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REVIEW

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Regulation of epithelial ion channels by the cystic fibrosis transmembrane conductance regulator*

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Abstract Abstract: In most epithelia ion transport is tightly regulated. One major primary target of such regulation is the modulation of ion channels. The present brief review focuses on one specific example of ion channel regulation by the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR functions as a cAMP-regulated Cl^- channel. Its defect leads to the variable clinical pictures of cystic fibrosis (CF), which today is understood as a primary defect of epithelial Cl^- channels in a variety of tissues such as the respiratory tract, intestine, pancreas, skin, epididymis, fallopian tube, and others. Most recent findings suggest that CFTR also acts as a channel regulator. Three examples are discussed by which CFTR regulates other Cl^- channels, K^+ channels, and epithelial Na^+ channels. From this perspective it is evident that CFTR may play a major role in the integration of cellular function.

Key words Cystic fibrosis · Cl^- channel · K^+ channel · Na^+ channel · Respiratory tract · Colon

Abbreviations *CF* Cystic fibrosis · *CFTR* Cystic fibrosis transmembrane conductance regulator · *IBMX* Isobutylmethylxanthine · *ICOR* Intermediate conductance outwardly rectifying · *MDR* Multidrug resistance protein

Introduction

The pathophysiological basis of cystic fibrosis (CF) was first correctly understood by Schulz and Frömter [67] some 28 years ago, when they measured the transepithelial voltage of sweat gland ducts in normals and patients with this disease. Their data is summarized in Fig. 1. The transepithelial lumen negative voltage is generated by rheogenic (electrogenic) Na^+ absorption via, as we now know, epithelial Na^+ channels [12, 53], and its magnitude depends on the shunting of the voltage by the counterion Cl^-. Because the voltages in CF patients tended to be higher than in normals, they argued that this indicates a defect in Cl^- permeability in these patients. This conclusion became popular only after essentially the same finding was reported by Quinton [61] 15 years later. By that time a more rigorous analysis of the cellular basis for this impermeability for Cl^- became possible with the availability of new techniques such as the patch clamp method [31].

Shortly after Quinton's rediscovery patch clamp analysis in epithelia identified an intermediate conductance Cl^- channel, named by ourselves intermediate conductance outwardly rectifying (ICOR) [32, 74] Cl^- channel (also named ORDIC or ORCC by others). Some groups have claimed that this type of channel is defective in CF [23, 37, 52]. Our own findings, however, indicated that this type of channel is activated only by patch excision, and that its incidence is equally frequent in excised patches of normal and CF respiratory epithelial cells [45]. We concluded that this channel itself has little if anything to do with CF and argued that it may be tonical-

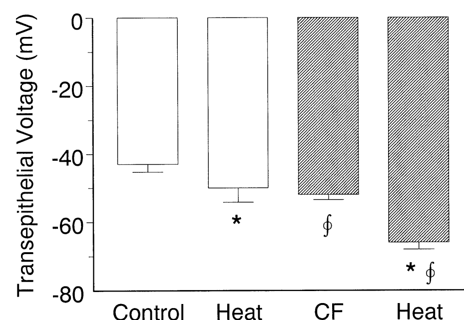

Fig. 1 Transepithelial voltage measurements in sweat gland ducts from normal individuals (*open bars*, *n*=6) and patients with CF (*hatched bars*, *n*=11). Sweat secretion was stimulated by heat exposure. Note the significant increase in voltage by heat exposure (*asterisks*). The values in CF patients are significantly higher than those in controls (§). (Data summarized from [67])

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ly inhibited on the intact cell by a cytosolic inhibitor [42, 43, 46]. Most groups lost interest in this Cl^- channel when the gene responsible for CF, the cystic fibrosis transmembrane conductance regulator (CFTR), was cloned by Tsui's group [66] 7 years ago, and when it became clear from expression studies that CFTR induces a cAMP-regulated Cl^- current of much smaller conductance [5, 24, 65, 72]. This Cl^- channel induced by CFTR expression has a linear current voltage curve, a conductance of 6–8 pS or perhaps even less [47], and a conductance sequence of $Br^- \geq Cl^- > I^-$. Unlike the ICOR channel, it is relatively insensitive to all known blockers of Cl^- channels [11, 27, 44, 74].

Very rapidly, overwhelming evidence accumulated that CFTR is in fact a Cl^- channel and not a regulator thereof. This evidence can be summarized briefly as follows: (a) cRNA or cDNA coding for CFTR and injected or transfected into a variety of cells (HeLa, fibroblasts, SF9, CHO, *Xenopus laevis* oocytes) monotonically produce a small cAMP-regulated Cl^- channel [24, 65]; (b) pathophysiologically relevant mutations of CF, such as ΔF508 produce much less if any Cl^- current; (c) mutations in the 6th membrane spanning domain of CFTR which, on the basis of modeling, are thought to alter ion selectivity in fact do so [2, 73]; and (d) the incorporation of CFTR into lipid bilayers produces the characteristic Cl^- currents [5]. Many more findings, which cannot be cited in this context, support this view [24, 65]. Especially the points (c) and (d) of this list are generally accepted strong criteria. Unfortunately, these studies (c and d) have been performed in only a few laboratories. Our own attempts to reproduce two of these studies [2, 73] in *X. laevis* oocytes injected with cRNA for wild-type or mutated CFTR failed in asmuch as all site-directed mutations in the sixth membrane spanning domain of CFTR examined in our laboratory resulted in similar ion selectivities [35]. This does not disprove the conclusion that CFTR is a Cl^- channel, but it does question some of the supportive arguments.

Current controversies

CFTR is present in many cells of our body. It is not surprising that it is present in the tissues mentioned above, which are also affected in disease, but it is puzzling why it is also found in other cells, which do not show an obvious abnormality, such as kidney or heart [25]. Furthermore, knockout mice, of which several types have been developed over the past few years [4, 14, 16, 60, 69, 76], show various degrees of abnormality which do not completely fit to what was expected from experience in humans. Some even show mucous oversecretion in the gut.

While originally it appeared likely that frequent mutations such as ΔF508 (a mutation in the first nucleotide binding fold) destroyed normal channel function, it was found later that ΔF508 functions quite well as a Cl^- channel [51], but that the protein is normally held back in the "quality control" in the endoplasmic reticulum and degraded there [13, 17, 40, 55, 65, 77]. It must still be acknowledged that we have little understanding of the correlation of genotype and phenotype [18] for most of the more than 400 mutations which have been described for this disease. Obviously most puzzling are the mutations that truncate the protein but show fairly mild clinical symptoms.

The similarity of CFTR and the multidrug resistance protein (MDR) was noted early and has provided the basis for speculations that CFTR is an ATP-driven pump rather than a Cl^- channel, or perhaps both, and, conversely, that MDR might act as a volume-activated Cl^- channel [26, 33, 34, 64, 75]. The latter conclusion could not be confirmed by ourselves or others [49, 62]. However, the similarity of both classes of proteins (extending to the whole class of ATP-cassette binding proteins) remains an intriguing observation and leaves open a wide field for speculation and, hopefully, corresponding experimentation.

One recent suggestion links CFTR function to the above ICOR (ORCC) type Cl^- channel: it has been proposed that CFTR exports ATP, and that ATP, possibly through what now would be called a P_{2x} receptor, opens luminal ICOR type channels [68]. This concept is difficult to reconcile with our own observations that the ICOR-type channel is very rarely seen on the cell, even after stimulation by cAMP [45, 48], and with those of others showing that the cAMP-activated Cl^- conductance has properties that differ from the ICOR type channel [15]. In addition, most recent findings question the role of CFTR as an ATP-conducting pore [63].

Another intriguing suggestion has been made on the basis that CFTR is required in endosomes as a Cl^- channel to secure their acidification [3]. An acid pH appears to be required for appropriate sialylation of proteins, and a defect in this processing might lead to altered (sulfated) surface proteins in respiratory epithelial cells, which have a high binding affinity for pathological bacteria such as *Pseudomonas aeruginosa* [38]. This then would lead to an invasion of the respiratory tract by hard-to-treat pathological bacteria.

New perspectives

Cl^- channel regulation and exo-/endocytosis

CFTR has been implicated in the process of exocytosis and endocytosis. The evidence for this comes from various types of findings: CFTR has been shown to control membrane traffic [9, 29]; activation of Cl^- currents by increases in cytosolic Ca^{2+} and by cAMP cooperate inasmuch as the Ca^{2+} effect on conductance is stabilized by cAMP [1]; increases in cytosolic Ca^{2+} enhance the membrane capacitance in colonocytes abruptly, and cAMP does so much more slowly [28, 29]; cytoskeletal inhibitors such as cytochalasin D or inhibitors of exocytosis such as clostridial toxin C2 attenuate or abolish Cl^- channel activation [36]. Figure 2 shows an example of

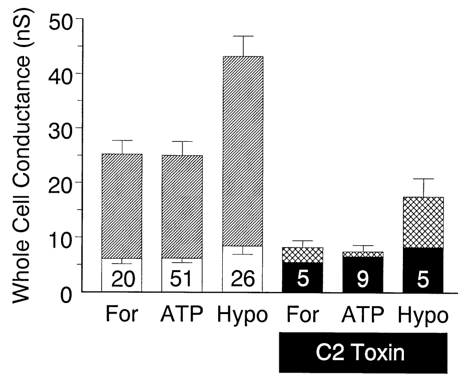

Fig. 2 Whole-cell conductances in bronchial epithelial cells dialyzed with clostridial toxin (C2, 10 nmol/l, kindly provided by Prof. Dr. K. Aktories). *For*, Forskolin, 10 µmol/l; ATP, 0.1 mmol/l; *Hypo*, hypotonic solution, 150 mosm/l, all added to the bath to increase whole-cell conductance. Mean values ± SEM (number of cells). *Open bars*, controls; *hatched bars*, activated conductances. In the presence of C2 toxin the increase in whole-cell conductance by all three activating pathways is significantly attenuated. (Data from [36])

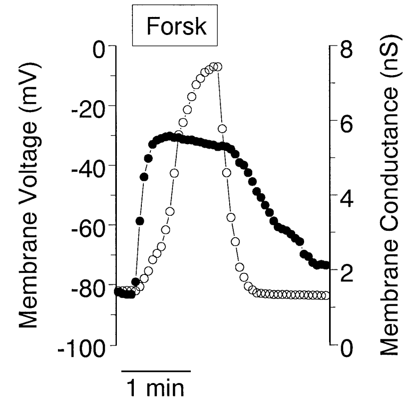

Fig. 3 Whole-cell patch clamp analysis of a rat distal colonic crypt base cell. Forskolin (*Forsk*, 10 µmol/l, bath) depolarizes the membrane voltage (*closed circles*) very rapidly. The increase in conductance (*open circles*) is much slower. In the continued presence of forskolin the voltage starts to repolarize, while the conductance continues to increase. This suggests that forskolin activates two types of conductances: first and very strongly, a Cl^- conductance and, secondly and less pronounced, a K^+ conductance. The effects of forskolin are reversible. (Data from [19])

the effect of clostridial toxin (C2) pretreatment of bronchial epithelial cells. While three types of mechanisms activate Cl^- channels under control conditions – (a) hypotonic cell swelling, (b) Ca^{2+} mobilizing agonists, and (c) cAMP – all three mechanisms of activation are defective after clostridial toxin dialysis via the patch clamp pipette.

It is not clear at this stage how all these findings can be put together. One hypothesis which has been put forward by ourselves proposes that CFTR, when activated by cAMP, slows endocytosis [28, 29]. One salient assumption in this hypothesis is that exocytosis proceeds as a Ca^{2+}-activated process. This is in accordance with the abrupt increments in membrane conductance and membrane capacitance induced by Ca^{2+}-mobilizing agonists. The hypothesis can also explain the slow increase in membrane conductance and membrane capacitance by cAMP, and it would account for the cooperativity of the two messenger pathways (Ca^{2+} and cAMP). The most stringent confirmation of this hypothesis is the demonstration that inhibitors of exocytosis also prevent agonist-induced conductance increases. The acute (20-min) effects of C2 toxin and cytochalasin D seem to support the hypothesis [36]. However, corresponding membrane capacitance measurements are still not available. This involves technical difficulties in measuring very small changes in membrane capacitance against the substantial background noise produced by very substantial increases in membrane conductance [29]. Another issue not easily reconciled with this hypothesis regards quantitative aspects. In HT_{29} colonocytes the agonist-induced increments in membrane capacitance are only 0.3–1 pF (i.e., 2–10% of total capacitance), yet the increases in membrane conductance can easily be by 20–40 nS (i.e., tenfold). The exocytosis hypothesis assumes that a very small area of vesicle membrane inserts extremely densely packed Cl^- channels into the luminal membrane. Furthermore, it must be kept in mind that many laboratories have shown a more direct means of Cl^- channel (CFTR) activation involving protein kinase A dependent phosphorylation [65].

Regulation of K^+ channels by CFTR

An interesting type of K^+ channel modulation by CFTR has been suggested recently [59]. In this study CFTR and ROMK2, an inwardly rectifying renal K^+ channel of intermediate conductance, have been coexpressed in *X. laevis* oocytes. This confers an increase in glibenclamide sensitivity to this K^+ channel. The underlying mechanism is unclear. This is one example of a new concept suggesting the interaction of two distinct membrane proteins to generate a functional unit. This concept has very recently gained additional momentum by a study showing that a subunit of a K^+ channel and the sulfonylurea receptor form an ATP-regulated K^+ channel [39].

It has long been known that agonists acting via cAMP can lead to biphasic voltage responses in some epithelia such as colon and respiratory tract [19, 58, 79]. An example is shown in Fig. 3. After the addition of forskolin to activate adenylate cyclase and increase cytosolic cAMP, the membrane voltage of colonic crypt base cells depolarizes and then repolarizes slightly while forskolin is still present. During this phase the membrane conductance continues to increase. These data indicate that the initial depolarization reflects mostly an increased Cl^- conductance (CFTR), and in addition the delayed repolarization corresponds to the additional activation of a K^+ conductance [21].

Another kind of K^+ channel regulation, as occurs, for example, in the colonic crypt base cell, has recently been

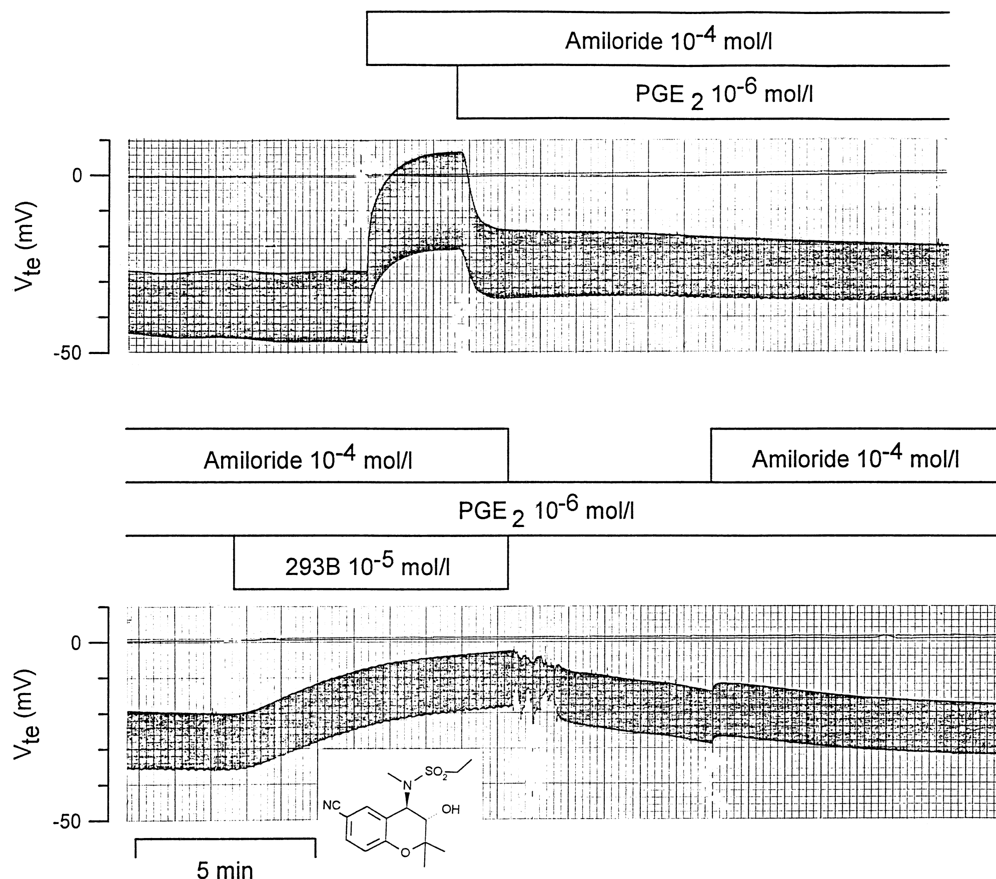

Fig. 4 Ussing chamber experiment in rabbit distal colonic mucosa. Transepithelial voltage (*V_{te}*) and transepithelial resistance (voltage excursions for constant current pulses) are recorded continuously. The initial lumen negative V_{te} of –26 mV collapses after the addition of amiloride to the luminal solution. Then secretion is stimulated by prostaglandin E_2 (*PGE_2*, blood side). Successful stimulation is apparent from the increase in lumen negative voltage to –19 mV and the reduction of transepithelial resistance. The secretory current is inhibited almost completely by the K^+ channel blocker 293B (blood side). This effect is reversible. *Inset*, Chemical structure of the chromanol 293B. (Data from [54])

examined in detail by ourselves [6, 19, 21, 22, 78]. We found that the normal resting conductance is determined largely by a small Ca^{2+} regulated K^+ channel of approximately 15 pS. This channel is strongly Ca^{2+} dependent in the physiological range of cytosolic Ca^{2+} activities ($[Ca^{2+}]_i$) which are in the range of 50–500 nmol/l [6]. Agonists such as acetylcholine or carbachol activate this channel strongly by elevating $[Ca^{2+}]_i$. This channel is inhibited by Ba^{2+} but not by the chromanol 293B (see below). This type of channel is closed after the addition of forskolin because the cytosolic Ca^{2+} activity is reduced by forskolin. The reduction in cytosolic Ca^{2+} by forskolin in turn is caused by the marked depolarization [6, 19, 78]. During this phase of cAMP-mediated stimulation colonic NaCl secretion would (paradoxically) come to a halt because the membrane voltage approaches E_{Cl^-}, and no driving force is left for Cl^- to leave the cells across the luminal membrane. In this phase, however, another type of K^+ channel is activated and takes over the whole cell K^+ current [54, 78]. This K^+ channel is of very small conductance (probably <3 pS). It appears to share some similarities with the so-called IsK channel [10, 71].

The very small colonic K^+ channel is Ca^{2+} independent and inhibited by a new class of chromanols (Fig. 4) related chemically to cromakalim. The pivotal role of this channel in cAMP-mediated Cl^- secretion (caused by prostaglandin E_2 in this case) is easily apparent from Fig. 4. In this Ussing chamber study the chromanol 293B inhibited cAMP mediated Cl^- secretion almost completely. The concept of this regulation of colonic K^+ channels is depicted in Fig. 5. These cells possess at least two types of K^+ channels: the small type, activated by Ca^{2+} and agonists such as acetylcholine, and the very small one, coming into play when Cl^- secretion is activated by cAMP. This concept also can explain quite easily why acetylcholine and cAMP-mediated stimulation act cooperatively: (a) acetylcholine enhances cytosolic Ca^{2+} and activates the respective K^+ channels and (b) probably also Cl^- channels, and (c) cAMP activates Cl^- channels and (d) also, with some delay, another type of K^+ channel. The most important conclusion from these studies is that cAMP activation of CFTR type Cl^- channels in itself would not suffice to drive secretion if there were not the secondary activation of another K^+ channel which is not demonstrable under resting conditions. It is unclear how cAMP, after CFTR activation, enhances the conductance of this specific type of K^+ channel.

Recently this issue has been examined in *Xenopus* oocytes injected with cRNA for wild-type CFTR or ΔF508 [57]. The basic observation is depicted in Fig. 6. CFTR-expressing oocytes have a strongly increased conductance (here depicted as outward current) when activated by membrane permeable cAMP (chlorophenylthio

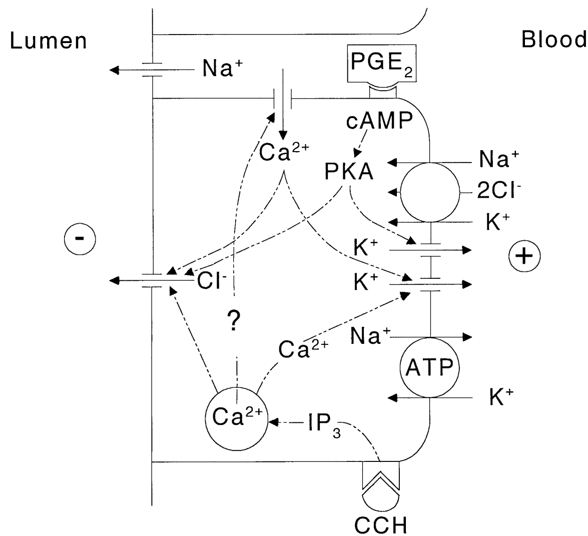

Fig. 5 Scheme of Cl^- secretion in a colonic crypt cell. *Solid arrows*, ion channels; *circle*, carrier; *circle with ATP*, (Na^++K^+)-ATPase. Cl^- is taken up by the $Na^+2Cl^-K^+$ cotransporter. K^+ recycles via K^+ channels. Na^+ is pumped out by the (Na^++K^+)-ATPase. Cl^- leaves the cell via luminal Cl^- channels. The luminal Cl^- extrusion and the basolateral K^+ recycling generate a lumen negative voltage, which drives Na^+ through the paracellular pathway into the lumen. Secretion can be stimulated by agonists such as carbachol, acting through inositol 1,4,5-triphosphate and Ca^{2+} release from respective stores. Ca^{2+} store depletion activates Ca^{2+} entry via respective Ca^{2+} channels. The increase in cytosolic Ca^{2+} ($[Ca^{2+}]_i$) activates K^+ channels in the basolateral membrane, which have a single channel conductance of 15 pS [78]. In addition, increased $[Ca^{2+}]_i$ also activates Cl^- channels, which may be different types of Cl^- channels [15] or be identical with CFTR [44]. Prostaglandin E_2 (*PGE_2*) and other agonists such as vasoinhibitory peptide enhance cytosolic cAMP. This leads to the activation of CFTR type Cl^- channels. In addition, another type of very small conductance K^+ channel is activated [78]. This type of K^+ channel is essential for cAMP driven Cl^- secretion because the depolarization caused by the opening of Cl^- channels reduces $[Ca^{2+}]_i$ and inactivates the Ca^{2+} regulated K^+ channel. This scheme explains the cooperativity of the two agonists

cAMP) or by the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX). This conductance is caused principally by Cl^-, as the voltage of activated oocytes closely follows E_{Cl^-} in Cl^- replacement studies. Addition of the K^+ channel inhibitor 293B has little effect on the voltage and conductance of control oocytes but reduces the conductance by 15–25% and depolarizes the zero current voltage in IBMX-activated oocytes [57]. The depolarization of the zero current voltage clearly indicates that 293B inhibites a K^+ and not a Cl^- conductance. Oocytes injected with the mutated form of CFTR (ΔF508) and pretreated by IBMX show only a very limited increase in Cl^- current (10–20% of wild-type CFTR) and no significant 293B inhibitable K^+ conductance. These data indicate that cAMP activates not only the exogenous CFTR injected into oocytes but also an endogenous K^+ conductance. As with that in colonic crypt cells, this K^+ conductance is not of the resting type and is inhibited by the chromanol 293B.

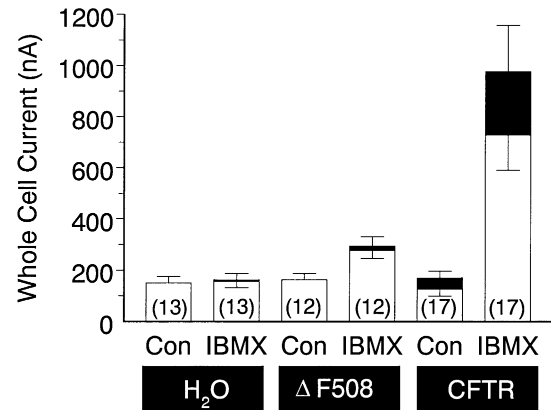

Fig. 6 Whole-cell outward currents of *X. laevis* oocytes clamped to a voltage of +30 mV. Data taken from ref. 57. Mean values ± SEM (number of oocytes). *H_2O*, Water-injected oocytes; *ΔF508*, ΔF508 cRNA injected oocytes; *CFTR*, CFTR cRNA injected oocytes. *Open bars*, baseline current and Cl^- current; *black bars*, 293B-inhibitable K^+ current. Note that 293B has little effect in water and ΔF508, injected oocytes. The effect is also small in "resting" CFTR-injected oocytes but is increased markedly when CFTR is activated by isobutylmethylxanthine (IBMX, 1 mmol/l). Note also that IBMX enhances the Cl^- conductance of ΔF508-injected oocytes only slightly, but strongly in wild-type CFTR-injected oocytes

Regulation of Na^+ channels

It has been known for the past 10–15 years that the transepithelial voltage and conductance in respiratory epithelia of CF patients has an increased amiloride-inhibitable component [7, 8, 41]. It is concluded from these findings that the more viscous mucus in the respiratory tract and the reduced mucociliary clearance of CF patients have two causes: (a) the reduced NaCl and water secretion and (b) the enhanced (amiloride inhibitable) NaCl absorption. More recently these data have found direct support from intracellular recordings in respiratory epithelial cells from CF patients [50]. It was found that the amiloride effect on membrane voltage and membrane conductance is higher in cells from CF patients than in those from controls. This is summarized in Fig. 7. The underlying mechanism is unclear. Two basic explanations could account for these findings: the defect in CFTR (such as ΔF508) could upregulate the endogenous amiloride inhibitable Na^+ conductance, or wild-type CFTR would normally suppress this Na^+ conductance, and defective CFTR loses this effect.

With the cloning of the epithelial Na^+ channel [12, 53] a more detailed analysis became possible. In one study CFTR was expressed in MDCK cells and it was found to suppress the cAMP upregulated Na^+ conductance indicating that CFTR has a regulatory influence on Na^+ channels [70]. In another study wild-type CFTR or ΔF508 were coexpressed with all three subunits (α, β, γ) of the amiloride inhibitable epithelial Na^+ channel in *X. laevis* oocytes [56]. It was found (Fig. 8) that these oocytes possessed the expected amiloride inhibitable Na^+

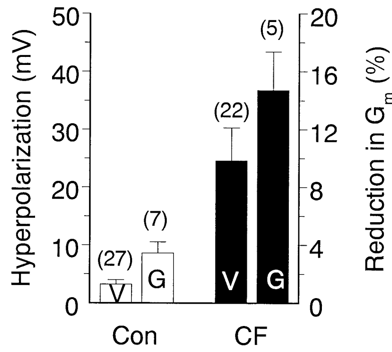

Fig. 7 Patch clamp studies in nasal epithelial cells from healthy individuals (*open bars*) and those from CF patients (*black bars*). Mean values ± SEM (number of cells). The hyperpolarization (*V*) and fall in conductance (G_m, *G*) caused by 10 µmol/l amiloride are shown. Note that the effect of amiloride is more marked on V and G in CF patients. (Data from [46])

conductance and the cAMP activated Cl^- conductance in wild-type CFTR, but very little Cl^- current in ΔF508 injected oocytes. The surprising finding was that, in strictly paired studies, the amiloride inhibitable Na^+ conductance was reduced sharply when wild-type CFTR was activated by isobutylmethylxanthine (IBMX, leading to increased cytosolic cAMP). In ΔF508 expressing oocytes IBMX had no such effect, i.e., the amiloride inhibitable Na^+ conductance was high both, in the absence and presence of IBMX. These data allow three interrelated conclusions: (a) there is an inverse relationship between the cAMP-dependent Cl^- conductance and the Na^+ conductance, (b) this finding, when extrapolated to epithelia with bidirectional Na^+ transport, might explain the determination of the direction of NaCl transport (see below), and (c) these data also explain why Na^+ absorption might be enhanced in respiratory epithelia of CF patients as the defective CFTR loses the inhibitory effect on Na^+ channels.

This function of CFTR may play a pivotal role in the overall regulation in NaCl-transporting epithelia. It has long been an open question of whether luminal Cl^- channels, required for NaCl secretion, and Na^+ channels, required for NaCl absorption, can exist in one type of cell, or whether they are spatially separated in different types of cells. Recent data suggest that both properties can be found in the same cell or strictly electrically coupled cells of the respiratory tract and the colonic crypt [22, 50]. This raises the serious problem of how the direction of NaCl transport can be defined. cAMP activation of cells possessing luminal Na^+ channels and CFTR-type Cl^- channels residing in the same membrane would certainly enhance NaCl uptake (absorption), but could not cause NaCl secretion unless at the same time the Na^+ channel were be closed. This issue has been examined very recently in the middle part of the rat colonic crypt. When treated by glucocorticoids (or mineralocorticoids), these cells absorb NaCl vigorously [22], and this absorption is inhibited completely when the luminal Na^+ channels are blocked by amiloride. The amiloride-inhibitable

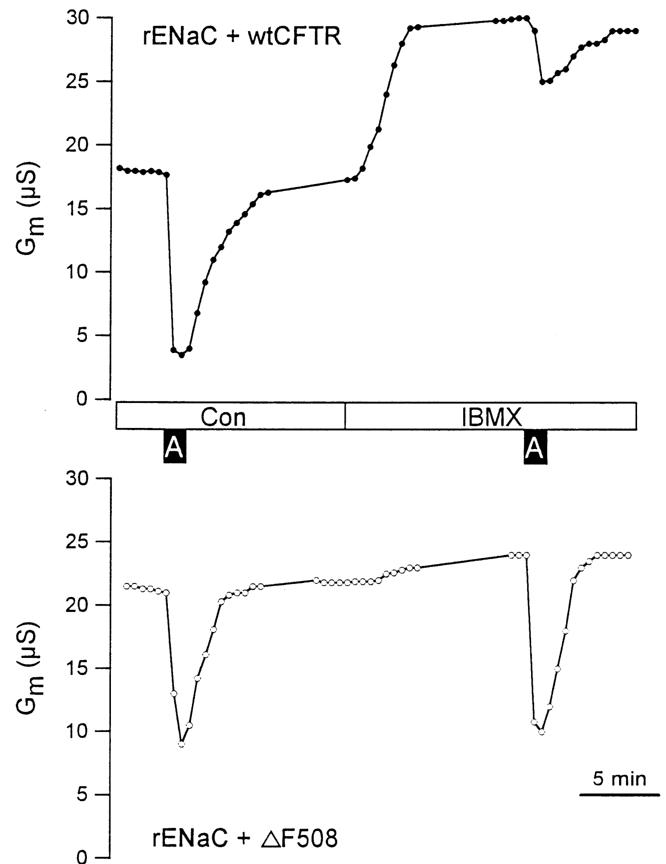

Fig. 8 Conductance (G_m) measurements in *X. laevis* oocytes expressing renal epithelial Na^+ channels (*rENaC*) and wild-type CFTR (*wtCFTR*, *upper panel*) or ΔF508 (*lower panel*). *Con*, Control conditions; *A*, amiloride 10 µmol/l; *IBMX*, isobutylmethylxanthine 1 mmol/l. Note the strong reduction in G_m by amiloride in wtCFTR-injected oocytes in the absence of IBMX. IBMX increases G_m. Now amiloride has only a very small effect. In ΔF508-injected oocytes IBMX has very little effect on G_m, and the amiloride effect is large and comparable in the absence and presence of IBMX. This indicates that activated wtCFTR, but not ΔF508, attenuates the coexisting Na^+ conductance. (Data from [56])

Na^+ current is almost abolished when the same cell is pretreated by agonists increasing cAMP, and when Cl^- secretion is induced [20]. This indicates that, as in the *X. laevis* oocytes expressing both transport proteins, there is a reciprocal regulation: whenever CFTR is activated by cAMP this leads to the "closure" of Na^+ channels in the luminal membrane. NaCl transport is thus redirected from the absorptive to the secretory direction.

Conclusion

CFTR functions as a small conductance Cl^- channel present in a variety of Cl^- transporting epithelia. In addition, it has a number of other functions which are not yet fully understood. The present brief review has addressed its putative function in membrane traffic, its possible function as an ATP exporter, and its close relationship to MDR. Most recent and surprising evidence

points to its role in the regulation of other ion channels. Two examples have been considered in some detail: CFTR, when stimulated by cAMP, upregulates an IsK-type K^+ channel and downregulates Na^+ channels present in the same cell. The mechanistic aspect of this regulation is not clear. While the interaction with Na^+ channels could be based on a direct (protein-protein) interaction in the same membrane (K.K., unpublished), the upregulation of K^+ channels is more difficult to envisage because Cl^- and K^+ channels are located in opposing cell membranes, the Cl^- channel in the luminal membrane and the K^+ channel in the basolateral membrane. At this stage it would not come as a surprise if CFTR were to interact with as yet other membrane transport proteins.

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