channels. We will summarize what is known about the function of these important ion transporters at the single channel level, at the single protein level, and at the molecular level. In our discussion of both sodium and chloride channels, we will point out how dysfunctions in the operation of these channels are reflective of various pathological conditions, specifically cystic fibrosis. We will conclude by discussing our view of what important questions lie ahead.

Epithelial sodium channels

Epithelial sodium channels have been localized and identified in many Na^+ reabsorbing epithelia either by patch clamp or immunocytochemistry, or both. They are usually situated in the apical or luminal membrane of epithelia and act as the regulatory control point for adjusting the rate of transepithelial Na^+ transport. Some examples of epithelia in which these channels occur are renal distal[9] and collecting tubules,[10,11] sweat ducts,[12] toad and mammalian urinary bladder,[13,14] stomach,[15-17] colon,[18] nasal epithelia, lung alveolar type II cells,[19] embryos,[20] and even many sensory transduction organs like the tongue,[21,22] olfactory epithelia,[23] and the hair cells of the inner ear.[24]

Much of our knowledge concerning the properties and regulatory mechanisms of these important apical channels comes from electrophysiological experiments on model epithelia such as frog skin, toad urinary bladder, and cultured A6 cells, an epithelial cell line, most probably of renal distal tubule origin, derived from the kidney of the African clawed toad, *Xenopus laevis*.[25] Only recently have the biochemistry and molecular biology of these epithelial ion channels been approached.[26]

One distinguishing feature of epithelial sodium channels is their sensitivity to inhibition by the potassium-sparing diuretic compound amiloride. Total apical sodium current, I_{Na}, is given by

$$I_{Na} = \gamma_{Na} N P_o (V_a - E_{Na})$$

Where γ_{Na} is the single channel unit conductance, N is the number of sodium channels per unit area of membrane, P_o is the open state probability, V_a is the apical membrane potential, and E_{Na} is the equilibrium potential for Na^+ across the apical membrane. Theoretically, total Na^+ entry rate can be modulated by altering any one, or a combination of the above variables, notably single channel conductance (γ_{Na}) or density of open channels (NP_o).

Although the hormonal and intracellular regulation of epithelial Na^+ channels is well documented, the molecular events underlying these phenomena are far from being understood. For example, both the mineralocorticoid hormone aldosterone and the peptide hormone antidiuretic hormone (ADH) cause a 2–4-fold increase in the rate of transepithelial Na^+ reabsorption. This increase is brought about by a 2–4-fold increase in the density of apical Na^+ channels without an appreciable alteration in single channel kinetic properties. Aldosterone causes de novo protein synthesis, but it has not yet been shown that the newly synthesized proteins are Na^+ channels. Data do exist suggesting that aldosterone-induced proteins (AIP) act to activate pre-existing apical Na^+ channels.[27,28] On the other hand, ADH increases Na^+ transport by activating adenylate cyclase and elevating intracellular cyclic AMP (cAMP) levels.[29] Recently, our laboratory has shown that the catalytic subunit of protein kinase A (PKA) in vitro and in vivo can directly phosphorylate one subunit component of an epithelial Na^+ channel (namely, a 300 kDa subunit, see below). It is tempting to speculate that this phosphorylation may shift dormant apical Na^+ channels into an active state, because the increased level of phosphorylation is directly correlated with the increased rate of Na^+ transport.[30] Furthermore, addition of the catalytic subunit of PKA plus ATP to the bathing solution of an excised patch of toad urinary bladder or A6 apical membrane activate Na^+ channels. In contrast, Lester et al.[31] reported that incorporation of cAMP–dependent PKA plus ATP and cAMP or protein phosphatases had no effect on the rate of amiloride-sensitive Na^+ uptake into toad bladder vesicles. The reason(s) for this disparity is (are) unknown. What is clear, however, is that ADH and aldosterone act via different mechanisms and do not share a common pool of channel precursors.[32]

The complexity of epithelia makes it difficult to address the issues of ion channel structure, selectivity, and regulation in an unambiguous manner. As in many other systems, better insight into molecular mechanism can be achieved by studying channels in isolation, either in membrane vesicles, excised membrane patches, or preferably by the isolation, purification, and reconstitution of these channels into a well-defined and controllable membrane system. We will briefly summarize what is known about epithelial sodium channels from a single channel and biochemical point of view.

Single channel characteristics of epithelial Na^+ channels

Currents through single Na^+ channels were first recorded in the bilayer experiments of Benos and coworkers.[33] In these experiments, native apical membrane vesicles from the amphibian renal cultured cell line A6 were incorporated into planar phospholipid bilayer membranes, and discrete quantal current jumps due to Na^+ flow through individual channels were recorded.The range of single channel conductances was 4–80 pS under conditions of symmetrical 200 mM NaCl, with a mean value of 35 pS. Both γ_{Na} and P_o were found to be voltage-independent in the range ±60 mV. The channels were found to be perfectly cation selective; however, the ability of these channels to discriminate between Na^+ and K^+ was poor. The measured permeability ratio of Na^+/K^+ was 2–3 : 1.

In single channel experiments on a myriad of Na^+ transporting epithelial cells, several different amiloride-blockable channels have been reported (*Ta-*

Table 1 Properties of single amiloride-sensitive Na^+ channels in different tissues

Source	Open-state conductance ([NaCl] ≥100mM)	P_{Na}/P_K selectivity	Amiloride K_i	Reference
A6 cells (bilayers)	4–80 pS	2–3	0.1μM	33
A6 cells (patch)	3–10 pS	4–5/<20	≤0.5μM	45
Rat cortical collecting tubules	5–8 pS	≥10	<0.5μM	10
Rabbit straight proximal tubules	12 pS	≥20	10μM	34
Rabbit inner medullary cells	28 pS	1	<0.5μM	11
Rat and pig brain endothelium	28 pS	1.5	10μM	47
Pig thyroid cells	3 pS	1.2	0.15μM	35
Human sweat duct cells	15 pS	2–4	<0.5μM	12

Table 2 Cationic selectivity of amiloride-blockable sodium channels in different epithelia

Tissue	Sequence	$P_{Na}:P_K$	Method	Reference
Toad urinary bladder	H > Li > Na > K	670:1	macroscopic	46
Frog skin	Li = Na > K > Rb > Cs	100:1	macroscopic	48
Rat colon	Na > Li > K > Rb > Cs	25:1	macroscopic	49
Rabbit urinary bladder	Na > K	30:1	macroscopic	50
Rabbit urinary	Na > K	2.6–9:1	macroscopic	51
A6 cells	Na > K	20:1	single channel recording	45
A6 cells	Na > K	3–4:1	single channel recording	45
Rabbit late proximal tubules	Na > K	19:1	single channel recording	34
A6 cells	Na > K	2:1	single channel recording	33

ble 1). In many cases, particularly in cultured A6 cells, conditions can be manipulated such that only one specific type of channel can be observed, e.g., a small unit conductance/high Na^+ over K^+ selective channel. Do these observations mean that there are different proteins displaying different behaviors, or can perhaps a single protein complex exhibit varied kinetic characteristics depending upon its physicochemical configuration? This is, perhaps, one of the most intriguing questions that the current state of knowledge permits us to ask.

The evidence that is summarized in *Table 1* leads to the conclusion that there may not be a single class of amiloride-sensitive Na^+ channels, but rather a family of epithelial channels, based on both kinetic and pharmacological characteristics. Amiloride-sensitive Na^+ channels can be broadly classified as having high or low sensitivity to the drug (i.e., an apparent equilibrium inhibitory dissociation constant of less than or greater than 1 μM). Further, within each group, the channels display either high or low Na^+ versus K^+ selectivity. Examples of high amiloride affinity/highly Na^+ selective channels are found in A6 cells and rat cortical collecting tubules; high affinity/low Na^+ selective channels are found in A6 cells, LLC-PK1 cells, rabbit renal inner medullary cells, human sweat ducts, and pig thyroid cells. Low amiloride affinity/high Na^+ selective channels are located in rabbit pars recta and blastocysts, while low affinity/low Na^+ selective channels are found in rat and pig brain endothelium.

Interestingly, cation selectivity seems also to be associated with channel open state conductance: highly Na^+ selective channels are usually associated with low conductance channels, while poorly selective channels have a higher conductance, although this statement is not always true.[34,35] In fact, very few detailed measurements of alkali metal ion selectivity through apical amiloride-sensitive channels have been performed. *Table 2* summarizes the information currently available. Based on the composite data for the highly Na^+ selective channel in toad urinary bladder, frog skin, and rat descending colon, the ion selectivity of this channel corresponds to that of Eisenman's selectivity sequence *X*. Thus, the channel must possess a very high electric field strength, and most or all of the water molecules associated with Na^+ in bulk solution must be stripped prior to ion translocation.

Biochemical characteristics of epithelial Na^+ channels

Benos and co-workers have purified a high amiloride affinity epithelial Na^+ channel from A6 cells and bovine kidney papilla using conventional biochemical techniques.[36] The general protocol for the purification of this amiloride-sensitive Na^+ channel consisted of first preparing an enriched apical membrane fraction, then treating this fraction with the zwitterionic detergent CHAPS. The CHAPS solubilized material was then passed over a lectin chromatography column, and the protein that was bound to this column purified by gel filtration, namely, size exclusion, high performance liquid chromatography (HPLC). At each of these stages of purification, the equilibrium binding of [^{3}H]methylbromoamiloride was measured, and func-

tional activity assessed in reconstitution studies. In all cases, nearly 100% of the specific binding sites were recovered, and high affinity amiloride-sensitive Na^+ transport was maintained. At final purification, over a 5000-fold enrichment in amiloride-sensitive Na^+ channels was achieved. This enrichment represents only 0.002% of the total protein present in the starting material. The specific activity of binding in the purified fraction is on the order of 1300 pM/mg protein. Assuming that one molecule of amiloride binds to one molecule of the protein, and assuming a molecular mass of 730 kDa (see below), this purification scheme attains more than 90% homogeneity because the theoretical binding limit is 1400 pM/mg protein. When the HPLC-purified material is labeled with ^{125}I separated on a non-reducing SDS gel, and an autoradiograph made of the gel, the protein runs as a single band at 730 kDa. SDS-PAGE of the native channel under reducing conditions reveals that it is composed of at least six major polypeptides with average molecular mass values of 315, 150, 95, 70, 55, and 40 kDa. This is a very reproducible finding for this protein isolated from both the A6 cells and the bovine kidney.

Experiments were designed to identify the subunit of this epithelial Na^+ channel protein to which amiloride binds. For these experiments, the irreversible binding properties of the synthetic compound [^{3}H]methylbromoamiloride were exploited. Solubilized apical membrane protein was irradiated with 350 nm ultraviolet light in the presence of 1 μM [^{3}H]methylbromoamiloride, either in the absence or presence of a 100-fold excess of bromoamiloride. The total protein was run on a 3–12% polyacrylamide gel, and then the gel was cut and examined for radioactivity. The gels were run under non-reduced and reduced conditions. Under non-reduced conditions, all of the counts were located in a region corresponding to molecular mass of 740 kDa, consistent with the molecular mass of a channel protein isolated by the conventional biochemical procedures outlined above. If the protein were run under reduced conditions, all the counts moved to a region corresponding to 150 kDa. On this basis, we concluded that the primary binding site for amiloride is located on the 150 kDa subunit. Kleyman and colleagues[32,37] prepared anti-amiloride antibodies, which act against amiloride and some of its congeners. In preliminary experiments, purified Na^+ channel protein was irradiated in the presence of 5 nM NMBA (2′–methoxy–5′–nitrobenzamil), run on an SDS gel, transferred to nitrocellulose, and probed with the anti-amiloride antibodies. These antibodies were made against the pyrazine ring moiety of amiloride. The anti-amiloride antibody recognized the 150 kDa band. On the basis of these independent approaches, we conclude that it is the 150 kDa subunit to which amiloride binds.

An amiloride binding protein that presumably represents an amiloride-sensitive Na^+ channel has been identified, purified, and cloned in pig and human kidney cortex vesicles using another amiloride analog ([^{3}H]benzamil) by Barbry and coworkers.[38] SDS-PAGE of the purified phenamil binding protein under non-reduced conditions revealed a single polypeptide of molecular mass of 180 kDa, whereas when examined under reducing conditions, the results suggest that the native protein may be a dimer composed of two identical subunits, each with a molecular mass of 90–100 kDa. Interestingly, this channel from pig kidney, when reconstituted into lipid vesicles, displayed conductive $^{22}Na^+$ uptake that was inhibited by both phenamil and/or amiloride with a K_i' of around 10 μM. This channel protein has recently been cloned and sequenced. Surprisingly, while there are glycosylation sites, there are no consensus phosphorylation sites nor any hydrophobic spanning regions based on hydropathy plots. If this gene coding for this protein is transfected into mammalian cells or into *Xenopus* oocytes, amiloride binding activity can be recovered, but there is no amiloride-sensitive Na^+ transport observed in patch clamp studies. The authors suggest that their amiloride binding protein may be a component of a larger molecular weight complex. Thus, on the basis of these data, it would appear that both high and low amiloride affinity Na^+ channels may share some common biochemical features. Cloning of high amiloride affinity Na^+ channels is in progress in several laboratories.

An amiloride-sensitive Na^+ channel has recently been localized to the apical microvilli of renal epithelial cells using a polyclonal antibody generated against this channel protein.[39,40] In addition, our laboratory has examined the possibility that the Na^+ channel is linked to the actin-rich cytoskeleton of the microvilli.[41] Incubation of A6 renal epithelial cells in cytochalasin D, followed by fluorescence double-labeling using the Na^+ channel antibody and rhodamine phalloidin (which is specific for actin) reveals a clustering of actin and Na^+ channels, suggesting that the two are co-localized. Treatment of A6 cells with Triton X-100, followed by immunoelectron microscopy, indicates that the Na^+ channels remain associated with the detergent-insoluble, actin-rich cytoskeleton. Immunoblotting of partially purified Na^+ channel proteins demonstrates that ankyrin, fodrin, and actin co-purify with the channel, suggesting that the channel may be linked to actin via ankyrin and fodrin. To determine if actin binds to the Na^+ channel, purified Na^+ channel was incubated with the ^{125}I–labeled erythrocyte isoform of ankyrin and immunoprecipitated using the anti–Na^+ channel antibody and protein A. SDS-PAGE and autoradiography of the immunoprecipitate reveals the presence of ankyrin. These results suggest that ankyrin binds directly to the Na^+ channel at the 150 kDa subunit, the same subunit that binds amiloride. These results are consistent with the idea that ankyrin links the Na^+ channel to the underlying cytoskeleton. Further support of this notion comes from lateral diffusion meaurements of apical Na^+ channels. These measurements indicate that the Na^+ channels have a very small lateral diffusion coefficient, approximately 2×10^{-11} cm^2/s, suggesting that these channels have a very restricted lateral mobility.

Recent experiments have demonstrated that Na^+ movement through amiloride-sensitive Na^+ channels in the cultured renal epithelial A6 and LLC PK_1 cell lines and cultured rat renal inner collecting duct medullary cells is activated by a pertussis toxin–sensitive G protein.[11,42,43] Further, single Na^+ channel activity can be modified in excised patches of A6 apical membranes by the addition of activated $\alpha_{i\text{-}3}$ subunit of G protein.[42] Based upon these observations and because of the availability of a biochemically homogeneous preparation of epithelial Na^+ channel protein, Ausiello et al.[44] have undertaken studies to define G protein function and identify with this protein complex. These investigators found that two of the channel subunits, namely, the 95 and 40 kDa polypeptides, are specifically ADP-ribosylated in the presence of pertussis toxin. One of these channel subunits (the 40 kDa one) has been identified as the $\alpha_{i\text{-}3}$ subunit of the G protein on Western blots with specific $\alpha_{i\text{-}3}$ antibodies. Hence, it appears that the Na^+ channel itself contains a modulatory G protein. This may well represent a unique single autotransduction pathway in epithelia, perhaps responsive to both intracellular and extracellular signals.

Epithelial chloride channels

Although much information concerning the electrophysiological properties of epithelial Cl^- channels is known, very little information exists concerning its biochemistry, in fact, much less than that for Na^+. Epithelial Cl^- transport can be divided into two components: absorption and secretion. These processes occur in varying proportions along the length of the gastrointestinal (GI) tract, as well as in other systems, e.g., liver, kidney, and pancreas. For example, secretion and absorption of Cl^- ions in the GI tract appear to take place in different regions. Secretion of Cl^- during digestion primarily originates in the crypts of Lieberkuhn,[52] whereas Cl^- absorption is achieved by the surface epithelium.[53] Of the two mechanisms, secretion of Cl^- is more clearly understood. The transport is under humoral control and is essential for the correct maintenance of fluid and electrolyte balance. In each case, it is important to recognize that the vectoral transport of fluid and electrolytes depends on the polarized distribution of conductive, electroneutral, and active transporting moieties in individual cells.

The cellular secretion of Cl^- has been studied in a wide variety of epithelia, including the GI tract,[54] exocrine glands,[55] trachea,[56] and cornea,[57] as well as in cultured epithelial cell lines.[58-60] Much of our understanding of secretory Cl^- transport has also been developed from non-GI or non-mammalian models, e.g., airways, flounder intestine, and shark rectal gland. Electrophysiological recording has been instrumental in the development of models for secretion and absorption of Cl^-. Patch clamping has enabled the precise measurement of individual ionic conductances across a patch of membranes sealed to the tip of a micropipette. A variation of this technique, the whole-cell clamp, enables the macroscopic currents flowing through the cell to be quantified. These methods are analogous to the voltage clamp of nerve axons.

The fundamental transport moiety that provides the electromotive driving force for the isotonic secretion or reabsorption of fluid and electrolytes in leaky epithelia such as the GI tract is the Na^+/K^+ ATPase. This transporter, which, with a few exceptions, is situated on the basolateral membrane of all epithelial cells, is responsible for maintaining the non-equilibrium distribution of Na^+ and K^+ ions and, consequently, the electrical potential difference across the cell membrane. This pump, which transports Na^+ out of the cell in exchange for extracellular K^+ in a ratio of 3 Na^+:2 K^+ per hydrolysis of one ATP molecule, provides the driving force for the accumulation of Cl^- ions in the cell above their equilibrium concentration, as predicted by the Nernst equation (see *Figure 1*). Experimental observations using ion-sensitive microelectrodes established that intracellular Cl^- activities in a variety of transporting epithelia were higher than that predicted by an equilibrium distribution.[61-64] The link between the Na^+/K^+ ATPase and the high resting intracellular Cl^- concentration is due to the presence of a $Na^+/K^+/2Cl^-$ or Na^+/Cl^--cotransporter (located

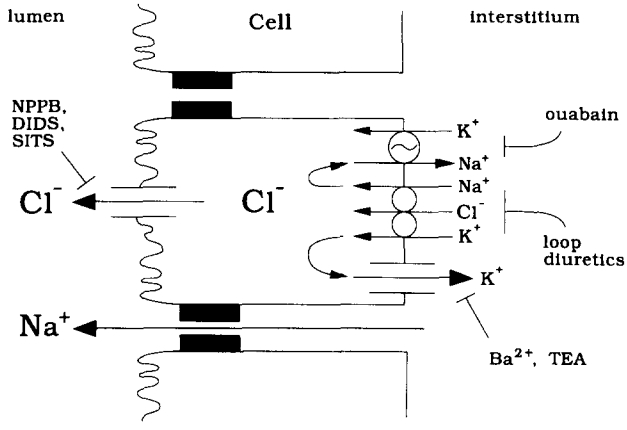

Figure 1 Schematic model of a typical Cl^- secretory epithelial cell in a leaky epithelium. The Na^+/K^+ ATPase, $Na^+/2$ Cl^-/K^+ co-transporter, and a K^+ channel are all localized to the basolateral membrane. The removal of intracellular Na^+ by the ouabain sensitive Na^+/K^+ ATPase, creates an inwardly directed electrochemical gradient for Na^+. When the cell is stimulated, the basolateral K^+ channel opens, permitting efflux of K^+ to the interstitium. This K^+ channel may be inhibited by Ba^{2+} and tetraethylammonium (TEA). The Na^+ gradient is also exploited by the $Na^+/2$ Cl^-/K^+ co-transporter, permitting the cycling of Na^+ and K^+ across the basolateral membrane. The operation of this co-transporter results in the net accumulation of intracellular Cl^-. The co-transporter is in turn sensitive to the loop diuretics, bumetanide, piretanide, and furosemide. Chloride ions leave the cell down their electrochemical potential energy gradient via an apically located Cl^- channel. This channel is sensitive to a variety of inhibitors including 5 nitro-2-(3-phenyl-propylamino)-benzoate, (NPPB), 4, 4'diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), and 4 acetamido-4'-isothiocyanatostilibene 2, 2'-disulfonic acid (SITS). The increased apical efflux of Cl^- requires an ion for charge compensation and sodium crosses the epithelial barrier via the paracellular pathway. Water can follow via either the trans- or paracellular route, resulting in secretion of an isotonic sodium chloride solution. See text for further details.

on either the luminal or basolateral membrane, depending on whether the cell is absorptive or secretory) and a basolateral K^+ channel. The co-transport, which is electrically silent, has been identified in many epithelia that transport Cl^- against an electrochemical gradient.[65] Sodium enters the cell down its electrochemical gradient that has been created by the operation of the Na^+/K^+ ATPase. Its entry is accompanied by Cl^- and, in some cases, K^+. Although at rest there is little driving force for K^+ accumulation by the cells, a K^+ channel opens at the basolateral membrane when the cell is stimulated. The subsequent efflux of K^+ enhances the driving force for the operation of the co-transport system. Potassium and Na^+ thus cycle in opposite directions between the co-transporter and the Na^+/K^+ ATPase. The net result of these ion movements is the accumulation of intracellular Cl^-. Because the net cellular accumulation of Cl^- requires the hydrolysis of ATP by the Na^+/K^+ ATPase, its transport is sometimes referred to as being secondarily active. The final component of the system is the Cl^- channel. This protein is located on the apical membrane of secretory epithelia or on the basolateral membrane of absorptive epithelia. In a secretory cell, the net result of these ion movements is the secretion of Cl^- into the gut lumen. This creates a lumen negative potential and Na^+ flows down the electrochemical gradient via the paracellular pathway. The increase in osmotic pressure draws water into the lumen via the paracellular or transcellular pathway. In an absorptive cell, the same principle works in reverse, although the paracellular pathway for ion and water movement is somewhat less important. The principal ion in absorption is Na^+, whereas in secretion Cl^- is more important.

One exception to the generalized model for Cl^- secretion/absorption appears to be the rat ileum. Isolated ileal brush border membrane vesicles were found to accumulate Cl^- in a pH-dependent manner. As the pH of the medium fell, intravesicular proton concentration and Cl^- accumulation were increased.[66] This increase in intravesicular Cl^- concentration could be inhibited by SITS and furosemide, two known inhibitors of Cl^-/OH^- exchange mechanisms. In contrast, experiments designed to demonstrate the presence of Na^+/Cl^- co-transport in these vesicles were unsuccessful.[67] It was therefore proposed that Cl^- absorption by the ileum is achieved by a parallel arrangement of an Na^+/H^+ and a Cl^-/OH^- antiporter transporting salt into the cell driven by a pH gradient across the luminal membrane. At the basolateral membrane, Cl^- is suggested to leave by a conductance pathway, while Na^+ is removed by the Na^+/K^+ ATPase.

Chloride channels have now been extensively studied by the patch clamp technique in a wide variety of tissues. Many studies have focused on isolated cells maintained either in primary or in continuous cell culture. This is particularly true in the case of human tissue where access to surgical or autopsy specimens is often severely limited. The majority of studies in gastrointestinal epithelia have been performed in colonic carcinoma cells maintained in culture. Both T-84 and HT-29 cells possess at least two distinct Cl^- channels in the apical membrane. These channels differ from each other in their conductance, their current/voltage relationships, and their susceptibility to Cl^- channel blockers.[59,60,68,69] Channels with similar properties have been identified in trachea,[70,71] shark rectal gland,[72] and in pancreatic duct epithelia.[73] The detailed electrophysiological characteristics of epithelial Cl^- channels in general, and secretory Cl^- channels in particular, are given in three excellent recent reviews.[72,74,75]

Regulation of chloride channels in epithelia

Chloride transport in epithelia is subject to regulation by the autonomic nervous system and can be in turn influenced by the central nervous system (CNS). Several different humoral factors and/or neurotransmitters, including agents such as epinephrine, histamine, neuropeptides, and acetylcholine, can affect the secretory or absorptive status of the tissue. The two predominant intracellular signaling pathways for Cl^- channel activation are calcium and cAMP. Cyclic AMP, which is formed following receptor activation, e.g., β adrenergic receptors in the trachea or secretin receptors in the pancreatic ducts, activates PKA by causing dissociation of the regulatory and catalytic subunits of the enzyme. Agents that are known to increase Cl^- channel activity in T-84 cells via this pathway include vasoactive intestinal peptide (VIP), prostaglandin E_2 (PGE_2), dibutryl cAMP (a membrane permeable analog of cAMP), and forskolin, which directly activates adenylate cyclase.[59,76] The use of the patch clamp technique in airway epithelia has shown that the catalytic subunit of PKA can directly activate Cl^- channels in the presence of ATP.[77,78] Control experiments in which the subunit was added to the cytoplasmic surface of the membrane in the absence of ATP did not result in Cl^- channel activity,[77] thus implicating a role for PKA-mediated phosphorylation at a step proximal to channel activation. Cohn[79] has observed that cytosolic proteins of M_r (in kDa) 17, 18, 23, and 37 were phosphorylated following stimulation of T-84 cells with either VIP or the catalytic subunit of PKA. Phosphorylation of these proteins occurs with the same time course as does the stimulation of Cl^- secretion. However, as these proteins are soluble, it is unlikely that they are components of the membrane bound channel complex.

The second major pathway of Cl^- channel regulation epithelia is by modulation cytosolic calcium activity. Agonists that increase intracellular calcium activity include acetylcholine (via muscarinic receptors), neurotensin, and cholecystokinin (CCK). The source of calcium is 2-fold: (a) from the extracellular medium and (b) from internal calcium stores. Agonist-induced rises in Ca^{2+} have been demonstrated in (among others) T-84 cells,[80] intestinal 407 cells (an embryonic small intestinal cell line),[81] and in tracheal airway cells.[82] In electrically excitable cells, e.g., nerve or

muscle, the entry of calcium across the membrane is regulated by voltage-sensitive calcium channels. However, in cells that do not conduct action potentials, the mechanism underlying calcium entry is unclear. One suggestion for Ca^{2+} entry is through channels regulated by the inositol polyphosphates IP_3 and IP_4.[83,84] IP_3 is additionally involved in the mobilization of calcium from intracellular stores.[85] IP_3-sensitive calcium pools are found in close proximity to the plasma membrane.[86,87] One of the further consequences of IP_3 production, via the receptor-mediated activation of phospholipase C on phosphotidylinositol 4-5-bisphosphate, is the concomitant production of diacylglycerol (DAG). DAG binds to protein kinase C (PKC), thereby increasing the affinity of the enzyme for calcium. When activated by calcium and diacylglycerol, PKC transfers a phosphate group to its target protein, thereby influencing a wide variety of cellular processes. Application of analogs of DAG, the phorbol esters (phorbol myristate acetate [PMA] or phorbol dibutyryl acetate [PDB], can activate PKC directly.[88] PDB causes a rapid increase in short circuit chloride current in rabbit small intestine.[89] In the rat, PMA given in vivo stimulates fluid secretion while PKC in vitro phosphorylates enterocyte microvillar membranes.[90] In the canine trachea, PMA stimulated a chloride-dependent short circuit current, as well as ^{36}Cl efflux.[91] The effects of PMA are not due to changes in cAMP level. However, at high doses of phorbol ester, the cell becomes refractory to stimulation, even to cAMP-mediated agonists. When purified PKC was added to human or canine airway cells in the presence of ATP and phorbol ester, two effects were noted. At low calcium concentrations (<10 nM), PKC activated Cl^- channels in excised inside/outside patches.[92,93] However, if the calcium was increased to >10 μM, PKC had the opposite effect, inactivating previously active channels and preventing voltage activation or activation by PKA.[92] The exact mechanisms underlying the dual regulation of chloride channels by PKC remains to be elucidated. A further consequence of IP_3 production in the cell is the production (via the cleavage of DAG) of arachidonic acid. It has been recently suggested that arachidonic acid and other *cis*-unsaturated fatty acids can directly inhibit Cl^- channels in cultured airway cells when applied to the cytosolic side of a membrane patch.[94,95] These effects of arachidonic acid are not due to phosphorylation or to metabolism to other active byproducts. In fact, some metabolites of arachidonic acid increase Cl^- channel activity.[96] It has been suggested that arachidonic acid may directly interact with the channel protein to influence its activity.[95] At present, however, this hypothesis remains to be established.

Absorption of chloride and sodium by the gut is also influenced by cytosolic calcium concentration. In most cases, calcium inhibits NaCl transport while causing a concomitant increase in electrogenic chloride secretion, as seen above.[97] These effects of calcium on absorption can be reversed in the rabbit ileum by the addition of calmodulin (CaM) inhibitors such as trifluroperazine, promethazine, or the napthelene sulfonamide W13.[98] Promethazine inhibits the phosphorylation of microvillar proteins in the rabbit ileum by Ca^{2+}-CaM–dependent kinase and decreases short circuit current.[99] W13 stimulated linked NaCl absorption, while having no effect on Na^+-dependent D-glucose absorption. In contrast, calcium-stimulated Cl^- secretion in the rabbit ileum does not involve a Ca^{2+}-CaM complex, as secretion induced by calcium ionophore or cAMP was not affected by W13.[99] In T-84 and cultured airway cells, multifunctional CaM-kinase is associated with activation with chloride conductance, as measured by whole-cell recordings.[100] Addition of inhibitors of CaM-kinase, CaM or heat-inactivated CaM-kinase all suppressed chloride conductance.

Biochemistry

In contrast to the amiloride-sensitive sodium channel described above, little is known about the biochemistry of epithelial anion channels. Chloride conductance in T-84 cells are characterized by at least three distinct kinetics that may reflect either three separate conducting proteins or three conformational variants of the same protein.[69] Activation of these conductances is either by calcium, cAMP, or cell swelling, and they have a differing sensitivity to blockers. In shark rectal gland (secretory) and in rabbit urinary bladder (absorptive), at least two distinct chloride channels are present.[72,101] Purification of these channels theoretically should be possible by exploiting their differential sensitivity to anion channel blockers such as the disulfonic stilbenes. However, with the exception of the ligand gated GABA (γ-amino-butyric acid) and glycine chloride channels, the available chloride channel ligands are of insufficiently high affinity to make purification practicable. Other approaches, including the production of antibodies to synthetic peptides as well as classical biochemical approaches to protein purification, have also been applied. Purification of epithelial chloride channels is made difficult, not only due to the differing channels that may be present in a single cell, but also because of the low abundance of these channels in the membrane. The turnover number of a biological channel is in the range of 10^6–10^8 ions/s. By comparison, the maximal turnover number of valinomycin, an antibiotic K^+-ionophore is 10^4 ions/s, and for the Na/K-ATPase is only 5×10^2 ions/s. Hence, only a small number of channels are necessary to move an appreciable amount of ions in a given time period. For example, 10^2 to 10^4 times fewer Cl^- channels would be required to transport the same amount of Cl^- ions as would a co-transporter (e.g., $Na^+/K^+/2\ Cl^-$). Hence, the biochemical isolation of these low abundance proteins is formidable.

An initial approach to channel purification has been to isolate channel-containing cell membrane fractions,

and then to assay for channel activity. Putative channel proteins are reconstituted either into artificial membrane vesicles, where channel activity can be monitored by conventional flux assays, or into lipid bilayers, where channel characteristics can be examined electrophysiologically and compared to the native material. Isolation of chloride channel–enriched membrane fractions from the gut is complicated by the presence of several different cell types along the length of the gut. The specific isolation of crypt or surface cell membranes is not currently possible. Bridges has used a partially purified preparation of rat colonocyte membrane vesicles to study the properties of the chloride channel following its incorporation into an artificial bilayer.[102] A recent patch clamp study of native rat colonocytes revealed a channel of somewhat different properties to that observed by Bridges.[53] Hence, the possibility exists that the process of producing the membrane vesicles alters the properties of the native channel. However, when T-84 membrane vesicles were prepared and incorporated into the lipid bilayer in the same manner as the colonocytes, a channel was seen with the same characteristics as that found in the rat colonocytes (Venglarik, C. and Bridges, R. J., personal communication). Membrane vesicle fractions enriched from chloride transport activity have also been prepared from the bovine trachea.[103] The apical membrane fraction was solubilized in octyl glucoside and the soluble protein reconstituted into phospholipid vesicles.[104] These reconstituted vesicles exhibited several similarities to the native system. Reconstitution of apical membrane vesicles into planar lipid bilayers revealed the presence of three chloride channels[105] that were inhibited by DIDS. The predominant channel observed in these studies had a conductance of 71 pS, and could be activated by the catalytic subunit of PKA in the presence, but not in the absence, of ATP. This activation by PKA is similar to that observed for other chloride channels. However, the most noticeable feature of this study was the rapid rundown of channel activity, which was not observed in studies of channels on native cells. A slightly different approach to the study of reconstituted anion channels has been adopted by Miller and coworkers. In this technique, membrane vesicles from the electroplax organ of the electric ray *Torpedo californica* were fused to giant liposomes by sequential freezing and thawing. The channel proteins incorporated were then amenable to study by conventional patch clamp techniques.[106] Single chloride channels incorporated into these liposomes had identical properties to the native channel. Reconstituted systems are crucial for assaying the ability of any putative channel protein to transport ions. Comparing it to the native protein can additionally provide an opportunity for the easy screening of potential affinity ligands that may then be used for isolating the channel itself.

However, in none of the studies so far described was an attempt made to purify biochemically the putative channel protein free of other membrane components. To date the most rigorous attempt has been that of Ran and Benos.[107] In this study, apical membrane vesicles from bovine trachea were dissociated of extrinsic proteins and then solubilized with Triton X-100. The solubilized material was separated by passage over ion exchange resins. Channel activity, as assessed by DIDS-sensitive uptake of ^{125}I iodine into artificial lipid vesicles, was assayed after each purification step. Channel activity was found in a membrane fraction, which bound to the cationic but only loosely bound to the anionic exchanger. This observation is consistent with the protein of interest having a net positive charge as might be predicted for a protein that would conduct negatively charged moieties. Elution of the material bound to the cationic exchanger by stepwise increases in pH revealed a peak of activity eluted at a pH of 8.0 to 8.5. When this fraction was subjected to reduced SDS-PAGE analysis, a protein of M_r 38000 was found to be particularly enriched. A second purification over an anionic exchange column, followed by gel electrophoresis, revealed essentially pure 38 kDa polypeptide. Intriguingly, when the range of the gel encompassing M_r of 35-42 kDa was electroeluted and run a second time over another gel, an additional doublet of M_r 62-64 kDa was observed. This band was found to co-purify with the 35-42 kDa protein throughout all steps of the purification procedure. A possible explanation for this is aggregation following exposure to air and disulfide bond formation. In fact, following treatment of this band with 20 mM DTT, the 62-64 kDa doublet was successfully reduced to the lower molecular weight.

Sodium cholate solubilized protein isolated from membrane vesicles of the thick ascending loop of Henle of the pig kidney have yielded similar results.[108] In this study, membrane vesicles enriched for chloride transport were labeled by incubation with [^3H]DIDS. Two bands of molecular mass 65 and 31 kDa were labeled. In particular, the labeling of the 31 kDa band by the radioactive ligand could be prevented both by cold DIDS and by a second inhibitor of chloride channel activity 5-nitro-2-(3-phenyl propylamino)-benzoate (NPPB). Both these compounds inhibited chloride channel activity in the reconstituted liposomes. Dubinsky and co-workers have reported that three protein bands at 40, 67, and 200 kDa are associated with chloride channel activity as assayed by bilayer studies in bovine trachea, renal cortex, intestine, and pancreas.[109] However, the membrane fraction used is probably derived from subcellular organelles as the vesicles are insensitive to ouabain and do not possess alkaline phosphatase activity. A convenient alternative method of isolating any membrane protein is to use a compound that will bind reversibly to the protein of interest and that can then be used to retrieve the protein. Affinity ligands can be divided into two broad groups: chemical ligands (e.g., drugs, channel blockers) and biological ligands (e.g., lectins and antibodies). Both groups of compounds have found applications in the purification of anion channels.

Chemical ligands

As mentioned above, one of the difficulties in isolating chloride channels has been the lack of compounds with sufficient specificity and high affinity for the proteins concerned. H2-DIDS, which was one of the first compounds found to inhibit chloride transport proteins such as erythrocyte band 3 and chloride channels,[102] is an irreversible covalently active agent. Derivatives of DIDS, which bind reversibly to the channel and have high inhibition constants, are in the process of being developed and may prove to be useful ligands in the future.[102] NPPB, which is one of a group of anthranilic acid derivatives,[110] is a more potent blocker of absorptive than secretory chloride channels.[102] It has an inhibition constant of 8×10^{-8} M in the rabbit thick ascending loop of Henle, but this can vary by three to four orders of magnitude in different tissues. A third group of chloride channel blockers, the indanyloxy acetic acids (IAA compounds), has also been suggested as suitable affinity ligands for chloride channel purification. One member of this family, IAA-94, has been used by Al-Awqati and co-workers to isolate four membrane associated proteins from bovine trachea and kidney cortex microsomes.[111] In this approach, solubilized membranes were passed over an agarose column, derivitized with IAA-23. After extensive incubation and washing, the specifically bound proteins were eluted with a second compound IAA-94. After SDS-PAGE, the eluate contained several proteins and was found to be enriched in four proteins of M_r 97, 64, 40, and 27 kDa. When the eluted proteins were precipitated and reconstituted into artificial liposomes, three different chloride channels were observed. However, a major problem with this study was that these channels were insensitive, not only to the stilbene isothiocyanates and anthranilic acid derivatives, e.g., DIDS and NPPB, but also to the IAA compounds, such as IAA-94, that were used to competitively elute the proteins from the column. The reason for this anomaly remains unclear.

Biological ligands

One group of ligands that has offered much promise for the isolation of low abundant proteins in cells is antibodies. These have the required specificity (up to 10^{12} M), their binding is reversible, and they have multiple applications. Both polyclonal and monoclonal antibodies have been used in attempts to purify chloride channels. Jentsch et al.[112] raised antibodies to a stilbene disulfonate, SITS. Membrane vesicles prepared from the electroplax organ of *Torpedo marmorata*, a rich source of voltage-activated chloride channels, were then labeled with SITS, solubilized, and passed over a WGA lectin affinity column. Western blot analysis using the anti-SITS antibody as a probe revealed bands of molecular weights 93 and 105 kDa. As the band of 93 kDa bound in addition ^{32}P ATP and an antibody raised against the Na^+/K^+ ATPase, it is probable that this protein was the α subunit of this ATPase. Polyclonal antibodies raised against the 105 kDa protein were then employed to screen a cDNA library and the subsequent isolation of a 6 Kb clone. Unfortunately, the RNA coding for this protein failed to induce chloride channel activity when expressed in *Xenopus* oocytes. When the mRNA coding for the protein was selectively depleted from the total RNA preparation of *Torpedo*, the oocytes still did not express normal chloride channel activity. These authors were forced to conclude that the isolated protein was not a chloride channel component. A DIDS binding protein of similar molecular mass has also been previously identified in the electric organ of *Narke japonica*.[113]

A different approach to isolate the channel is to raise antibodies against short synthetic peptides whose amino acid sequences correspond to a defined region of the protein. This technique is applicable if the amino acid sequence of the protein is already known, as is the case when the gene for that protein has been identified. However, even when this is not the case, antibodies can be raised to regions of a known protein where there is good evidence that it shares a common feature with the protein of interest. This strategy has been adopted by Sorscher et al., who have raised a polyclonal antibody against a synthetic peptide that corresponds to a region in erythrocyte band 3, suggested to be involved in DIDS binding.[114] This antibody recognized an approximately 60 kDa protein in membrane preparations of T-84 cells. Intriguingly, immunocytochemistry revealed that the antibody labeled small vesicles inside the cells that were trafficked to the membrane following cell stimulation with forskolin. The time course of this movement was identical to that observed with the forskolin-induced stimulation of chloride transport in these cells. This suggests that cAMP-induced increases in chloride current observed in these cells may be at least in part due to a recruitment of new channels from the cytosol.

Finn and co-workers have identified two candidate chloride channel proteins in the gall bladder epithelium of the mud puppy *Necturus*.[115] Monoclonal antibodies were raised against whole-cell homogenates of *Necturus* gall bladder epithelial cells, and then screened for their ability to inhibit chloride conductance as assessed from microelectrode measurements. Five antibodies reduced the chloride conductance, one by as much as 83%. In Western blots, this latter antibody recognized two bands at 219 and 69 kDa. It is intriguing that several studies have focused on proteins in the 60–70 kDA range as being potential chloride conductors. It is not clear whether these proteins could act as a channel alone or with other as yet unidentified subunits as a heteromeric complex. Ran and Benos[116] have raised a rabbit polyclonal antibody to the 38 kDa protein. When solubilized tracheal membrane was subjected to non-reducing SDS-PAGE and subsequent Western blot analysis, this anti-P38 antibody recognized two major protein bands of 140 and 250 kDa. When subjected to reducing conditions (increase in DTT), the appearance of bands at 62-64 kDa

and 32 and 38 kDa was accompanied by a concomitant decrease in the higher molecular mass species. This finding suggests that the native protein exists as a heteromer connected by disulfide bonds. Electrophysiological studies have suggested that at least two chloride channels from *Torpedo* electroplax and rabbit renal cortical collecting duct may exist as dimers.[117,118] Biochemical analysis revealed a dimeric structure with disulfide linkages of the SITS binding protein of *Torpedo*.[112] Recently, the voltage-gated chloride channel of the *Torpedo* electric organ has been successfully cloned. Jentsch and colleagues,[119] using an expression cloning system, succeeded in cloning the cDNA for the channel. Electroplax RNA was size fractionated to yield the active fraction, i.e., that fraction that coded for functional chloride channel. The fraction was then used to construct a cDNA library. DNA derived from this library was in turn used to deplete total RNA from the complementary message. cDNA clones that reduced chloride conductance were isolated and sequenced. RNA transcribed from these cDNA clones were able to induce a DIDS- and DPC-sensitive chloride channel in *Xenopus* oocytes of similar properties to that expressed following injection of total RNA. The cDNA encodes for a protein of 805 amino acids with a predicted molecular weight of 89 kDa. A preliminary model of the protein predicts 9 and possibly 13 membrane spanning hydrophobic domains with a large cytoplasmic loop proximal to the C terminal. This loop also contains a consensus phosphorylation site for PKA (cAMP-dependent kinase). Whether this channel will prove to be unique, or simply the first member of a family of related channels including those in epithelial cells, remains to be determined.

Cystic fibrosis and secretory diarrhea

Cystic fibrosis. Cystic fibrosis is the most common lethal genetic disease in Caucasians affecting one in 2000 live births.[120] It is characterized by a secretory incompetence that affects the airways, pancreas, and GI tract. The movement of chloride out of secretory epithelial cells is essential in maintaining the fluid layer and in propagating fluid secretion. In the absence of chloride transport, the secretions are thick and viscous, blocking the airways and preventing the flow of digestive enzymes from the pancreas. It was first noted by Quinton that in the reabsorptive sweat duct, the potential difference across the perfused CF ducts was approximately 10 times more negative than in control (non-CF ducts). However, if permeant chloride ions were replaced by impermeant sulfite ions in the bathing medium, then the potential difference in the control ducts approached that of the CF tissue, thus linking the CF defect with a reduced chloride permeability.[121] Subsequently, work by Knowles[122] and Widdicombe[123] confirmed this reduced chloride permeability in cultured airway epithelia. Frizzell and co-workers, and Welsh and Leidtke,[124,125] using the cell-attached patch recording configuration, demonstrated that β adrenergic agents could activate chloride channels in normal, but not in CF, epithelia. The defect is, however, distal to the production of cAMP as there is no difference in the levels of cAMP attained following stimulation of normal or CF cells.[125,126] Application of the catalytic subunit of PKA to excised membrane patches of human trachea activated chloride channels in normal but not in CF-derived material.[77,78] However, the lack of activity in CF tissues is not due to a lack of chloride channels in the membrane, as these channels can be activated by calcium and voltage, but not by PKC,[92,93,127] although these may not be all the same protein. In any case, these observations implicate a regulatory protein in maintaining chloride channel activity. This protein was identified with the cloning of the gene for CF in 1989,[128,129] and designated the CFTR or cystic fibrosis transmembrane regulatory protein. The protein consists of 1,480 amino acids, and the predicted structure based on an hydropathy analysis consists of 12 membrane-spanning domains interspersed by three large cytoplasmic regions, with a predicted weight of 170 kDa (without glycosylation). The molecule comprises two repeating motifs, consisting of six helical membrane-spanning segments and a hydrophilic domain that possesses a consensus sequence for ATP binding. The deletion of a single phenylalanine residue at position 508 in the first nucleotide binding fold gives rise to CF in 70% of patients affected by the disease.[130] The two halves of the molecule are joined by a highly charged region containing many possible phosphorylation sites known as the R-domain. The R-domain has to date no known counterpart, whereas the rest of the molecule resembles the p-glycoproteins and a family of ATP binding transport proteins in its domain organization.[131] Even though the gene for CF was cloned in 1989, we are little closer to understanding what the CFTR protein does or how its dysfunction has such a devastating effect on fluid secretion. Complementation studies in which a corrected version of the gene is introduced into cells carrying the CF defect have been successful in restoring Cl^- transport competency in airway cells and in a pancreatic adenoma cell line expressing the CF phenotype.[132,133]

Until recently, it was thought unlikely that CFTR was itself a chloride channel. Its homology with a family of unidirectional transport proteins that hydrolyze ATP differentiates it from the structure of known channels.[119,134,135] However, the transfection of the CFTR gene into cells that do not normally possess Cl^- channels was marked by the appearance of a cAMP responsive chloride permeability.[136] This result lends itself to two possible explanations: either (a) the introduction of the gene reveals previously inactive cryptic channels, or (b) the protein is itself a Cl^- channel. In either case, further studies need to be performed to establish the physiological role for the protein.

Although the primary focus of recent physiological research in CF has been on the chloride transport defect, it is now generally accepted that CF is also a disease of defective Na^+ transport. Respiratory epithelia from CF patients are characterized not only by

an abnormally low Cl^- permeability, but also by an increase in Na^+ conductance of the apical membrane. This Na^+ conductance is sensitive to amiloride, which caused a much greater fall in transepithelial conductance in CF than in normal tissues.[137] Aerosolized amiloride has recently been tested in clinical trials as a therapeutic agent in the treatment of CF. In patients treated with the drug, sputum viscosity and lung elasticity significantly improved as compared to the control group.[138] Treatment of the chloride channel defect in CF will, however, require a further understanding of the relation between CFTR and Cl^- channel function.

Secretory diarrhea

Secretory diarrheas such as those caused by *Vibrio cholerae* and *Escherichia coli* can be viewed as being a complementary condition to CF. They are characterized by a switch from absorption to secretion of fluid by the small intestine. This, combined with the decrease in absorption of water by the colon, combined with other manifestations of the disease such as vomiting, can lead, in severe cases, to the loss of fluid equivalent to the weight of the patient. The loss of this much fluid results in severe electrolyte imbalance and in systolic hypotension, hypokalemia, and hyponatremia. Fatal complications include metabolic acidosis, due to the high excretion of bicarbonate, and hypovolemic shock. If untreated, death results in 50% to 70% of cases.[139]

The cause of cholera is the toxin produced by the organism. The toxin has two components. The B subunit, which is actually composed of five identical polypeptides, binds irreversibly to a cell surface receptor. The B subunits surround the A subunit, which is composed of two different polypeptides and is the active component of the toxin.[140] The A subunit enters the cell and activates adenylate cyclase by binding irreversibly to a G protein, which couples the enzyme to its receptor. The G protein, in a quiescent state, exists as a heterotrimer composed of α, β, and γ subunits. When an agonist binds to its receptor (e.g., VIP), the conformation of the α subunit, which at rest binds GDP, changes so that the α subunit can bind GTP. The binding of GTP causes dissociation of the heterotrimer, allowing the G-α-GTP complex to bind and switch on adenylate cyclase. Under physiological conditions, the α subunit hydrolyzes the GTP to GDP and the enzyme is switched off; the heterotrimer is reformed and awaits the next cycle. However, cholera toxin will transfer an ADP-ribose group from NAD^+ to the α subunit of the G protein, so that it can no longer hydrolyze the bound GTP. This results in a continued activation of adenylate cyclase and thus the continous production of cAMP.[141] As described above, cAMP is a potent activator of chloride channels and reduces the absorptive capacity of the gut, which together results in a shift from net absorption to net secretion. Other toxins, such as those derived from *E. coli*, share several similarities with cholera toxin. The active subunit of the heat labile toxin of *E. coli* is also an ADP-ribosyltransferase. Other bacterial toxins (e.g., Shiga toxins) responsible for causing diarrhea do not act via an increase in cAMP, but instead are cytotoxins, destroying the absorptive cells of the gut.[142]

Summary and prospectus

Many human diseases including hyper- and hypoaldosteronism, cystic fibrosis, and secretory diarrhea result from alterations in the proper functioning of epithelial Na^+ and/or Cl^- channels. Despite the wealth of electrophysiological information concerning the characteristics and regulatory properties of both epithelial Na^+ and Cl^- channels, virtually nothing is known about their biochemistry and molecular biology. Extrapolating from a very limited data base, it may be that both epithelial Na^+ and Cl^- channels belong to a super family of ion channel proteins, certain ones existing in a particular tissue for a particular physiological purpose. An elucidation of this provocative suggestion is a major challenge to researchers in this area. The developmental aspects of transport functions present another potentially fruitful area for research. What are the genetic signals and/or inherent cellular programming for the expression of such transport systems?

Another interesting question concerns the apparent ubiquity in the nature of oligomeric transport proteins functioning as ion channels. Almost all of the voltage dependent ion channels, as well as the epithelial ones, conform to this pattern. A possible explanation for multiple subunit composition may be redundancy; for example, one ion-binding or translocation subunit may be subjected to different extrinsic regulatory inputs, depending upon its location (e.g., kidney, lung, sweat duct, inner ear hair cells, etc.) or lipid-protein microenvironment. While this idea is speculative, it certainly will be tested in years to come.

It is evident that a thorough understanding of epithelial ion channel structure and function is essential for at least two important reasons. First, this knowledge will provide a much clearer path for solving and treating some of the more devastating human diseases that occur, not only by intervention through a regulatory pathway but also through the use of more appropriate and specifically designed drugs. Second, an understanding of the basic structure of epithelial ion channels will permit a more seminal appreciation of physiological function at the molecular, cellular, and organ levels.

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