Chloride channels in the apical membrane of normal and cystic fibrosis airway and intestinal epithelia

MATTHEW P. ANDERSON, DAVID N. SHEPPARD, HERBERT A. BERGER, AND MICHAEL J. WELSH
Howard Hughes Medical Institute, Departments of Internal Medicine and Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, Iowa 52242

Anderson, Matthew P., David N. Sheppard, Herbert A. Berger, and Michael J. Welsh. Chloride channels in the apical membrane of normal and cystic fibrosis airway and intestinal epithelia. Am. J. Physiol. 263 (Lung Cell. Mol. Physiol. 7): L1-L14, 1992.—Cl⁻ channels located in the apical membrane of secretory epithelia play a key role in epithelial fluid and electrolyte transport. Dysfunction of one of these channels, cystic fibrosis transmembrane conductance regulator (CFTR), causes the genetic disease cystic fibrosis (CF). We review here the properties and regulation of the different types of Cl⁻ channels that have been reported in airway and intestinal epithelia. We begin by describing the properties of the CFTR Cl⁻ channel and then use those properties as a point of reference. We focused particularly on the evidence that localizes specific types of Cl⁻ channel to the apical membrane. With that background, we assess the biological function of various Cl⁻ channels in airway and intestinal epithelia.

cystic fibrosis transmembrane conductance regulator; secretion; intestine; adenosine 3',5'-cyclic monophosphate-regulated channels; calcium-activated channels; volume-regulated channels; outwardly rectifying channels; anion channel

CHLORIDE CHANNELS located in the apical membrane play a key role in the secretion of fluid and electrolytes across epithelia. In Cl⁻-secreting epithelia, a series of basolateral membrane transport processes accumulate Cl⁻ intracellularly at a concentration above electrochemical equilibrium (127). Then on activation, apical membrane Cl⁻ channels allow Cl⁻ to exit passively from the cell, moving down a favorable electrochemical gradient. Apical Cl⁻ exit is followed by paracellular Na⁺ flow, and the secretion of water ensues. Thus Cl⁻ channels provide a pathway for CA⁻ exit from the cell and a key point at which to regulate the rate of transepithelial Cl⁻ secretion.

The discovery that adenosine 3',5'-cyclic monophosphate (cAMP) fails to activate a Cl⁻ conductance in the apical membrane of cystic fibrosis (CF) epithelia and the development of powerful electrophysiological techniques to study ion channels has lead to a considerable increase in research on Cl⁻ channels. CF, the most common lethal genetic disease in Caucasians, is caused by mutations in a single gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (41, 70, 93, 96, 99). Amino acid sequence analysis and comparison with other proteins suggested that CFTR consists of five domains (96) (Fig. 1): two membrane-spanning domains, each composed of six transmembrane segments; an R domain, which contains several consensus phosphorylation sequences; and two nucleotide-binding domains (NBDs). Sequence similarity in the NBDs and the predicted topology of CFTR (with the exception of the R domain) suggest that CFTR belongs to a family of proteins variously called the traffic adenosinetriphosphatases (ATPases) (1), the ABC transporters (67), or the TM6-NBF family (95). Most members of this family are ATP-dependent transporters, including periplasmic permeases in prokaryotes and P-glycoprotein responsible for multidrug resistance in higher eukaryotes. However, recent studies (see below) indicate that CFTR is a Cl⁻ channel, regulated by cAMP-dependent phosphorylation and by intracellular ATP. The cytoplasmically located NBDs and the H domain distinguished CFTR from the structure of known voltage- and ligand-gated ion channels (64), indicating that CFTR may represent the first identified member of a new family of ion channels.

Mutations in CFTR eliminate the apical membrane cAMP regulated Cl⁻ permeability in CF secretory epithelia. This defect likely contributes to the pathogenesis and pathophysiology of the disease (16, 19, 89). However, it is not yet clear how mutations in CFTR result in other aspects of the CF phenotype, such as increased Na⁺ absorption in CF airway epithelia (20) and increased sulfation of glycoconjugates (25). An appealing hypothesis that may explain some of the multiple abnormalities in CF is that defective acidification of intracellular compartments, resulting from reduced Cl⁻...
We begin by discussing the properties of Cl\textsuperscript{−} channels generated by expression of recombinant CFTR. We then proceed to compare those properties with the properties of previously described epithelial Cl\textsuperscript{−} channels. Table 1 summarizes the data and organization of the review.

**Cyclic AMP-regulated Cl\textsuperscript{−} Channels**

*Whole cell studies in cells expressing recombinant CFTR.* Recombinant CFTR was first expressed in CF airway epithelial cells (57, 90) and a CF pancreatic epithelial cell line (CFPAC-1) (41). In those studies the CF defect in Cl\textsuperscript{−} conductance was corrected; cAMP-activated Cl\textsuperscript{−} currents appeared when the normal CFTR gene was expressed. This result suggested that CFTR was either a Cl\textsuperscript{−} channel itself or it functioned to regulate such channels. To ascertain its function, CFTR was expressed in a number of nonepithelial mammalian cells lacking endogenous CFTR, including NIH/3T3 fibroblasts (3), Chinese hamster ovary cells (4,109), HeLa cells (4), mouse L cells (98), and Vero cells (33). CFTR was also expressed in nonmammalian cells, such as the Sf9 insect cell line (69) and Xenopus oocytes (11, 42). In each case, expression of CFTR generated a unique Cl\textsuperscript{−} current that was activated by cAMP agonists [forskolin, 3-isobutyl-1-methyl-xanthine (IBMX), and membrane-permeant cAMP analogues]. Such currents were not observed in mock-transfected cells.

The whole cell Cl\textsuperscript{−} currents displayed similar properties in all cells expressing recombinant CFTR (Table 1). One defining property is channel regulation. Under baseline conditions there is little, if any, Cl\textsuperscript{−} current, but, after addition of cAMP agonists, a dramatic increase in Cl\textsuperscript{−} current ensues. The increase in Cl\textsuperscript{−} current did not appear to be dependent on intracellular Ca\textsuperscript{2+} because in several studies the pipette solution used to dialyze cells...
was strongly buffered with ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N' -tetraacetic acid (EGTA) [estimated concentration of intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) < \(10\) nM]. Membrane potential did not appear to modulate channel activity because there were few time-dependent effects of hyperpolarizing or depolarizing voltages (Fig. 2A). (A small, slowly activating component at depolarized potentials was seen in some cells expressing recombinant CFTR.) The lack of voltage-dependent regulation contrasts with Ca\(^{2+}\)-activated and volume-regulated Cl\(^-\) channels (see below and Fig. 2, D and E).

The biophysical properties of Cl\(^-\) currents generated by expression of CFTR were also similar in the different cell types studied. The current-voltage (I-V) relationship of the cAMP-regulated Cl\(^-\) current was linear in the presence of symmetrical Cl\(^-\) concentrations. Reversal potential measurements with different NaCl concentration gradients indicate that the channel is selective for anions over cations.

The anion selectivity sequence is a distinguishing feature of CFTR Cl\(^-\) currents because it differs from the reported anion selectivity sequences of many other epithelial Cl\(^-\) channels (46, 52). The anion permeability sequence of CFTR-generated Cl\(^-\) currents recorded in 3T3 fibroblasts and HeLa cells is Br\(^-\) > Cl\(^-\) > I\(^-\) (Tables 1 and 2). Mutation of basic residues in the transmembrane domains of CFTR to acidic residues (Lys-95 mutated to Asp or Lys-335 mutated to Glu) altered the permeability sequence to I\(^-\) > Br\(^-\) > Cl\(^-\) (Table 2) (3). Mutation of two other basic residues (Arg-347 and Arg-1030) to acidic residues did not alter the selectivity sequence. The ability to change the properties of the conduction mechanism by altering specific amino acids within CFTR provides the most compelling evidence that CFTR is itself a cAMP-regulated Cl\(^-\) channel. In Sf9 insect cells expressing

<table>
<thead>
<tr>
<th>Table 2. Relative anion permeability of cAMP-regulated channels in apical membrane and in cells expressing wild-type and mutant CFTR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>cAMP</td>
</tr>
<tr>
<td>3T3 fibroblasts CFTR</td>
</tr>
<tr>
<td>HeLa cells CFTR</td>
</tr>
<tr>
<td>K95D</td>
</tr>
<tr>
<td>K335E</td>
</tr>
<tr>
<td>R347E</td>
</tr>
<tr>
<td>R1030E</td>
</tr>
<tr>
<td>Human airway epithelia Apical</td>
</tr>
<tr>
<td>T84 epithelia Apical</td>
</tr>
<tr>
<td>CF airway epithelia Apical</td>
</tr>
<tr>
<td>Data are mean values calculated from currents in individual cells stimulated with cAMP. Permeability ratios, (\frac{P_X}{P_{Cl^-}}) where X is I(^-) or Br(^-), were calculated from reversal potential measurements using Goldman-Hodgkin-Katz equation, and conductance ratios (\frac{G_X}{G_{Cl^-}}) were calculated from the slope of I-V relationship. [Modified from Anderson et al. (3).]</td>
</tr>
</tbody>
</table>
CFTR, the channel was reported to be slightly more permeable to I$^-$ than to Cl$^-$ (69). However, other properties were unaltered, including regulation by cAMP, the voltage dependence, the linear $I$-$V$ relationship, the single-channel conductance (see below), and the blocker sensitivity (see below). This difference in anion permeability sequence from mammalian cells does not appear to be due to the state of glycosylation of CFTR (membrane proteins expressed in Sf9 cells are poorly glycosylated) because when a CFTR mutant that lacks the glycosylation sites (Asn 894 and Asn 900 mutated to Gln) (27, 58) was expressed in HeLa cells, Cl$^-$ was still more permeable than I$^-$ (Sheppard and Welsh, unpublished data). Moreover, in other unpublished studies, we have found that the conditions used to study CFTR in Sf9 cells do not alter the anion selectivity sequence of CFTR expressed in mammalian cells (Anderson and Welsh, unpublished data). Thus the difference in selectivity cannot be accounted for by differences in solutions or pH. Future studies aimed at resolving this difference may reveal interesting characteristics of the Sf9 insect cells.

The effect of Cl$^-$ channel inhibitors on CFTR has not been extensively examined to date. In part, this can be attributed to the absence of specific blockers with which to discriminate the different types of epithelial Cl$^-$ channels. CFTR Cl$^-$ currents are blocked by high concentrations of diphenylamine-2-carboxylate (DPC) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), with stronger inhibition by DPC observed at negative potentials (4, 87). Extracellular 4,4$'$-diisothiocyanatostilbene-2,2$'$-disulfonic acid (DIDS), a stilbene-disulfonic acid derivative, blocks several types of epithelial Cl$^-$ channels (Table 1); however, its inability to block CFTR Cl$^-$ channels has proved a characteristic signature (69, 103). Blockers were added to the mucosal bath for epithelia expressed in Airway, and T84 intestinal epithelia (T84 Int), and in 3T3 fibroblasts stably expressing CFTR (CFTR). Blockers were added to the mucosal bath for epithelia and the extracellular bath for 3T3 fibroblasts.

### Single-channel studies in cells expressing recombinant CFTR

Single-channel studies have also been performed on cells expressing recombinant CFTR (Tables 1 and 3). All reports identify a low-conductance Cl$^-$ channel with a linear $I$-$V$ relationship that is selective for anions over cations. In agreement with the whole-cell patch-clamp studies, single-channel studies using cell-attached and excised, inside-out membrane patches revealed that channel open-state probability ($P_o$) was relatively independent of voltage. The anion permeability sequence was Cl$^-$$>$ I$^-$ in mammalian cells expressing CFTR (14, 33). Similar results were obtained when membrane vesicles from cells expressing CFTR were incorporated into planar lipid bilayers (114); the channels had a low single-channel conductance, a linear $I$-$V$ relationship, no time-dependent voltage activation, and a greater permeability for Cl$^-$ than for I$^-$.

In cell-attached patches, the channel was reversibly activated by addition of cAMP agonists (11, 14, 33, 69, 109), and in excised, inside-out patches, it opened when the catalytic subunit of cAMP-dependent protein kinase (PKA) and ATP were added to the solution bathing the internal (cytosolic) surface of the membrane (14, 109). CFTR Cl$^-$ channels in planar lipid bilayers were also activated by PKA and ATP (114). Protein kinase C also activated the channel in excised inside-out patches, although it was less efficacious than PKA (109). Interestingly, PKC was also found to increase the magnitude and/or rate of increase in $P_o$, by subsequently added PKA. In addition, Tabcharani et al. (109) have shown that alkaline phosphatase closes PKA-activated CFTR Cl$^-$ channels in excised inside-out patches. These results suggested that phosphorylation of CFTR causes the channel to open.

Direct evidence that PKA phosphorylates CFTR on the R domain (Fig. 1) to open the Cl$^-$ channel was provided by two additional studies. First, PKA phosphorylated CFTR in vivo on four serine residues located within the R domain, and simultaneous mutation of those four residues to alanines prevented cAMP-stimulated activation (28). Second, deletion of the majority of the R domain (including 3 of 4 phosphorylation sites) of CFTR produced a channel that was constitutively open, even without an increase in cAMP (94). Residual cAMP regulation above the constitutive activity was also observed; however, this residual regulation was abolished when the remaining phosphorylation site (serine 660) was mutated to alanine (28, 94).

### Table 3. Single-channel properties of Cl$^-$ channels in cells expressing recombinant CFTR

<table>
<thead>
<tr>
<th>Cell</th>
<th>Method</th>
<th>Conductance $P_{i^-}/P_{Cl^-}$</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH 3T3</td>
<td>Cell-attached</td>
<td>8.6</td>
<td>14</td>
</tr>
<tr>
<td>Sf9</td>
<td>Cell-attached</td>
<td>7.5-8.4</td>
<td>69</td>
</tr>
<tr>
<td>CIIO</td>
<td>Cell-attached</td>
<td>9.6</td>
<td>109</td>
</tr>
<tr>
<td>Vero</td>
<td>Cell-attached</td>
<td>4.9</td>
<td>33</td>
</tr>
<tr>
<td>Xenopus</td>
<td>Cell-attached</td>
<td>7.7</td>
<td>11</td>
</tr>
<tr>
<td>NIH 3T3</td>
<td>Excised</td>
<td>10.4</td>
<td>14</td>
</tr>
<tr>
<td>CHO</td>
<td>Excised</td>
<td>0.42</td>
<td>109</td>
</tr>
</tbody>
</table>

Some differences in single-channel conductance may be explained by differences in temperature, Cl$^-$ concentration, and the pH buffer (HEPES) (14). $P_{i^-}/P_{Cl^-}$ permeability ratio of I$^-$ and Cl$^-$; $I$-$V$ relationships are linear for all channels.
More recent studies have shown that the CFTR Cl⁻ channel is also regulated by nucleoside triphosphates such as ATP (2); once phosphorylated by PKA, the channel requires cytosolic ATP to remain open. ATP activates the channel by a mechanism independent of both PKA and the R domain. Regulation at the nucleotide binding domains of CFTR is an attractive explanation for this effect (Fig. 1).

Studies of the apical membrane in polarized epithelia expressing endogenous CFTR. For a Cl⁻ channel to directly govern Cl⁻ secretion, it must be located in the apical membrane. A large body of evidence has shown that the apical membrane of airway and intestinal epithelia contains a CAMP-regulated Cl⁻ conductance (12/1). Moreover, this membrane has been identified as the site of the CF defect (89). Therefore, it is important to examine the properties of apical membrane Cl⁻ channels in epithelia expressing endogenous CFTR to see if they match the properties of recombinant CFTR in nonepithelial cells. To be certain that the properties of the apical membrane are measured, cells must be grown on permeable filter supports so that they differentiate, form tight junctions, and polarize, thereby segregating apical and basolateral proteins. Only under these conditions can the transepithelial Cl⁻ secretion found in native epithelia be observed. Because transepithelial current is determined by apical and basolateral membranes in series, the properties of a single membrane cannot be resolved by measuring current across the intact epithelium. One method of studying the apical membrane in isolation is to functionally eliminate the basolateral membrane. This can be achieved by permeabilizing the basolateral membrane with nystatin, which forms pores permeable to monovalent ions (6, 34, 65). The apical membrane Cl⁻ conductance can then be measured as the current generated by a Cl⁻ concentration gradient when transepithelial voltage is clamped to 0 mV.

In both normal airway and in several intestinal epithelial cell lines [T84 (80), Caco-2 (53), and HT-29-clone 19A (8)], addition of cAMP agonists increases the apical membrane Cl⁻ conductance (6). When transepithelial voltage is varied, the biophysical properties of these Cl⁻ channels can be assessed. As observed for recombinant CFTR, the I-V relationship is linear, the current shows no time-dependent voltage effects (Fig. 2C), and the anion permeability sequence is Cl⁻ > I⁻ (Table 2). A similar transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91). Studies of radiolabeled intact sweat gland duct epithelium, which also manifests no time-dependent voltage effects (Fig. 2C), and the transepithelial anion selectivity has been observed in the intact sweat gland duct epithelium, which also manifests the CF Cl⁻ channel defect (91).

That conclusion is also supported by the finding that, in CF airway epithelia, CAMP failed to increase the apical membrane Cl⁻ conductance (6, 18, 31, 89, 132). Similarly, in heterologous expression systems, cells expressing most CF-associated mutant forms of CFTR fail to respond to cAMP stimulation (4, 58, 93). Although a recent study in Vero cells (33) suggested that CFTR containing the most common mutation, deletion of phenylalanine at position 508 (CFTRΔF508), retained some residual function, such function was only ~5% of wild type CFTR. It was estimated that CFTRΔF508 channels were observed at ~25% the frequency of wild-type channels, and each CFTRΔF508 channel had ~20% I₀, of wild-type channels. Although similar currents were not observed in previous whole cell patch-clamp studies of mammalian cells expressing recombinant CFTRΔF508, it is possible that activity of 5% of wild type would fall below the sensitivity of the expression system and assay used or alternatively. CFTRΔF508 may be processed differently by Vero cells (4, 58, 93). When CFTRΔF508 was expressed in Xenopus oocytes, a CAMP-regulated Cl⁻ current was also generated (42), but the current was a much larger percentage (60%) of that observed with wild-type CFTR when compared with the results observed in mammalian cells. Similar results were obtained with SF9 insect cells (12). This difference may result from differences in processing of mutant protein by amphibian oocytes and insect cells.

Patch-clamp studies of single cells expressing endogenous CFTR. Intestinal epithelial cell lines have been the cells of choice for epithelial patch-clamp studies because they express a large CAMP-regulated Cl⁻ conductance and relatively large amounts of CFTR. They are also technically easier to study than acutely isolated cells. Cliff and Frizzell (30) made the first comprehensive whole cell study of CAMP-regulated Cl⁻ conductances in T84 cells. T84 cells possess a CAMP-regulated Cl⁻ current with properties similar to those observed in cells expressing recombinant CFTR and in the apical membrane of polarized epithelia expressing endogenous CFTR. The current had a linear I-V relationship, showed no appreciable time-dependent voltage effects, was inhibited by DPC (but not DIDS), and had an anion permeability sequence of Cl⁻ > I⁻ (6, 30).

The whole cell properties of the CAMP-regulated Cl⁻ conductance in intestinal epithelia resembled the single-channel properties of a CAMP-regulated Cl⁻ channel in the apical membrane of rat and human pancreatic duct cells (54, 55). The channel had a small single-channel conductance (4-7 pS) and a linear I-V relationship. However, the reported anion permeability sequence (Br⁻ > I⁻, Cl⁻) (56) was different from that of whole cell CAMP-regulated Cl⁻ channels. This difference may be attributed to the difficulty of studying anion selectivity using the cell-attached configuration of the patch-clamp technique in cases in which the intracellular Cl⁻ concentration and cell membrane potential are unknown. Subsequent studies identified similar low-conductance, CAMP-regulated Cl⁻ channels in T84 and Caco-2 intestinal cells (13, 111). These low-conductance Cl⁻ channels possess the properties predicted from single-channel studies of cells expressing recombinant CFTR. Furthermore, these properties...
are also consistent with those of cAMP-regulated Cl− currents in the apical membrane and whole cell patch-clamp studies of native epithelial cells, both of which express endogenous CFTR.

**Identity of the CFTR Cl− channel.** Several results indicate that CFTR is a cAMP-activated Cl− channel located in the apical membrane of Cl− secretory epithelia. First, the data show that the regulatory and biophysical properties of cAMP-regulated Cl− currents are similar in cells expressing recombinant CFTR, in epithelial cells expressing endogenous CFTR, and in the apical membrane of Cl− secretory epithelia. Second, specific alterations of the amino acid sequences of CFTR alter the anion-selectivity sequence of the cAMP-activated Cl− channels. Third, site-directed mutations and deletions within CFTR alter the regulation of the Cl− channel. Fourth, CFTR has been immunocytochemically localized to the apical region of several epithelia (32, 38, 82) and is present in the apical membrane of Cl−-secreting intestinal epithelial cell lines (38). Fifth, CF-associated mutations in CFTR abolish or severely reduce the cAMP-regulated Cl− conductance in the apical membrane of secretory epithelia and in cells expressing recombinant protein.

The conclusion that the CFTR Cl− channel is in the apical membrane places it in a location where its activation by PKA-dependent phosphorylation allows Cl− to exit from the cell, thus completing the final step in cAMP-stimulated Cl− secretion. Regulation of this channel by ATP may also be important in coupling the cellular metabolic status to the rate of transepithelial Cl− secretion. Metabolic status to the rate of transepithelial Cl− secretion at the basolateral membrane of polarized epithelial monolayers with nystatin and study the apical membrane in isolation, as described above. This method could also be used to compare the properties of these channels with those activated by cAMP. When [Ca2+]i was increased with Ca2+ ionophores or inflammatory mediators in nystatin-permeabilized airway epithelia a transient Cl− current was activated (6). In contrast to the cAMP-regulated Cl− current, the Ca2+-activated Cl− current was present in both normal and CF airway epithelial monolayers, suggesting but not proving that cAMP and Ca2+ activate different Cl− channels.

**Ca2+-activated Cl− channels in the apical membrane of polarized epithelia.** Several studies have shown that an increase in [Ca2+]i stimulates transepithelial Cl− secretion in airway epithelia. Microelectrode studies and the use of blockers have suggested that an increase in [Ca2+]i activates both apical Cl− channels and basolateral K+ channels in airway epithelia (17, 85, 86, 130, 133). A more sensitive method of determining if the apical membrane contains Ca2+-activated Cl− channels is to permeabilize the basolateral membrane of polarized epithelial monolayers with nystatin and study the apical membrane in isolation, as described above. This method could also be used to compare the properties of these channels with those activated by cAMP. When [Ca2+]i was increased with Ca2+ ionophores or inflammatory mediators in nystatin-permeabilized airway epithelia a transient Cl− current was activated (6). In contrast to the cAMP-regulated Cl− current, the Ca2+-activated Cl− current was present in both normal and CF airway epithelial monolayers, suggesting but not proving that cAMP and Ca2+ activate different Cl− channels.

When similar studies were performed with cultured intestinal epithelial cell lines (T84, Caco-2, and HT-29 clone 19A), no Ca2+-activated Cl− current was observed in the apical membrane (6). A previous 36Cl−-flux study of polarized T84 cells also failed to observe an A23187-stimulated increase in apical Cl− transport (81). The results indicate that, unlike airway epithelia, the apical membrane of these intestinal cells does not contain Ca2+-activated Cl− channels.

Vaandrager et al. (119) reported Ca2+-stimulated 36Cl− efflux from both apical and basolateral membranes of HT-29 clone 19A intestinal epithelia grown on permeable supports for 7–9 days. On the basis of these results and previous microelectrode studies, they suggested that Ca2+ activates Cl− channels in both membranes. These results at first appear to contradict the results described above; however, in our original studies we used epithelia grown for >13 days. In subsequent studies, we also found that cultures of HT-29 clone 19A grown for <9 days display a small Ca2+-activated apical Cl− current but that this Cl− current disappears with time in culture (Anderson and Welsh, unpublished observations). This result suggests that...
that this epithelial cell line may take time to fully differentiate once seeded on a permeable filter support. This conclusion is also supported by the lack of polarization of the Ca\(^{2+}\)-induced \(^{36}\)Cl\(^{-}\) efflux (119).

The observation that the apical membrane of these intestinal epithelial cells contain cAMP- but not Ca\(^{2+}\)-activated Cl\(^{-}\) currents also suggested that cAMP and Ca\(^{2+}\) activate different channels. However, that result could also be explained by differences in the regulatory pathways present in the two cell types. Three additional studies in airway epithelia provided more direct evidence that cAMP and Ca\(^{2+}\) activate different Cl\(^{-}\) channels (6).

1) In contrast to cAMP-regulated Cl\(^{-}\) currents, Ca\(^{2+}\)-activated Cl\(^{-}\) currents were more permeable and more conductive to I\(^{-}\) than to Cl\(^{-}\) (Table 2).

2) DIDS inhibited Ca\(^{2+}\)- but not cAMP-activated Cl\(^{-}\) currents.

3) An increase in both second messengers caused an additive increase in Cl\(^{-}\) current. Table 1 summarizes the properties of Ca\(^{2+}\)-activated apical membrane Cl\(^{-}\) currents.

These studies also suggested that an increase in intracellular Ca\(^{2+}\) does not acutely regulate the CFTR Cl\(^{-}\) channel. In T84 monolayers, with a nystatin-permeabilized basolateral membrane, Ca\(^{2+}\) ionophores in the presence of high extracellular Ca\(^{2+}\) concentrations did not activate apical Cl\(^{-}\) channels under basal conditions (6) nor did they acutely increase or decrease current already activated by cAMP (Anderson and Welsh, unpublished observations). The finding that Ca\(^{2+}\) and cAMP produce additive, rather than synergistic, increases in the apical membrane Cl\(^{-}\) conductance of airway epithelia also supports this conclusion (6).

If intestinal cells do not contain Ca\(^{2+}\)-activated Cl\(^{-}\) channels in the apical membrane, how then does an increase in Ca\(^{2+}\) stimulate transepithelial Cl\(^{-}\) secretion? As suggested in previous studies of T84 cells (81), such stimulation likely occurs when Ca\(^{2+}\) activates basolateral membrane K\(^{+}\) channels (39). The resulting hyperpolarization increases the driving force for Cl\(^{-}\) exit across the apical membrane. Such a sequence of events has been previously shown to occur in airway epithelium (85, 86, 117). If some apical Cl\(^{-}\) channels are open under basal conditions in the intestine, Cl\(^{-}\) secretion would result. It is interesting to note that canine airway epithelium differs from human airway epithelium in that it has few if any Ca\(^{2+}\)-activated Cl\(^{-}\) channels in its apical membrane (Anderson and Welsh, unpublished observations). As animal models of CF are developed, it will be interesting to determine whether the absence of apical membrane Ca\(^{2+}\)-activated Cl\(^{-}\) channels in the airway epithelia correlates with an increased severity of lung disease.

These data and conclusions also provide an explanation for the previously puzzling observation that Ca\(^{2+}\) fails to stimulate Cl\(^{-}\) secretion in CF intestine but does stimulate secretion in CF airway. The results suggest that CF does not cause defective regulation of Ca\(^{2+}\)-activated Cl\(^{-}\) channels. Rather, the Ca\(^{2+}\)-activated Cl\(^{-}\) channels that circumvent the CF defect in airway epithelia are not present in the apical membrane of intestinal epithelia. We would speculate that Ca\(^{2+}\) stimulates Cl\(^{-}\) secretion through cAMP-activated apical membrane Cl\(^{-}\) channels that are open under basal conditions in normal intestine.

The defect in Ca\(^{2+}\)-stimulated secretion in the CF intestine would then result from a defect in these cAMP-activated apical membrane Cl\(^{-}\) channels.

**Whole cell patch-clamp studies of Ca\(^{2+}\)-activated Cl\(^{-}\) channels in epithelial cells grown on impermeable supports.** In the last section we discussed Ca\(^{2+}\)-activated Cl\(^{-}\) channels that are located in the apical membrane of polarized epithelia grown on filters. We now turn to studies of cells grown on impermeable supports in which cell polarity is undefined. In an attempt to learn more about the properties and regulation of Ca\(^{2+}\)-activated Cl\(^{-}\) channels, a number of investigators have used the whole cell patch-clamp technique to study airway or intestinal cells grown on impermeable supports (6, 30, 122, 137). Under these culture conditions, Ca\(^{2+}\)-activated Cl\(^{-}\) currents have a linear I-V relationship, are more permeable to I\(^{-}\) than to Cl\(^{-}\) (Table 1), and are inhibited by DIDS. These properties are the same as those of Ca\(^{2+}\)-activated Cl\(^{-}\) currents located in the apical membrane of airway epithelia (Table 1).

However, two observations hint that Ca\(^{2+}\)-activated Cl\(^{-}\) channels in the apical membrane are different from those in cells grown on impermeable supports (Fig. 4). First, a striking property of Ca\(^{2+}\)-activated Cl\(^{-}\) channels in cells grown on impermeable supports is the time-dependent increase in current seen at depolarizing voltages (Fig. 4E). Interestingly, large, prolonged increases in Ca\(^{2+}\) sometimes cause this voltage dependence to disappear (Anderson and Welsh, unpublished observations). The time dependence has been observed both in conventional whole cell patch-clamp studies (6, 30, 122, 137) and in studies using the perforated-patch, whole cell recording configuration (65), which prevents the loss of important intracellular mediators that occur during conventional whole cell recordings (Ref. 6 and Anderson and Welsh, unpublished observations). Moreover, time-dependent currents were also observed when intracellular Ca\(^{2+}\) was increased with Ca\(^{2+}\) ionophores or with the inflammatory mediator ATP. In sharp contrast, the Ca\(^{2+}\)-activated Cl\(^{-}\) channels in the apical membrane of intestinal epithelium differ from human airway epithelium in that they have few if any Ca\(^{2+}\)-activated Cl\(^{-}\) channels.

---

**Fig. 4. Model of Cl\(^{-}\) channels in airway and intestinal epithelia.** A: polarized cells in an epithelium (grown on filters) with Cl\(^{-}\) channels in apical membrane. B: single cells grown on plastic for studies with whole cell patch-clamp technique. Different shading (open, hatched, and solid) refer to different Cl\(^{-}\) channel types. [Modified from Anderson and Welsh (61).]
current located in the apical membrane did not display any time-dependent voltage effects (6).

Second, although a number of researchers have reported Ca\(^{2+}\)-activated Cl\(^{-}\) channels in intestinal cells grown on impermeable supports (the Ca\(^{2+}\)-activated currents had properties similar to those found in airway epithelia), these currents are not present in the apical membrane of polarized intestinal epithelia. What happened to those Ca\(^{2+}\)-activated Cl\(^{-}\) channels? The present data suggest that they are not found in the basolateral membrane (6). We speculate that this particular Ca\(^{2+}\)-activated Cl\(^{-}\) channel may not be expressed in the apical or basolateral membrane of polarized intestinal or airway epithelial cells. Such a conclusion would be consistent with the observation that the expression of many epithelial proteins is dependent on the substrate. That conclusion is also consistent with the observation that Ca\(^{2+}\)-activated Cl\(^{-}\) currents with similar properties have been observed in many nonepithelial cells (23, 88, 97). Perhaps these voltage-dependent Ca\(^{2+}\)-activated Cl\(^{-}\) channels are not specialized for transepithelial Cl\(^{-}\) secretion but instead serve some other cellular function.

These observations raise an important question: are the Ca\(^{2+}\)-activated Cl\(^{-}\) channels studied in the plasma membrane of airway epithelial cells grown on impermeable supports the same as those in the apical membrane of airway epithelia grown on permeable filters? At present, the data are insufficient to answer the question with certainty. The question is important, however, because of the potential for bypassing the CF defect. Whereas the data described above suggest that cAMP activates the same Cl\(^{-}\) channel in epithelial cells independent of the culture conditions, this may not be true of Cl\(^{-}\) channels activated by Ca\(^{2+}\). Whereas it is possible that apical Ca\(^{2+}\)-activated Cl\(^{-}\) channels are expressed in cells grown on impermeable supports, if the results of such studies are to be interpreted as describing the Ca\(^{2+}\)-activated Cl\(^{-}\) channels that mediate transepithelial Cl\(^{-}\) secretion, it is necessary to demonstrate that those particular Ca\(^{2+}\)-activated Cl\(^{-}\) channels are expressed in the apical membrane.

The mechanism by which Ca\(^{2+}\) activates Cl\(^{-}\) currents in cells grown on impermeable supports may be via the multifunctional Ca\(^{2+}\)-calmodulin-dependent protein kinase (CaM-kinase); the currents were activated by the kinase, and activation was inhibited by kinase inhibitors. Similar studies implicating a role for CaM-kinase have been performed by Sole and Wine (106) in normal and CF airway and sweat duct epithelial cells and in T84 cells; whole cell Cl\(^{-}\) currents in the different cells studied were indistinguishable. In cells exposed to hyposmotic extracellular solutions, Cl\(^{-}\) channels were found with properties similar to those predicted by the whole cell studies; they had an outwardly rectifying I-V relationship and were inactivated by depolarizing voltages. The single-channel conductance was 50 pS at 0 mV.

It has been suggested, based on the observation that a hyposmotic extracellular solution stimulates transepithelial Cl\(^{-}\) secretion in canine tracheal epithelium (84), that the volume-regulated Cl\(^{-}\) channel exists in the apical membrane. However, the response was relatively small when compared with cAMP agonists. An alternative explanation for the stimulation of current is that basolateral membrane K\(^{+}\) channels were activated by cell swelling (22). The resulting cell hyperpolarization could drive Cl\(^{-}\) secretion through apical Cl\(^{-}\) channels that are open under basal conditions. In T84 cells, whole cell volume-activated Cl\(^{-}\) currents are larger than cAMP-activated Cl\(^{-}\) currents, yet exposure of T84 monolayers to hyposmotic extracellular solutions causes very little, if any, increase in short-circuit current (Anderson and Welsh, unpublished observations). On the other hand, evidence that the apical membrane of native intestinal epithelia contains volume-regulated Cl\(^{-}\) channels comes from conventional and ion-selective microelectrode studies of Necturus small intestines (49, 50). Such studies showed that the apical membrane of Necturus small intestine was dominated by a cAMP-activated Cl\(^{-}\) conductance (50). However, measurements of Cl\(^{-}\) fluxes during Na\(^{+}\)-coupled L-alanine transport (which causes cell swelling) detected an increase in the apparent apical membrane Cl\(^{-}\) permeability (49). Moreover, cell swelling, induced by exposure of enterocytes to hyposmotic mucosal solutions, also activated an apical membrane Cl\(^{-}\) conductance (51).

It is probably safe to suggest that these volume-activated Cl\(^{-}\) channels might play some role in the regulatory volume decrease that follows cell swelling. Although it is tempting to speculate beyond this assertion, at present there are insufficient data to support general or specific conjecture about the biological function of the channels. An unequivocal determination of their location would be of considerable help.

### VOLUME-REGULATED Cl\(^{-}\) CHANNELS

Whole cell patch-clamp studies of single airway and intestinal epithelial cells grown on impermeable supports identified a large Cl\(^{-}\) current that exhibited voltage-dependent inactivation at depolarizing voltages (84, 100, 106, 186). These currents were augmented by hyposmotic and inhibited by hyperosmotic extracellular solutions. Although the corresponding changes in cell volume have not been measured, these effects presumably result from cell swelling and shrinkage, respectively. A number of properties distinguish the volume-regulated Cl\(^{-}\) channels from CFTR Cl\(^{-}\) channels (Table 1). The volume-regulated Cl\(^{-}\) channels display a permeability sequence of I\(^{\to}\) > Cl\(^{-}\), an outwardly rectifying I-V relationship, block by DIDS, and time-dependent current inactivation at depolarizing voltages (Fig. 2D).

Single channel studies of volume-regulated Cl\(^{-}\) channels have been performed by Solc and Wine (106) in normal and CF airway and sweat duct epithelial cells and in T84 cells; whole cell Cl\(^{-}\) currents in the different cells studied were indistinguishable. In cells exposed to hyposmotic extracellular solutions, Cl\(^{-}\) channels were found with properties similar to those predicted by the whole cell studies; they had an outwardly rectifying I-V relationship and were inactivated by depolarizing voltages. The single-channel conductance was 50 pS at 0 mV.

It has been suggested, based on the observation that a hyposmotic extracellular solution stimulates transepithelial Cl\(^{-}\) secretion in canine tracheal epithelium (84), that the volume-regulated Cl\(^{-}\) channel exists in the apical membrane. However, the response was relatively small when compared with cAMP agonists. An alternative explanation for the stimulation of current is that basolateral membrane K\(^{+}\) channels were activated by cell swelling (22). The resulting cell hyperpolarization could drive Cl\(^{-}\) secretion through apical Cl\(^{-}\) channels that are open under basal conditions. In T84 cells, whole cell volume-activated Cl\(^{-}\) currents are larger than cAMP-activated Cl\(^{-}\) currents, yet exposure of T84 monolayers to hyposmotic extracellular solutions causes very little, if any, increase in short-circuit current (Anderson and Welsh, unpublished observations). On the other hand, evidence that the apical membrane of native intestinal epithelia contains volume-regulated Cl\(^{-}\) channels comes from conventional and ion-selective microelectrode studies of Necturus small intestines (49, 50). Such studies showed that the apical membrane of Necturus small intestine was dominated by a cAMP-activated Cl\(^{-}\) conductance (50). However, measurements of Cl\(^{-}\) fluxes during Na\(^{+}\)-coupled L-alanine transport (which causes cell swelling) detected an increase in the apparent apical membrane Cl\(^{-}\) permeability (49). Moreover, cell swelling, induced by exposure of enterocytes to hyposmotic mucosal solutions, also activated an apical membrane Cl\(^{-}\) conductance (51).

It is probably safe to suggest that these volume-activated Cl\(^{-}\) channels might play some role in the regulatory volume decrease that follows cell swelling. Although it is tempting to speculate beyond this assertion, at present there are insufficient data to support general or specific conjecture about the biological function of the channels. An unequivocal determination of their location would be of considerable help.

### OTHER Cl\(^{-}\) CHANNELS

In the intestinal epithelial cell line HT-29 clone 19A, a Cl\(^{-}\) channel has been described that is activated by addition of guanosine 5'-O-(3-thiotriphosphate) (GTP$\gamma$S, 10 μM) to the internal surface of excised, inside-out membrane patches (113). The I-V relationship was inwardly
rectifying in the presence of symmetrical Cl\textsuperscript{-} concentrations and the single-channel conductance was ~20 pS. The permeability to I\textsuperscript{-} was greater than to Cl\textsuperscript{-}. A GTP\textgamma{}S-activated Cl\textsuperscript{-} permeability was also found in vesicles prepared from the apical membrane of HT-29 monolayers, suggesting that the channel may be present in the apical membrane of the native intestinal epithelium. It will be interesting to learn whether such channels contribute to the Cl\textsuperscript{-} secretion associated with cholera. In Necturus gastric oxyntic cells a cAMP-activated Cl\textsuperscript{-} channel with similar biophysical properties has been reported (37).

Other anion-selective channels have also been reported in airway epithelia, but their physiological significance is uncertain. An anion channel of ~20 pS has been observed in isolated canine (104) and human (43, 44, 47) airway epithelia. There have been conflicting reports about its Ca\textsuperscript{2+} dependence and the effects of voltage on \( P_\text{o} \). There are suggestions that it may be defectively regulated in CF cells. A large-conductance (250–400 pS), poorly anion selective channel has also been reported in canine (104) and human (43) airway epithelia and mouse pulmonary alveolar type II cells (72). In transformed renal epithelial cells a similar channel has been recently shown to be regulated by reagents that affect the cytoskeleton, suggesting a possible role in cell volume regulation (107).

The planar lipid bilayer technique has also been used to study epithelial Cl\textsuperscript{-} channels. Cl\textsuperscript{-} channels reconstituted from bovine airway epithelium, including one activated by PKA, have large single-channel conductances (75, 120). In intestinal membrane vesicles outwardly rectifying Cl\textsuperscript{-} channels have been reported (21, 92). Because the properties of these Cl\textsuperscript{-} channels are different from those of Cl\textsuperscript{1+}R studied using the planar lipid bilayer patch-clamp technique, it seems unlikely that they are the CFTR Cl\textsuperscript{-} channel.

**DEPOLARIZATION-ACTIVATED, OUTWARDLY RECTIFYING CL\textsuperscript{-} CHANNELS**

**Properties of the channel.** Early patch-clamp studies on secretory epithelial cells identified a Cl\textsuperscript{-} channel with a characteristic outwardly rectifying \( I-V \) relationship in excised membrane patches bathed by symmetrical Cl\textsuperscript{-} solutions. The slope conductance measured at 0 mV was in the range of 25–50 pS. This channel has been identified in many epithelia and, interestingly, it has also been found in nonepithelial tissues, including fibroblasts and lymphocytes (10, 24). Its biophysical properties have been characterized in detail (see Refs. 128 and 46 for review). The channel was more permeable to \( \text{Cl}\textsuperscript{-} \) than to Cl\textsuperscript{-} and was inhibited by DIDS, DPC, and a large number of newly developed blockers (105, 116, 129). The channel was also inhibited by N-2-hydroxyethylpiperazine-N\textsuperscript{-} 2-ethanesulfonic acid (HEPES) and related biological buffers (62); such inhibition may contribute, in part, to the outward rectification of the \( I-V \) relationship. Variable effects of membrane potential on channel \( P_\text{o} \) have been reported; \( P_\text{o} \) has been shown by some to increase at moderately depolarized potentials (in the range of +50 mV) compared with equivalent hyperpolarized potentials (59, 83). However, others have reported that it is independent of membrane potential (48) or that \( P_\text{o} \) decreases with large depolarization (61, 84).

Early studies of the outwardly rectifying Cl\textsuperscript{-} channel indicated that it can be activated by cAMP agonists in cell-attached membrane patches (47, 59, 61, 63, 73, 125, 126) or by the catalytic subunit of PKA or PKC in excised, inside-out patches (36, 66, 68, 79, 101) from airway or intestinal epithelia. Moreover, such regulation was reported to be defective in CF, which in turn focused a great deal of attention on the channel. Regulation of this channel by PKA has not, however, been consistent nor always reproducible (48, 110, 135) and at least in the cell-attached mode appeared to occur in only a small percentage of patches (125, 135).

A curious and distinguishing feature of the channel was that, in excised membrane patches, the channel could often be activated by sustained strong membrane depolarization (79, 101, 110, 129); sustained strong hyperpolarization could then inactivate it. Several reports indicate that the channel is also activated by membrane patch excision at elevated temperatures (37°C vs. 20–23°C) (73, 129), by application of trypsin to the internal membrane surface (129), by bathing the internal surface with solutions containing high salt concentrations or an increased pH (110), and by the electrolyte composition of the solution (110). It was proposed that in the intact cell the channel was tonically inhibited and that patch excision combined with the various interventions released inhibition. This speculation prompted a search for a cytosolic inhibitor of Cl\textsuperscript{-} channel activity, and two groups (71, 74) have identified putative inhibitors. In addition, arachidonic acid (5) and protein kinase C in the presence of elevated Ca\textsuperscript{2+} concentrations and ATP (78) inhibited outward rectifiers in excised inside-out patches.

One common feature of several interventions that activate outwardly rectifying Cl\textsuperscript{-} channels is that they would be predicted to destabilize membrane proteins (110, 129). Although most of the interventions have not been tested in the cell-attached patch-clamp configuration, two of them have. Of these, strong membrane depolarization fails to activate the outward rectifier (59, 129), and cAMP agonists appear to activate these channels in only a low percentage of patches (125, 135). These observations led Wine and his colleagues (135) to question whether the regulation of this channel in excised membrane patches really reflects its modulation in intact cells. For the same reasons Tabcharani and Hanrahan (110) suggested that many different interventions, including the addition of kinase, might activate the channel in excised patches not through phosphorylation but rather by somehow altering the stability of the channel.

**Questions about the channel.** These observations raise several questions about the depolarization-activated outwardly rectifying Cl\textsuperscript{-} channel. Is the CFTR Cl\textsuperscript{-} channel the same as the depolarization-activated, outwardly rectifying Cl\textsuperscript{-} channel? We think not. Although the possibility that the two would be identical was an attractive hypothesis at one point, their very different biophysical and regulatory properties seem to preclude that possibility. Could the outwardly rectifying Cl\textsuperscript{-} channel be a CFTR Cl\textsuperscript{-} that has in some way been
modified or altered? That seems very unlikely, based on the striking divergence of their properties, the lack of any precedent for such a dramatic change in other channels, and the lack of correlation between the presence of CFTR mRNA and the expression of depolarization-activated, outwardly rectifying Cl− channels in a variety of cell types (124).

What then is the physiological function of the depolarization-activated, outwardly rectifying Cl− channel? There are several points to consider. First, because the properties are different from those of the cAMP- and Ca2+-activated apical membrane Cl− currents, we conclude that it is not directly involved in transepithelial Cl− secretion stimulated by these second messengers.

Second, because it shares many properties with volume-activated Cl− channels, the two may be one and the same. Despite these similarities, Solec and Wine (106) have shown that the volume-activated Cl− channels display less rectification, a higher P0 at negative voltages, a greater degree of run-down, a longer mean open duration, and less open-channel noise at positive voltages than outwardly rectifying Cl− channels. Whether these differences in kinetics result from different modes of activation of the same channel or from a completely different channel will probably not be resolved until the channel(s) are cloned.

Third, the location of the channel in polarized epithelium is not known; such knowledge is essential for any attempt to decipher its physiological function in epithelia. A patch-clamp study of the apical membrane of surface cells from native rat colonic epithelium reported the presence of outwardly rectifying Cl− channels (40). However, the channel was only observed in excised patches, and it is not clear whether the cells were Cl−-secreting cells. We also considered the possibility that the channel may not be expressed in epithelial cells once they polarize and differentiate (see the caveats discussed above for Ca2+-activated Cl− channels). Furthermore, the channel may not have a function unique to epithelia; such speculation might explain the fact that the channel is observed in a wide variety of nonepithelial cells.

What accounts for the reports of defective regulation of the depolarization-activated, outwardly rectifying Cl− channels in CF? We do not know. Based on our current level of understanding of the CFTR Cl− channel and the uncertainties about the function of the outwardly rectifying Cl− channel, such a defect is difficult to explain (see also Ref. 108). The problem is perhaps similar to attempts to understand how a defect in the CFTR Cl− channel produces defective regulation of apical Na+ channels and other phenotypic abnormalities in CF. In this regard it is interesting to recall that some forms of myotonia are caused by a defect in a single gene encoding a muscle Cl− channel, yet there are several other abnormalities in the membrane of affected cells (108).

CONCLUSION

The study of ion channels has been revolutionized by the development of the patch-clamp technique (60). However, epithelial cells have the unique property of being polar, with clearly defined apical and basolateral membranes, each with its own complement of channels, transporters, pumps, and regulatory systems. This property adds an additional complexity to the study of the ion channels that mediate vectorial fluid and electrolyte transport in epithelia. In such studies it is important to remember that changes in the culture conditions, which are often required for successful patch-clamping endeavors, may result in the disappearance of relevant channels or the appearance of irrelevant channels. Fortunately, the cAMP-regulated Cl− channel that is defective in CF can be found under a variety of culture conditions and displays unique properties that distinguish it from other epithelial Cl− channels. Much of this realization resulted from the cloning of CFTR. It may take a similar combination of approaches using recombinant DNA and cellular electrophysiological techniques to resolve the identity and function of other channels involved in epithelial function.

We thank our laboratory colleagues and collaborators for their help, suggestions, and criticism. We thank Dr. Jeffrey J. Wine for discussions and for reviewing the manuscript and Theresa Mayhew for typing the manuscript.

Work from the author’s laboratory was supported in part by the Howard Hughes Medical Institute, the National Heart, Lung, and Blood Institute, and the National Cystic Fibrosis Foundation.

Address for reprint requests: M. J. Welsh, 500 EMRB, Dept. of Internal Medicine, Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City, IA 52242.

REFERENCES


