM #600791) is the second most common cause of hearing loss worldwide [20]. In both cases, patients present with a malformation of the temporal bone called enlarged vestibular aqueduct (EVA), accompanied by a malformation of the membranous labyrinth, which displays an enlargement of the endolymphatic sac and duct. A cochlear incomplete partition type II (Mondini malformation) may occasionally be found (reviewed in [21]).

Up to now, 840 sequence alterations of the *SLC26A4* gene have been reported (https://www.ncbi.nlm.nih.gov/clinvar, accessed on the 15.12.2021) and only in part characterized concerning their clinical significance. Two hun-

dred and eighty of them are missense mutations generating protein variants of which 125 have been classified as pathogenic/likely pathogenic and may be retained in the endoplasmic reticulum. Pathogenic protein variants deriving from nonsense mutations, altered splicing, insertions, deletions, and partial gene duplications have been also described [22]. Abnormal and high expression of pendrin has been correlated with lung disorders [23,24]. Conversely, specific inhibition of renal pendrin has been suggested to face hypertension and the condition of diuretic-resistant water overload [25]. Based on the crystal structure of the transmembrane domain of *Deinococcus geothermalis* fumarate transporter (SLC26Dg), it has been proposed that pendrin holds fourteen transmembrane alpha-helices with cytosolic N-terminal and C-terminal domains [26]. The C-terminal region contains a Sulfate Transporter and Anti-Sigma Factor Antagonist (STAS) domain and a putative consensus protein kinase A (PKA) phosphorylation motif (714)RKDT(717). Deletion mutants lacking the putative PKA consensus site displayed a lower basal functionality and a reduced insertion in the plasma membrane [27]. Interestingly, additional putative phosphorylation sites for other kinases including PKC can be predicted by bioinformatics (https://services.healthtech.dtu.dk/service.php?NetPhos-3.1; NCBI Reference Sequence: NP_000432.1). In this respect, additional studies would be needed to clarify the possible role of phosphorylation on pendrin function and subcellular distribution.

In this review, we focus on the functional interaction between the anion exchanger pendrin and the CFTR channel in orchestrating the anion transport as well as the control of the pH and volume of the luminal fluids in epithelia of various organs. We summarize the evidence of a clear interplay between these two molecular entities in the airways and kidney, and information supporting a similar scenario in the inner ear, thyroid, and liver. Tissue-specific pendrin expression and function is also reviewed; for information concerning the structure, expression, and function of CFTR in different organs as well as clinical aspects of cystic fibrosis (CF; OMIM #219700) we refer to recent excellent reviews [28–30] and other publications of this same Special Issue.

2. The lung and airways

2.1 Pendrin expression and function in the airways

In the last few years, the involvement of pendrin in different respiratory disorders has been widely described [31–33]. In the airways, pendrin is expressed mainly in secretory non-ciliated cells, while ciliated cells lack significant pendrin expression [34,35]. In a mouse model of acute lung injury (ALI) induced by lipopolysaccharide (LPS), pendrin expression increased [36]. Interestingly, treatment with YS-01, a selective pendrin inhibitor, mitigated the typical ALI inflammatory responses by downregulating the expression of proinflammatory mediators and Nuclear factor kappa B (NF-kB) activation [37]. Also, pendrin-deficient mice showed a reduced allergen-induced airway hyperreactivity with respect to control animals [23].

Pendrin is highly expressed in animal models of asthma and chronic obstructive pulmonary disease (COPD) [32] and in patients with chronic rhinosinusitis [38]. Asthma and COPD are chronic inflammatory diseases affecting the airways and causing airflow impairment possibly due to bronchoconstriction and mucus overproduction. Several interleukins (ILs), including IL4, IL13, and IL17 play key roles in the pathogenesis of asthma and COPD [4,32,35]. Indeed, IL13 and IL4 are involved in mucus overproduction [39]. In the respiratory system, the electroneutral antiporter pendrin mediates the transport of Cl^- against HCO_3^- or thiocyanate (SCN^-). Thiocyanate is subjected to oxidation generating hypothiocyanite ($OSCN^-$), which holds antibacterial functions in the airway surface liquid (ASL), but also exhibits pro-inflammatory activity [40]. In human bronchial epithelial cells, treatment with IL4 stimulated the expression of pendrin, which mediates SCN^- transport [4], possibly by inducing the demethylation of pendrin promoter [41]. Using the microarrays technology, the pendrin gene has been identified as an IL13-inducible gene in human airway epithelial cells. Interestingly, IL13-induced upregulation of pendrin was not found in mice deficient for the Signal Transducer and Activator of Transcription STAT6, likely suggesting that IL13 increased the expression of pendrin by stimulating a STAT6-dependent pathway [32]. Furthermore, *in vitro* studies showed that mutation of a STAT6 binding motif abolished the IL4/IL13 effects on pendrin promoter activity [24]. Studies from our group showed that stimulation with forskolin or treatment with IL13 increased the abundance of pendrin in the plasma membrane of bronchial NCI-H292 cells [42,43], which may contribute to modulating the intracellular pH [42].

2.2 Pendrin and CFTR functional interplay in the airways

In human bronchiolar cells, IL4/IL13 modulate the transport of different ions. In particular, these cytokines downregulate Na^+ transport via ENaC and activate CFTR and pendrin functionalities [23,44]. At the basolateral side, the NKCC1 cotransporter mediates the uptake of Cl^-, which is therefore secreted at the apical side through CFTR (Fig. 1) [30].

In the airways, chloride and bicarbonate homeostasis is crucial for pH regulation and the dynamic maintenance of ASL. ASL is generated by secretion from submucosal glands and transepithelial osmotic water transport. Physiologically, ASL pH is slightly acidic compared to the interstitium. The apical secretion of protons is mainly mediated by the H^+-ATPase and H^+/K^+-ATPase, which transport H^+ against the electrochemical gradient. By contrast, the apical secretion of bicarbonate mainly involves CFTR and pendrin [45] (Fig. 1).

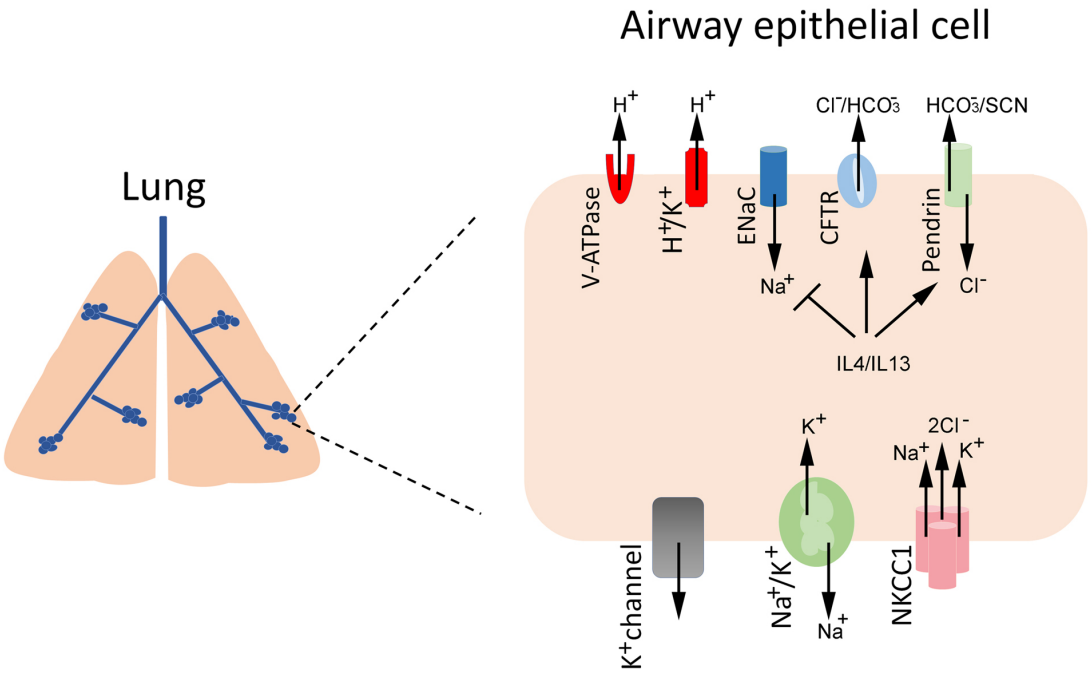

Fig. 1. Ion transport in the airway epithelial cells. On the apical membrane, airway epithelial cells express the vacuolar H^+-ATPase and H^+/K^+-ATPase, which secrete protons in the apical compartment. IL4/IL13 stimulate the antiporter pendrin and CFTR, which mediate the transport of Cl^- and HCO_3^-, and inhibit sodium uptake via ENaC. At the basolateral side, NKCC1 facilitates Cl^- entry accompanied by Na^+/K^+-ATPase function and Na^+ recycling. CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial Na^+ channel; NKCC1, Na^+ /K^+/$2Cl^-$ cotransporter; V-ATPase, vacuolar H^+-ATPase.

Altered mechanisms regulating ASL pH cause several respiratory disturbances [46]. CFTR controls bicarbonate efflux by transporting HCO_3^- directly. Loss of function of CFTR reduces the pH of ASL, thereby impairing bacterial killing [30,47,48]. Alternatively, bicarbonate secretion may also result from a coordinate function of CFTR and Cl^-/HCO_3^- exchangers of the SLC26 family. CFTR can bind members of the SLC26 family through a molecular interaction between the regulatory (R) and the STAS domains, respectively. Importantly, this interaction can be stabilized by the cAMP/PKA dependent phosphorylation of the R domain of CFTR [49].

Secretory and primary cells deriving from differentiated human airway epithelia express both pendrin and CFTR [50]. Studies in polarized Calu-3 cells that co-express CFTR and pendrin revealed that stimulation with forskolin potentiates HCO_3^- transport [51]. CFTR inhibition caused a significant decrease of fluid secretion. By contrast, knocking down pendrin functions, a reduction of the fluid pH with no relevant changes in the rate of fluid secretion was found. These findings suggest that pendrin is involved in controlling the fluid pH. Conversely, CFTR tightly modulates the rate of fluid secretion [51,52]. Importantly, the volume and the composition of ASL depends on a functional interplay between CFTR and ENaC, whose reabsorptive activity is downregulated in heathy conditions [47]. In addition, apical ion secretion via large-conductance, Ca^{2+}-activated, voltage-dependent K^+ (BK) channels and calcium-activated chloride channels (CaCC/TMEM16A) plays an important role in increasing ASL thickness [53,54].

Recent data revealed that stimulation of pendrin with IL4 or IL13 facilitates Cl^- secretion through CFTR [55]. Additionally, IL4-induced pendrin expression stimulates the activity of CFTR upon forskolin treatment [50].

Combined treatment with TNFα and IL17, which are key drivers of inflammatory airway diseases, augmented the expression level of CFTR and pendrin, resulting in a significant alkalinization of ASL [34]. Conversely, selective inhibition of pendrin in human bronchial epithelial cells expressing wild type CFTR causes a significant decrease of ASL pH similarly to that measured in cells expressing the F508del-CFTR mutant, strongly supporting the hypothesis that pendrin contributes to alkalinize the ASL [56].

Other studies, however, revealed that pendrin can play a role in controlling ASL volume and not in regulating ASL pH in IL13-stimulated cells. In IL13-treated airway epithelial cells isolated from healthy subjects or patients with cystic fibrosis, selective inhibition of pendrin significantly increased ASL volume, which might result from an impaired pendrin-dependent Cl^-/HCO_3^- exchange [57] accompanied by a downregulation of ENaC function in response to IL13. These findings would support the use of pendrin inhibitors in inflammatory lung diseases, including cystic fi-

brosis. Accordingly, in nasal epithelial cells isolated from deaf patients carrying biallelic pathogenic sequence alterations in the pendrin gene, ASL depth was thicker than controls. Importantly, stimulation with IL13 further increased ASL volume [58]. Overall, the actual contribution of pendrin in airway physiology is far to be defined. A coordinated function of CFTR and pendrin might play a key role in controlling the ASL pH and volume, possibly through the action of specific inflammatory cytokines, such as interleukins and TNFα. However, whether the combination of different interleukins with other inflammatory mediators may modulate pendrin function in relation to its ability to control ASL pH or volume remains to be established. Furthermore, more investigations are needed to clarify whether the combined activity of CFTR and pendrin may constitute a crucial defense mechanism counteracting inflammation.

3. The kidney

3.1 Pendrin and CFTR expression and function in the kidney

The distal tract of the nephron plays a key role in the regulation of the systemic pH owing to the unique property of secreting HCO_3^- or H^+ as required to counteract metabolic alkalosis or acidosis, respectively. In the kidney, pendrin is mainly expressed in the cortex and was localized on the apical membrane of β and non-α, non-β intercalated cells of the cortical collecting duct (CCD) and connecting tubule (CNT) [7,59,60]. The β intercalated cells represent a subpopulation of cells within the CCD that also express the H^+-ATPase on their basolateral membrane, but not apical aquaporin-2 or basolateral anion exchanger 1 (AE1), which are distinctive features of principal and α intercalated cells, respectively. The β intercalated cells are thought to mediate bicarbonate secretion [61] (Fig. 2).

Ectopic expression in HEK-293 cells showed that pendrin can function in the electroneutral Cl^-/OH^-, Cl^-/HCO_3^-, Cl^-/formate, and Cl^-/I^- exchange modes [62,63]. Seminal studies in perfused CCD tubules isolated from alkali-loaded pendrin knockout mice indicated that a main function of pendrin in the kidney is the excretion of bicarbonate [7]. Accordingly, pendrin expression and function is downregulated in metabolic acidosis (rats and mice subjected to oral NH_4Cl loading for 1–7 days [64–67]) and upregulated in metabolic alkalosis (rats and mice subjected to oral bicarbonate loading for 1–7 days [65–67]). Surprisingly, arterial pH, systolic blood pressure, and various parameters reflecting the kidney function, including chloride excretion, urine volume, and serum chloride and sodium concentrations were normal in pendrin knockout mice [68,69]. However, these mice produced an acidic urine and had an elevated serum HCO_3^- concentration compared to wild type mice, consistent with a decreased bicarbonate secretion [69].

Alterations in the kidney function of pendrin knockout mice become more obvious when these mice are exposed to aldosterone analogues, such as deoxycorticosterone pivalate (DOCP), or challenged by salt restriction. Pendrin knockout mice are resistant to DOCP-induced hypertension but develop metabolic alkalosis, denoting that (i) pendrin is upregulated by aldosterone in wild type mice and (ii) counteracts metabolic alkalosis in this setting [68]. Moderate NaCl restriction increased urinary volume and Cl^- excretion in pendrin knockout mice, which showed higher arterial pH, hematocrit, and blood urea nitrogen compared to wild-type mice, all consistent with contraction of the vascular volume. Under severe NaCl restriction, pendrin knockout mice become hypotensive [70]. These changes are strictly dependent on chloride rather than sodium, and chloride restriction can upregulate pendrin expression via redistribution to the apical membrane of β intercalated cells [71]. These findings highlight a fundamental role of pendrin in renal chloride conservation and regulation of blood pressure. Accordingly, pharmacological inhibition of pendrin potentiated the diuretic activity of furosemide in mice, further supporting the role of pendrin in NaCl reabsorption in the distal nephron [72].

The activity of pendrin in the kidney is under control of angiotensin II/aldosterone, which changes pendrin cellular abundance and distribution in part through a direct effect on the mineralocorticoid receptor of intercalated cells [68,73,74].

Interestingly, the activity of pendrin in the kidney is tightly connected to that of other ion-transporting systems. For example, in various mouse models, negative or positive changes in pendrin abundance are mirrored by changes in abundance and/or activity of the epithelial sodium channel ENaC in principal cells and the sodium-driven chloride/bicarbonate exchanger (Ndcbe) [74–77]. In contrast, gene ablation of the sodium-chloride cotransporter NCC in the distal convoluted tubule provoked a compensatory upregulation of pendrin [78], probably masking the effect of NCC loss of function on salt reabsorption; consequently, double knockout of pendrin and NCC caused severe salt wasting, volume depletion and metabolic alkalosis under baseline conditions [79].

CFTR mRNA and/or protein have been found in all nephron segments of the human and rat kidney, and are particularly abundant in the renal cortex and outer medulla. In the mouse kidney, CFTR was found in endosomes of the cells of the terminal part of the proximal tubule [80]. In humans, protein immunoreactivity was detected at the apical region of both the proximal and the distal renal tubules [81]; the expression starts during early embryogenesis [82]. In addition to wild-type CFTR, a truncated form (TNR-CFTR) containing only the first transmembrane domain, the first nucleotide-binding domain, and the regulatory domain is expressed in the kidney and demonstrated cAMP-dependent, PKA-stimulated Cl^- channel activity similar to that of wild type CFTR when expressed in *Xenopus* oocytes [83]. Abundant CFTR transcript levels were found in all

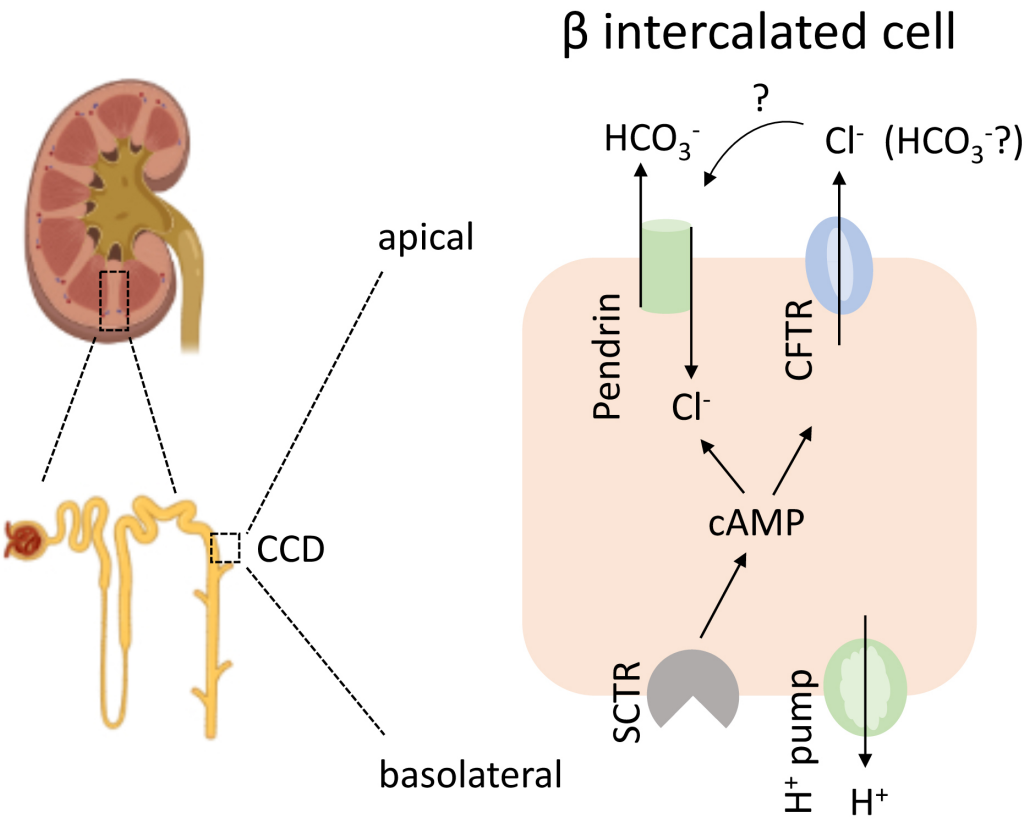

Fig. 2. Functional interplay of pendrin and CFTR in the distal nephron. In the kidney, pendrin is expressed in the cortical collecting duct and connecting tubule and co-localizes with CFTR in the apical membrane of β intercalated cells. These cells are characterized by expression of apical pendrin and basolateral H^+-ATPase and are considered HCO_3^--secreting cells. Increases in the intracellular concentration of cAMP following stimulation of the basolateral SCTR in these cells stimulate CFTR and pendrin activity and/or localization in the apical membrane and activate HCO_3^- secretion. HCO_3^- secretion occurs via pendrin, which also drives Cl^- reabsorption. Whether and to what extent bicarbonate secretion also occurs via CFTR is unknown. A functional CFTR is required for pendrin function. Whether CFTR directly stimulates pendrin or simply provides a pathway for Cl^- recycling remains to be established. CCD, cortical collecting duct; CFTR, cystic fibrosis transmembrane conductance regulator; SCTR, secretin receptor.

three main CCD cell types in rabbit (i.e., α and β intercalated cells as well as principal cells), with predominant expression in β intercalated cells [84]. In the mouse CCD, CFTR forms PKA-activated Cl^- channels in the apical membrane of principal cells [85]. The function of CFTR in the kidney has been extensively studied and includes the regulation of other ion transport pathways, including the renal outer medulla potassium (ROMK) channels and ENaC. Indeed, CFTR confers ATP-sensitivity to ROMK1 and CFTR activation inhibits ENaC (reviewed in [86]). Despite the abundant expression of CFTR in the kidney, primary renal disease is unusual in cystic fibrosis (CF) patients. However, lower molecular weight proteinuria has been described [87] and single case reports or small case series documented the occurrence of electrolyte imbalances in these patients [88].

3.2 Pendrin and CFTR functional interplay in the kidney

The first hint of a possible functional interplay between CFTR and pendrin in the kidney arose from experiments where the two proteins were expressed in HEK-293 cells. In these cells, co-expression with CFTR activated the Cl^-/OH^- exchange via pendrin by about five-fold, independently of stimulation of CFTR with forskolin [89]. In the same work, similar observations were made for other members of the SLC26 family, i.e., SLC26A3/DRA and SLC26A6. As mentioned above, the same research group later showed that the activation of SLC members DRA and SLC26A6 and CFTR is reciprocal and occurs via binding of the CFTR R domain to the STAS domain of SLC26 transporters, which is dependent on phosphorylation of the R domain by PKA [49]. If this also applies to pendrin was not explored.

The importance of studying the interplay between pendrin and CFTR in the kidney is linked to the fact that CF patients are prone to develop life threatening metabolic alka-

losis [88]. In this context, Soleimani and his research group first showed that the kidneys of CF mice have an impaired ability to excrete excess bicarbonate. Following a three-day oral bicarbonate load, CF mice showed a significantly more acidic urine as well as elevated bicarbonate and arterial blood pH compared to wild type animals, all indicative of the development of metabolic alkalosis. These effects were linked to transposition of pendrin towards the subcellular compartment of β intercalated cells, together with a decreased pendrin transcript and plasma membrane protein abundance. Although the mechanism of pendrin downregulation remained to be explored, these findings clearly indicate that metabolic alkalosis in CF patients may be the consequence of pendrin dysfunction [90].

Very recently, Berg *et al*. [91] dissected the molecular derangements leading to metabolic alkalosis in CF. By utilizing a global and a kidney tubule-specific CFTR knockout mouse as well as a pendrin knockout mouse, these authors found that bicarbonate excretion into the urine following i.p. injection of secretin or an acute oral load of bicarbonate is prevented in these mice [91]. Secretin, which is the first hormone that was discovered, is a peptide initially described as produced by the S cells of the duodenum following a drop of the duodenal content pH below a value of four. In addition to inhibiting the production of gastric acid from the parietal cells of the stomach, secretin also stimulates the secretion of bicarbonate from the pancreas. More recently, a direct effect of secretin on renal water reabsorption was found [92,93]. Berg *et al*. [91] found that secretin receptors are expressed on the basolateral membrane of β intercalated cells in native mouse kidney and stimulate bicarbonate secretion from these cells. Pendrin and CFTR colocalize on the apical membrane of these same cells. The secretin-dependent bicarbonate excretion requires functionally intact pendrin and CFTR, is stimulated by an elevation of cytosolic cAMP, is defective in CF patients, and is corrected by therapy with CFTR activators [91]. These findings denote that CFTR is required for pendrin function in the kidney and explain metabolic alkalosis in CF (Fig. 2).

The role of CFTR in governing the pendrin-mediated bicarbonate excretion into the urine was recently confirmed also in a mouse model of chronic base load (oral supplementation of $NaHCO_3$ for 7 days). Wild type mice responded to chronic base load with an elevation of urinary pH, which was absent in pendrin as well as in CFTR knockout mice. Instead, these mice developed metabolic alkalosis. Also, CFTR knockout mice failed to upregulate pendrin in their kidneys in response to base-loading, which corresponded to a pendrin loss of function [67].

If CFTR directly activates pendrin or simply provides a pathway for the apical recycling of chloride, thus enabling pendrin function, is currently unknown. One can imagine that a direct molecular interaction between pendrin and CFTR is necessary for pendrin activation and/or stability within the plasma membrane; it is tempting to speculate that this interaction might involve the STAS domain of pendrin and the R domain of CFTR, as it is reported for other members of the SLC family [49,94]. How CFTR can regulate pendrin protein abundance and whether this involves a possible transcriptional regulation is more difficult to envision and remains to be elucidated. Also, CFTR is capable of bicarbonate secretion in the pancreas and various other organs [95]; whether and to what extent CFTR itself participates in the renal bicarbonate excretion in the CCD independently of pendrin remains to be explored.

4. The thyroid

The thyroid gland contains two different endocrine units, the follicular and parafollicular cells. Follicular cells are involved in thyroid hormone generation. Thyroid hormone production is tightly controlled by the hypothalamic-pituitary-thyroid axis. Specifically, the thyrotropin-releasing hormone (TRH) promotes the pituitary thyroid stimulating hormone (TSH) release, which in turn stimulates thyroid follicles for protein synthesis and thyroxine (T4) and triiodothyronine (T3) secretion (Fig. 3). TSH is a pivotal modulator of thyroid cell functions. Thyroid hormones are involved in several metabolic functions including body temperature control, cardiovascular functioning, growth, and normal development. Therefore, the reduced activity of the thyroid gland observed in hypothyroidism can cause numerous dysfunctions such as bradycardia, cold intolerance, and fat accumulation. In the thyroid follicular cells, iodide uptake occurs via the sodium-iodide symporter (NIS), which is expressed at the basolateral membrane. This process requires the activity of the Na^+/K^+-ATPase that generates and maintains the necessary sodium gradient [96]. At the apical side, iodide is released into the follicular lumen, where it is oxidized and coupled to specific tyrosyl residues within the thyroglobulin (Tg). The iodinated thyroglobulin located into the colloid is internalized in the follicular cells through endocytosis and subjected to lysosome proteolysis to generate and release thyroid hormones [97].

Pendrin has been proposed to mediate the apical efflux of iodide [1]. Consistently, patients with Pendred syndrome can be affected by a partial defect in iodide organification that may lead to goiter development. Nevertheless, thyroid dysfunction is very variable even within the same family, with some patients having large goiters and others with minimal size increase of the thyroid. Importantly, in addition to pendrin, the calcium-dependent chloride channel anoctamin 1 (ANO1/TMEM16A) and CFTR [98,99] can also mediate the apical iodide efflux (Fig. 3).

These findings may explain why several patients with Pendred syndrome are euthyroid, although it cannot be excluded that these patients would develop thyroid dysfunction under conditions of dietary iodide deficiency. Also, the high variability in the clinical manifestations of thyroid disorders may be due to different dietary iodine intake be-

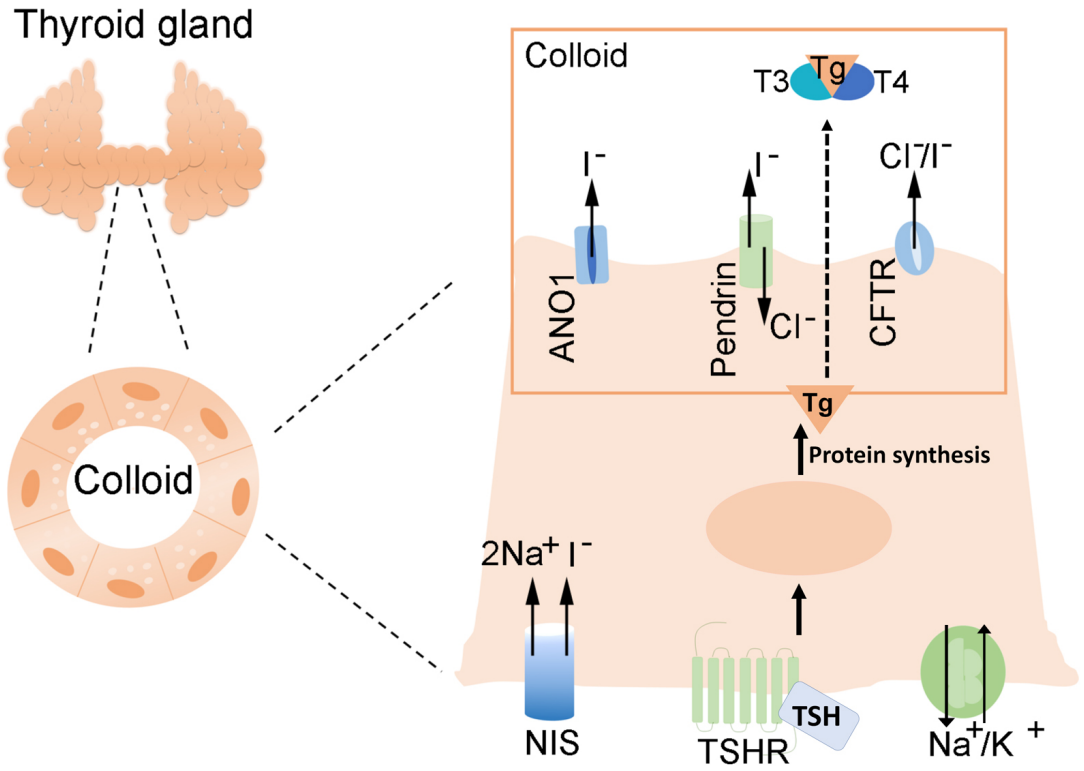

Fig. 3. Ion transport in the follicular cells of the thyroid. In the follicular thyroid cells, The TSH receptor (TSHR) is localized at the basolateral membrane, where it transduces signals resulting in an increased protein synthesis and thyroid hormones (T3, T4) production. Moreover, at the basolateral side, the cotransporter NIS is involved in sodium and iodide entry accompanied by Na^+/K^+-ATPase function. Pendrin is localized at the apical side, where it mediates the iodide efflux that is necessary for thyroid hormones generation. The apical transport of iodide can also occur via ANO1 and CFTR. ANO1, Anoctamin 1; CFTR, cystic fibrosis transmembrane conductance regulator; NIS, Na^+/I^- symporter; Tg, thyroglobulin; TSH, thyroid stimulating hormone; TSHR, thyroid stimulating hormone receptor.

tween patients [22]. Iodide transport to the luminal follicular side is strictly dependent on the iodide uptake at the basolateral side from the blood into the cytosol of thyrocytes (Fig. 3). The basolateral uptake is indeed useful to create a positive gradient for the apical iodide exit. In line, in Chinese hamster ovary cells (CHO) transfected either with NIS or with NIS and pendrin, iodide transport resulted significantly higher in pendrin positive cells [100]. Moreover, in COS-7 cells, the pendrin-dependent iodide efflux was potentiated by the increase of the extracellular chloride concentration, underlining the important role of the extracellular chloride levels in sustaining the iodide efflux [101]. As a matter of fact, co-expression of pendrin and NIS in polarized cells grown in a bicameral system promoted the transcellular flux of iodide into the apical compartment [102].

Physiologically, thyroid cells reabsorb sodium and release chloride in a cAMP-dependent manner. Indeed, in a porcine thyroid culture model, CFTR controlled the cAMP-dependent chloride secretion, raising the hypothesis that chloride may represent the counter-ion for pendrin-dependent iodide transport [8,103–105]. On the other hand, it has been proposed that CFTR may modulate the activity of pendrin by binding the STAS domain [49,106]. Interestingly, some patients with cystic fibrosis can develop mild hypothyroidism [107]. However, considering that CFTR can mediate either the apical efflux of iodide, similarly to pendrin, or the chloride secretion in the thyroid lumen, whether CFTR dysfunction contributes to hypothyroidism directly or following impairment of pendrin activity is still unclear.

5. The inner ear

In the inner ear pendrin is found in the apical membrane of epithelial cells of the cochlea, vestibular system, and endolymphatic sac and duct. Within the murine cochlea, pendrin is expressed in epithelial cells of the spiral prominence, outer sulcus cells, root cells, and spindle-shaped cells of the *stria vascularis* [108,109]. The *stria vascularis* is the structure devoted to the generation of the endocochlear potential, which is essential to the hearing function. In this location, pendrin-driven chloride reabsorption and bicarbonate secretion control the volume and pH of the endolymph, respectively; indeed, loss of pendrin function in knockout mice causes (i) an abnormal enlargement of the luminal volume and (ii) acidification of the endolymph, which are two events that lead to a complex scenario of

pathophysiological derangements, including impaired cell-to-cell communication, retarded development of the *stria vascularis* and organ of Corti, free radical stress, alteration of expression and function of other ion transporters, such as loss of expression of the Kcnj10 in the intermediate cells of the *stria vascularis* and BK K^+ channels in inner hair cells, as well as a greatly increased Ca^{++} concentration in the endolymph, possibly following inhibition of acid-sensitive epithelial Ca^{++} channels Trpv5 and Trpv6. All these pathophysiological changes lead to loss of the endocochlear potential and degeneration of the sensory hair cells, which eventually culminate in loss of hearing [110–112] (reviewed in [113]).

In the murine endolymphatic sac, pendrin is abundantly expressed in mitochondria-rich cells and in a subset of ribosome-rich cells, although at a much lower levels. In the endolymphatic sac, pendrin drives apical reabsorption of chloride followed by vectorial absorption of fluid [114]. Following pendrin gene ablation in mice, the main derangements seen in the cochlea, i.e. luminal enlargement and endolymph acidification, are also seen in the endolymphatic sac. Transgenic mice with pendrin expression restricted to the mitochondria-rich cells of the endolymphatic sac, but lacking pendrin expression in the cochlea or vestibular labyrinth, did not develop a luminal enlargement. Most importantly, hearing and balance were preserved in these mice [115]. These findings point to a specific function of pendrin in mitochondria-rich cells of the endolymphatic sac in controlling the volume of the endolymph.

In the cochlea, CFTR transcript and protein were found in the inner (IHCs) and outer (OHCs) hair cells of the organ of Corti of adult mice. In the lateral membrane of OHCs, CFTR establishes a direct molecular interaction with the motor protein Slc26a5/prestin and stimulates its activity [116]. An interplay between CFTR and pendrin in these sensory cells is unlikely, as pendrin expression appears to be restricted to epithelial cells. In addition to the organ of Corti, CFTR expression was also documented in the *stria vascularis*, spiral ligament, basilar membrane, and cochlear ganglion in guinea pigs and its expression was increased in a model of endolymphatic hydrops [117]. These findings are consistent with a role of CFTR in handling of ion composition and volume of the endolymph. A hypothesis of a functional interplay between CFTR and pendrin in the *stria vascularis* is tempting and would require the precise identification of the cell types expressing CFTR. Various degrees of hearing loss and alteration of the inner ear structures, including atrophy of the *stria vascularis*, have been described in CF patients [118]. Although these derangements have been linked to the use of ototoxic antibiotics, it has been suggested that exposure to aminoglycosides is not the only causal factor for hearing loss in CF [119]. It is plausible that loss of CFTR might affect proper pendrin function, thus contributing to hearing loss and/or increased sensitivity to ototoxic insults in CF.

In the intermediate portion of the rat endolymphatic sac, CFTR was found expressed on the apical membrane of epithelial cells and co-localized with ENaC, although the specific cell type was not determined in this study [120]. Recently, a newly discovered cell type, called the ionocyte, has been described as the main source of CFTR transcript and activity in mouse and human airways [121,122]. These cells share morphological and functional similarities with the intercalated cells of the kidney and mitochondria-rich cells of the inner ear [123]. Indeed, CFTR and pendrin transcripts are co-expressed in mitochondria-rich cells of the mouse endolymphatic sac [114]. CFTR is also expressed in the semicircular canal duct epithelium of rat, and seems to drive a cAMP-stimulated apical secretion of chloride; this may apply to other structures of the inner ear as well. These considerations lead to speculate that CFTR and pendrin might establish a functional interplay in the apical membrane of mitochondria rich cells of the endolymphatic sac in handling the ion composition, pH and volume of the endolymph.

6. The parotid duct

The parotid duct is a salivary duct emerging from the parotid gland, that is the major salivary gland. CFTR is expressed on the apical membrane of the ductal system of salivary glands, and its dysfunction contributes to the abnormal salivary secretion in CF [124]. In the parotid duct, pendrin and Slc26a6 are expressed in the luminal membrane and mainly mediate I^- secretion and Cl^-/HCO_3^- exchange activity, respectively. Silencing of CFTR in sealed ducts with dicer siRNAs attenuated the Cl^-/HCO_3^- exchange by Slc26a6, but had no effect on I^- secretion by pendrin. Accordingly, in single duct cells isolated from the parotid duct of pendrin knockout mice, CFTR currents were normal, whereas deletion of Slc26a6 facilitated the activation of CFTR [3]. Based on these findings, pendrin appears to have a minor role in luminal HCO_3^- transport in the parotid duct and a functional interplay with CFTR is unlikely.

7. The liver

The presence of pendrin transcript and protein in the mouse liver was first reported in 2011. The expression levels changes of pendrin in the liver during metabolic alkalosis and acidosis appeared to be opposite to those observed in the kidney, as pendrin was downregulated in metabolic alkalosis and upregulated in metabolic acidosis [8]. As the liver participates in the systemic acid-base balance by disposing of bicarbonate via the hepatic ureogenesis, it is conceivable that the acid-base status might regulate bicarbonate transporters in this organ; however, the function of pendrin in the liver needs to be further explored.

In the liver, CFTR expression is restricted to the apical membrane of the epithelial cells – the cholangiocytes - lining the biliary ducts and is not found in the hepato-

cytes [125]. In the liver, CFTR is essential to the transport of chloride, bicarbonate, and osmotically coupled water into the bile and to the biliary HCO_3^- secretion, which provides a protective shield to cholangiocytes against bile acids [126]. CF-related liver disease develops in about one third of CF patients, usually presents with focal biliary fibrosis and may lead to portal hypertension, which is one of the leading causes of death after lung failure and lung transplantation in these patients. The development of biliary fibrosis is thought to be a direct consequence of CFTR loss or reduction of function in the bile duct, leading to bile retention with consequent cellular damage and inflammation [127].

The precise cellular localization of pendrin in the liver is unknown; in addition, the main bicarbonate secretion pathway in cholangiocytes appears to be the AE2/SLC4A2-mediated Cl^-/HCO_3^- exchange [128]. However, being pendrin expression restricted to epithelial cells, an interplay of pendrin and CFTR in the regulation of bicarbonate secretion from cholangiocytes into the bile is possible. Of note, bile flow and biliary excretion of HCO_3^- and Cl^- are stimulated by secretin [129] and, as mentioned above, secretin stimulates pendrin activity in the kidney [91].

8. Conclusions and future perspectives

According to a number of cell-based and animal studies as well as clinical observations, the functional interplay between pendrin and CFTR seems to play an important role in the airways and in the kidney in physiological and pathological conditions including, and probably not limited to, CF and inflammation.

In the airways, pendrin and CFTR expression is driven by selective interleukins that are involved in the inflammatory responses and ASL physiology. In particular, the combined function of CFTR and pendrin may be important to control bicarbonate efflux that increases ASL pH and water secretion. However, the precise role of pendrin in terms of ion transport selectivity in CF and in other inflammatory diseases is still missing.

In the kidney, the pendrin-driven bicarbonate excretion relies on the functional and molecular integrity of CFTR and serves to counteract metabolic alkalosis. Whether and to what extent bicarbonate secretion also occurs via CFTR remains to be determined. Also, whether CFTR stimulates pendrin via a direct molecular interaction, favors its expression, folding and/or stabilization in the plasma membrane, or simply provides a pathway for Cl^- recycling across the apical membrane needs to be established. Although proof of a direct molecular interaction between the R domain of CFTR and the STAS domain of other members of the SCL family exists, this evidence is missing for pendrin, and the precise molecular mechanisms of this functional interplay remain elusive.

Several observations indicate that pendrin and CFTR might cooperate to control the apical anion transport as well as the pH and volume of the luminal fluid in other organs, including the inner ear and thyroid. Additional studies are needed to explore this intriguing hypothesis.

Author contributions

GT and SD conceived the study, performed the bibliographic research, prepared the figures, wrote the manuscript, contributed to editorial changes in the manuscript, read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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