12 Ion Channels in Epithelial Cells

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12.1 Ion Channels and Epithelial Function

Ion channels in epithelial cells serve to move ions, and in some cases fluid, between compartments of the body. This function of the transfer of *material* is fundamentally different from that of the transfer of *information*, which is the main job of most channels in excitable cells. Nevertheless the basic construction of the channels is similar in many respects in the two tissue types. This chapter reviews the nature of channels in epithelia and discusses how their functions have evolved to accomplish the basic tasks for which they are responsible. I will focus on three channel types: epithelial Na^+ channels, inward-rectifier K^+ channels, and CFTR Cl^- channels.

To appreciate the biological roles of these channels, it is necessary to consider the basic structure of an epithelial cell. These cells separate the major body fluid compartments from those which are essentially in contact with the outside world, including those fluids of the urinary tract, GI tract, and sweat. In the lung, epithelial cells separate the body from an air space, with a thin layer of fluid in between. All epithelia form layers of cells that are joined by so-called tight junctions. These junctions consist of an extracellular caulking material that helps to seal the layer and prevent material from leaking between the cells. The cells therefore have two very distinct membrane surfaces: one in contact with the internal body compartments (i.e., the interstitial fluid) and the other in contact with the external fluids. These are called the basolateral and apical membranes, respectively. A basic tenet of epithelial biology, originally emphasized by Ussing and colleagues (Koefoed-Johnsen and Ussing, 1958), is that the transport properties of these two membranes are very different. It turns out that many of the channels and other transport proteins that are specialized to epithelial cells reside in the apical cell membrane in contact with the urine, the intestinal contents, etc. while the transporters that are shared with other cells in the body are often expressed in the basolateral membranes.

Epithelial Na^+ channels reside in the apical membranes of epithelia in the kidney (mainly in the so-called distal nephron segments), the colon, the sweat and salivary ducts, and the lung. Their main job is to *reabsorb* Na^+ ions from the fluid within these organs. This term is used to denote the fact that most of the Na^+ in these fluids originates within the body and is either filtered into the urine or secreted by glands into the GI tract, sweat or saliva. Thus their importance is in the conservation of Na^+. (In one special but historically important case, the skin of

frogs, toads, and other amphibians, the channels may actually be used to obtain Na^+ from the environment.) This conservation process is critical, as in its absence the body would become depleted of Na^+ and of fluid, especially from the plasma and other extracellular compartments.

The basic arrangement is shown in Fig. 12.1A, which is a modern representation of the model of Koefoed-Johnsen and Ussing (1958). Na^+ ions enter the cells through the channels by the process of electrodiffusion. This movement is driven by a concentration difference for Na^+, which is lower within the cytoplasm than in the extracellular fluids, and by the electrical potential across the membrane, which is generally more negative within the cell. The membrane on the other side of the cell contains a "pump" which drives Na^+ out of the cell (toward a higher electrochemical potential) using ATP as an energy source. The net result is a transfer of Na^+ across the cell layer from outside to inside the body. The power underlying this process comes from the ATP-driven pump (or ATPase). It turns out, however, that the rate at which Na^+ is transported is determined, and regulated, by the channels. One other essential element in this system is the set of K^+ channels that operates in parallel with the pump in the basolateral membrane. These K^+ channels have two important functions with respect to epithelial Na^+ transport. First, they recycle the K^+ that enters the cells as part of the pump process as Na^+ is moved out. Furthermore, this conductance to K^+, together with the high concentration of K^+ inside the cells and the low concentration in the extracellular fluids, establishes the negative electrical potential within the cell. This provides part of the driving force for Na^+ to enter through the apical channels. As suggested above, neither the pump nor the K^+ channels in the basolateral membrane are special to epithelial cells; they are found in nearly every cell in the body and in each case their fundamental job is to keep intracellular Na^+ concentration low and the resting cell membrane voltage negative. In contrast, the Na^+ channels are found almost exclusively in epithelia and are always located in the apical membranes. This mechanism is predicated on the segregation of transport functions, with the channels on one side of the cell and the pumps on the other side. How these membrane proteins are sorted by the cell's biosynthetic machinery and maintained in different places within the cell is an important problem in epithelial biology that has not been completely solved (Caplan, 1997; Mostov et al., 2000; Campo et al., 2005). A discussion of this issue is beyond the scope of this chapter.

The net reabsorption of Na^+ shown in Fig. 12.1A would by itself lead to a separation of charge and the development of a large voltage difference that in turn would halt the process rather quickly. For Na^+ transport to continue the charge translocation must be balanced either by the movement of an anion (usually Cl^-) in the same direction or of a cation (usually K^+) in the opposite direction. To some extent this movement takes place between the cells, through the intercellular caulking which is not absolutely tight. This so-called paracellular transport is not very selective among small ions. There are, however, specific processes, some involving other ion channels, which mediate the transepithelial transport of both Cl^- and of K^+.

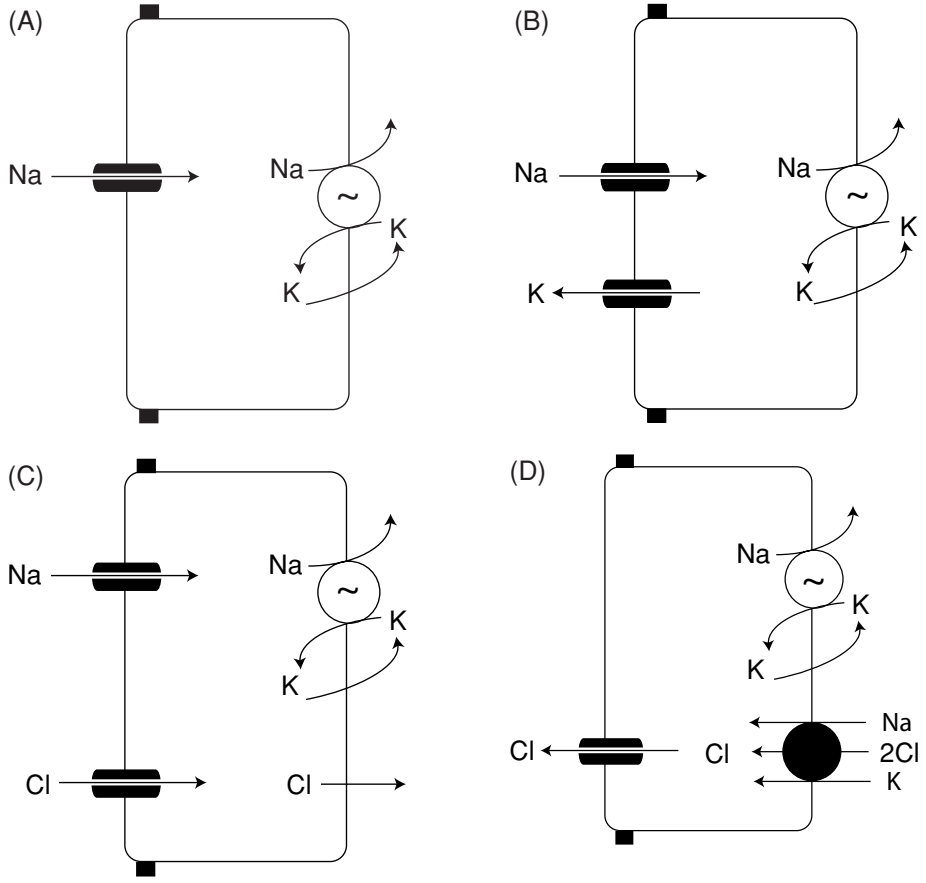

Fig. 12.1 Arrangement of ion channels and other transporters in epithelia. (A) Na^+-reabsorbing epithelium. Na^+ channels are expressed exclusively on the apical plasma membrane, which faces the urine, lumen of the gut etc., allowing Na^+ ions to enter the cell from the urine, the feces, or the ductal lumens. On the other side of the cell, called the basolateral membrane, a Na/K-ATPase or Na pump uses the energy of ATP to extrude Na^+ from the cell against unfavorable electrical and concentration differences. This completes the transport process and also keeps intracellular Na^+ concentrations low, maintaining the driving force for Na^+ to enter the cell through the channels. (B) K^+-secreting epithelia. The additional feature is a K^+-selective channel in the apical membrane, that allows the K^+ that enters the cell through the Na/K pump to exit the cell into the urine or the intestinal lumen. Na^+ and K^+ movements are electrically coupled since the movement of one creates a transmembrane voltage that increases the driving force on the other. (C) Cl^--reabsorbing epithelium. Here, Cl^- enters the cells in parallel with the movement of Na^+ through a separate channel. The Cl^- that enters the cells in this way leaves the cells across the basolateral membrane, either through another set of channels or in some cases through a cotransporter along with K^+. As in (B), the movements of Na^+ and Cl^- across the apical membrane are electrically coupled. (D) Cl^--secreting epithelium. The arrangement is very similar to the Cl^--absorbing cells in C, with two important differences. First, Na channels are absent. Second, a cotransporter that couples the inward movement of Na^+, K^+, and Cl^- is present on the basolateral membrane. These features combine to reverse the driving force on the Cl^- ion such that it moves out of the cells through the channels.

The generic case of K^+ transport is illustrated in Fig. 12.1B. Here, a single pathway involving an apically located K^+ channel has been added to the cartoon of the cell. Now some of the K^+ that enters the cells through the Na/K pump leaves across the apical membrane, resulting in the net *secretion* of the ion. This movement of K^+ can itself be desirable, since our diets are often rich in K^+ and the kidneys need to eliminate what is not required for replenishment or growth. In addition, as mentioned above, this transport helps to neutralize the charge that is moved when Na^+ is reabsorbed; transepithelial K^+ secretion stimulates the rate of Na^+ reabsorption and vice versa. This electrical coupling takes place to a large extent across the apical cell membrane. Efflux of K from the cell hyperpolarizes the membrane potential (makes it more negative), increasing the driving force for Na^+ entry into the cell.

As shown in Fig. 12.1C, addition of a channel for Cl^- movement across the apical cell membrane will also help to neutralize the charge movement associated with Na^+ transport. When combined with a mechanism for getting the Cl^- out of the cell across the basolateral membrane this will result in the reabsorption of NaCl. This efflux of Cl^- may be through channels or by cotransport with K^+. The net reabsorption of NaCl will decrease the osmolarity of the external fluid (e.g., the sweat). In epithelia which are permeable to water it results in the reabsorption of fluid along with the ions. As in the case for K^+ discussed above, the movements of Na^+ and of Cl^- across the epithelium as a whole, or across the apical membrane in particular, are electrically coupled. Increases in the flow of either ion will enhance the driving force for the other by changing the membrane potential.

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A slightly different arrangement of these transporters can result in the secretion of Cl^- and as a result of Na^+ and fluid. This is illustrated in Fig. 12.1D. Here, the apical Cl^- channel remains but the Na^+ channel has disappeared. In addition, a new transporter appears on the basolateral membrane that carries Na^+, K^+, and Cl^- into the cell at the same time. The direct coupling of the movement of Na^+ and Cl^- in this system is key, since it uses the forces pulling Na^+ into the cell to drive the accumulation of Cl^-. Now, in contrast to the case of Fig. 12.1C, the electrochemical activity of Cl^- is higher in the cell than it is in the external fluid, and the anion moves out of the cells through the channels. The charge movement is balanced by that of Na^+, probably moving between the cells. Thus the same apical Cl^- channels (CFTR, as we shall see) can mediate either the reabsorption or the secretion of NaCl and water. In some sea-dwelling animals this mechanism serves to rid the body of excess salt. Two examples are the rectal gland of the shark and the nasal gland of duck. In humans and other terrestrial vertebrates, this same mechanism is used to provide fluid for many glandular secretions including those of the pancreas and the salivary, sweat and tear (lachrymal) glands. It also governs the secretion of fluid by the intestine, which can provide lubrication for the digestive process under normal conditions but which leads to excess loss of fluids in some forms of diarrhea, especially those caused by cholera and other infectious agents.

12.2 Structural and Evolution of Epithelial Channels

The major ion channels that participate in the transport schemes, shown in Fig. 12.1, have been identified at the molecular level. They all belong to larger families of genes and proteins but in some, cases have evolved into more specialized functions.

12.2.1 Epithelial Na^+ Channels (ENaC)

The proteins comprising the epithelial Na channel were identified using an expression cloning approach (Canessa et al., 1993, 1994). There are three subunits, termed α, β, and γ ENaC (for *E*pithelial *Na C*hannel), all of which are necessary to reconstitute maximal activity in frog oocytes and other heterologous expression systems. Function is determined electrophysiologically as current or conductance that can be blocked by amiloride, a diuretic drug, which inhibits native channels with a K_d of around 100 nM. Only αENaC can produce channel activity by itself. However, all three subunits are homologous with each other and all three have the same predicted membrane topology, consisting of two predicted transmembrane helices, short cytoplasmic N- and C-termini and a large cysteine-rich extracellular domain (Fig. 12.2A). This suggests that the three subunits contribute in similar ways to the structure of the channel. The simplest models consist of a central pore surrounded by a pseudo-symmetrical arrangement of subunits. The nicotinic acetylcholine receptor provides a precedent for this structure, as it also consists of different but homologous membrane-spanning proteins constituting a single ion-conducting pore (Karlin, 2002). In addition, mutations in corresponding residues in the three ENaC subunits in some cases show some similar effects on ion conduction and block (Schild et al., 1997; Sheng et al., 2000). The stoichiometry of the channel is still a matter of debate. The most widely accepted structure, based on several experimental approaches, consists of 2α, 1β, and 1γ forming the holochannel (Firsov et al., 1998). However, a similar study suggested a larger complex with nine subunits and a 3:3:3 stoichiometry (Snyder et al., 1998). More recent evidence using fluorescence energy transfer is also consistent with a channel's having at least two of each subunit type (Staruschenko et al., 2005).

The ENaC proteins belong to a superfamily that includes channels which respond to mechanical and chemical stimuli. *Mec* and *deg* gene products were identified in *C. elegans* using screens of mutated worms having defects in their responses to touch (Chalfie, 1997). These proteins form channels when expressed in oocytes (O'Hagan et al., 2005). It is likely that in vivo they are sensory channels which open in response to a mechanical force, depolarizing neurons to initiate mechanosensory reflexes. The acid-sensing ion channels (ASICs) form another family of proteins with homology to ENaC (Waldmann et al., 1997) and are also considered to be sensory channels. They are expressed in neurons and are activated at low pH, presumably as a response to a noxious stimulus. FMRFamide-activated channels also belong to

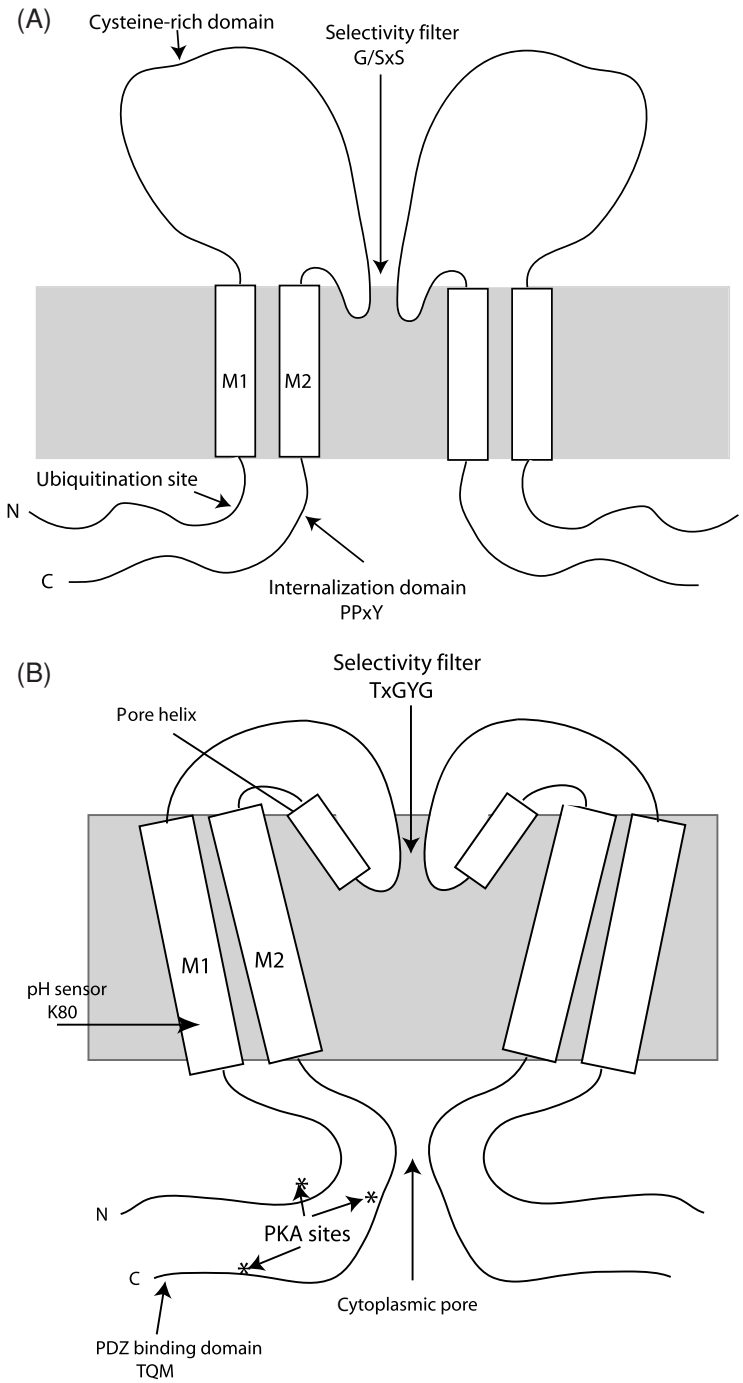

Fig. 12.2 Schematic drawings of the structures of ENaC (A), ROMK (B), and CFTR (C).

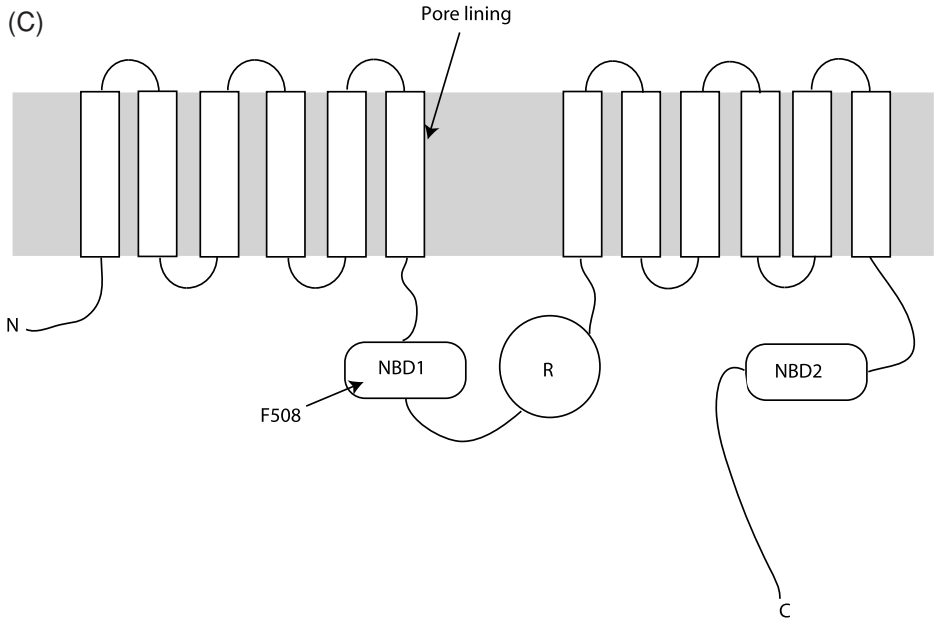

Fig. 12.2 (*continued*)

this superfamily. They are found in invertebrates such as snails and respond to low concentrations of peptides (Lingueglia et al., 1995). All of these channels appeared rather late in evolution; no bacterial orthologs have been identified. The sensory channels seem to be more widespread among the animal phyla than the ENaCs. It is possible that the latter were adapted from the former as the need for salt conservation and epithelial Na^+ transport arose. This might explain the large extracellular domains of the ENaC subunits, which have no clear role in epithelial transport but could be involved in the original function of sensing the external environment.

12.2.2 Epithelial K Channels (Kir1.1 = ROMK)

The K^+ channels residing in the apical membrane of renal epithelia (Fig. 12.1B) are part of a large superfamily that arose in bacteria. The function of these channels in bacteria remains obscure. However, in animals they have evolved into families that include voltage-gated, inward rectifier, and two-pore (background) channels. Each of these families contains a number of even more specialized channels which serve a huge range of physiological functions. All of the K^+ channels contain a signature sequence T-x-G-Y-G that forms the narrowest part of the conducting pore and that is responsible for establishing a high selectivity for K^+ over Na^+. The structure of several of these channels has been solved by crystallization and X-ray diffraction (Schild et al., 1997; Doyle et al., 1998; Sheng et al., 2000; Jiang et al., 2002; Kuo et al., 2003). These crystals clearly demonstrate the expected quaternary structure

of the proteins, with four usually identical subunits arranged around a central pore. They also indicate the selectivity filter near the interface of the membrane with the extracellular solution and a relatively large cavity in the center of the membrane. Both of these features had been predicted from hydrophobicity analysis and mutagenesis experiments.

Within this large group, the renal K^+ channels ROMK (for *R*enal *O*uter *M*edullary *K* channels) belong to the inward-rectifier (Kir) family of channels. In fact, they were the first of this family to be cloned (Ho et al., 1993) and are designated as Kir1.1. Other prominent members of this family include the anomalous rectifiers that set the resting membrane potential of skeletal muscle (Kir2), G-protein coupled channels that regulate the heart beat (Kir3) and ATP-sensitive channels that control insulin secretion by the pancreas in response to changes in plasma glucose concentrations (Bichet et al., 2003). Bacteria also have channels from this family, and a structure has been obtained for one of them (KirBac1.1) (Kuo et al., 2003). The basic selectivity filter and membrane cavity arrangement is similar to that of other K^+ channels. Each subunit contains two transmembrane helices, with a short extracellular loop containing the selectivity filter in between (Fig. 12.2B). The membrane cavity is formed mainly by the C-terminal helices. Two additional notable features of the channels are a cytoplasmic domain, formed by the N- and C-termini, that appears to form an extension of the transmembrane pore, and an additional helix on the N-terminus that lies at the interface between the membrane and the cytoplasm. The cytoplasmic portions of the channel proteins are involved in the regulation of channel function (see below).

Some Kir channels contain accessory subunits that may be required for proper activity. The best-studied case is that of Kir6. These form complexes with another set of transmembrane proteins, called sulfonylurea-binding proteins (SURs), named for their interactions with a class of antidiabetic agents (Inagaki et al., 1996). In this case the SURs are clearly necessary for full channel expression as well as for metabolic regulation by ADP. In the case of the renal K^+ channel, the Kir1.1 subunit appears to be sufficient to form a functional channel. However, there is evidence for interactions with SUR2b (Tanemoto et al., 2000) and with the SUR-related protein CFTR (McNicholas et al., 1996). It is not clear whether such interactions are important in the function of the channels in the kidney.

12.2.3 Epithelial Cl Channels (CFTR)

The most prominent of the apical Cl^- channels in epithelia is CFTR, the *c*ystic-*f*ibrosis *t*ransmembrane *c*onductance *r*egulator. This protein was identified as the site of mutations causing cystic fibrosis, a disease in which both the secretion and the absorption of fluids by epithelia can be defective (Riordan et al., 1989). CFTR is a larger and more complex protein than those formed by ENaC or Kir1.1 (Sheppard and Welsh, 1999). It is thought to have 12 membrane-spanning helices arranged in two domains. Each of these domains contains six helices and ends in a cytoplasmic region containing a nucleotide-binding domain (NBD) that interacts with ATP (Fig. 12.2C).

These features are shared by the family of ABC (ATP-binding cassette) transporters to which CFTR belongs. Curiously, CFTR is the only family member which clearly functions as an ion channel. The others generally carry out ATP-dependent active transport of nutrients and metabolites in bacteria, of a mating factor in yeast, and of xenobiotics, notably chemotherapeutic agents, in eukaryotic cells. CFTR has one structural feature unique to this family—a regulatory domain in the cytoplasm between the two sets of membrane-spanning helices. This is a site of phosphorylation by protein kinases that play an important role in the regulation of channel function.

The structure of the conducting pore in CFTR is unknown. The sixth transmembrane helix of the protein contains several positive charges which influence ion conduction, selectivity, and block. This segment very likely forms part of the lining of the pore. However, mutations in other transmembrane helices also have effects, and these may also contribute to the formation of the conduction pathway (Dawson et al., 1999; Sheppard and Welsh, 1999). The precise arrangement of the helices with respect to the pore is unknown. As with ENaC, the number of subunits required to form a functional channel is controversial. Evidence has been presented suggesting that a dimer is required (Zerhusen et al., 1999), but more recent data indicate that a single peptide can form a pore (Liu et al., 2004).

12.3 Functional Specializations of Epithelial Ion Channels

Epithelial ion channels are designed to transport solutes continuously over long periods of time. In contrast to many channels in excitable cells, they do not respond strongly to changes in membrane potential. Rather, they spend a significant fraction of time in the open state at all voltages. This makes a good deal of sense with respect to their physiological functions. One might also expect the conductances of individual channels to be relatively large, so as to minimize the number of proteins necessary to carry out these functions. This, however, turns out not to be the case, as the single-channel conductance of the epithelial ion channels discussed here tend to be at the lower end of the spectrum, which generally ranges from around 1 pS to several hundred pS for individual conducting units. In the section below I will review some of the basic permeation and selectivity properties of epithelial channels.

12.3.1 ENaC

ENaC channels are characterized by a single-channel conductance of about 5 pS when Na^+ is conducted at room temperature [see (Garty and Palmer, 1997) for a comprehensive review]. This is a small conductance even compared with voltage-gated Na^+ channels, which have around 15–20 pS unit conductance under similar conditions. At 37°C, the ENaC conductance increases to about 9 pS. One possible evolutionary advantage of the low conductance may be in conferring a high selectivity to the channels. They are almost perfectly selective for Na^+ over K^+; one estimate

was around 1000:1 compared to about 10:1 for voltage-gated Na^+ channels. Indeed, the only ions that have a measurable conductance through the channels are Na^+, Li^+, and under extreme conditions H^+. Based on these results, it has been proposed that ions may be almost completely dehydrated as they go through the channels, which could then have a selectivity filter that discriminates on the basis of the nonhydrated radius of the ion (Palmer, 1987; Kellenberger et al., 1999). This selectivity is clearly an important aspect of epithelial function. As can be appreciated in Fig. 12.1, any permeability or conductance of K^+ through the Na^+ channels will result in K^+ secretion and loss of K^+ from the body. Although as discussed above this is often desirable, the kidney and other epithelia need to be able to keep track of body Na and K contents individually and regulate these levels separately. Having such a narrow constriction and high selectivity may limit the rate at which ions can pass through the channel. Although, as discussed below, there is no clear correspondence between conductance and selectivity in K^+ channels, this may be the most efficient way to create a high level of discrimination for Na^+ over K^+. Na^+ passes through the channels with nearly equal ease in both directions. Current–voltage relationships under physiological conditions exhibit "Goldman" type rectification that reflects differences in the concentrations of permeant ions on the two sides of the membrane. With symmetrical concentrations, I–V relationships are approximately linear.

Like most channels, ENaC channels make abrupt transitions between open and closed states. The lifetimes of these states ~~is~~ are rather long—several seconds at room temperature—and to a first approximation insensitive to voltage. These kinetics, and indeed the open probability of the channels—are highly variable. In one study, P_o ranged from <0.05 to >0.95 under nominally the same experimental conditions (Palmer and Frindt, 1996). The reasons for this variability are not entirely clear but it suggests that the channels may be regulated through changes in P_o. As described in the next section, there is growing evidence that the major regulation of ENaC is through trafficking of the protein to and from the surface. Nevertheless several conditions modulate channel activity through alterations in P_o and gating kinetics. Conditions leading to increased P_o include strong hyperpolarization of the membrane, mechanical stress on the membrane, reduction of either extracellular or intracellular Na^+, and an increase in cytoplasmic pH (Garty and Palmer, 1997). The physical basis for the gating is unknown.

12.3.2 Kir1.1/ROMK

The unitary conductance of Kir1.1 channels is substantially higher than that of ENaC; with K^+ as the conducted ion the inward conductance is about 35 pS at room temperature and 45–50 pS at 37°C [see (Hebert et al., 2005) for a comprehensive review]. Furthermore, although again the channel's cycle between open and closed states, their open probability is quite high—around 0.9 independent of voltage. These two features seem well adapted to the physiological role of the channels. However, two aspects of the biophysical characteristics of the channels seem poorly suited to

their function. First, the conductance is considerably smaller than that seen for other K^+ channels, which can reach values as high as 200 pS for the Ca-activated maxi-K or BK channels. These high conductances can be achieved without any sacrifice in ion selectivity; a very high preference for K^+ over Na^+ is observed in all channels with the TxGYG signature sequence, regardless of the unitary conductance. The lower conductance of the Kir1.1 channels means that at least four times as many channels must be manufactured to carry out the function of K^+ secretion than would be necessary if channels of maximum conductance were employed. Even stranger, the conductance in the physiological direction out of the cell is substantially smaller than that for inward flow of ions. This is a general characteristic of the inward rectifier channel family and is responsible for the family name. The rectification arises from a voltage-dependent block of the channels by intracellular multivalent cations including Mg^{2+} and polyamines such as spermine and spermidine (Lopatin et al., 1995). Inward rectification is much more pronounced in many of the family members, particularly in the Kir2 and Kir3 types. These channels are expressed in skeletal and cardiac muscle fibers and help make the membrane potential bi-stable. That is, in the absence of a strong depolarizing stimulus the channels are open and the resting membrane potentials are strongly negative. When a sufficient stimulus arrives and the membrane depolarizes past its threshold, the K^+ channels shut off, allowing the action potential to proceed. Kir1 channels do not need to function this way. Indeed their role is to allow a steady-state diffusion of K^+ out of the cells. In fact, their outward conductance is considerably higher than in its Kir2 and Kir3 cousins. However, it is still 2- to 3-fold lower than the inward conductance, furthering the requirement for a larger number of channels. In this sense the inward rectifier family seems like a curious choice for the selection of a channel designed for epithelial K^+ secretion.

As discussed above, inward rectifier K^+ channels share the same basic selectivity filter structure with the voltage-gated K^+ channels, and the ability of these channels to discriminate between K^+ and Na^+ is very large. Other ions which can pass through the pore are Rb^+, Tl^+, and NH_4^+ (Choe et al., 1998). Of these, only NH_4^+ is of physiological importance. The physical basis for this selectivity is the precise arrangement of carbonyl oxygen atoms within the selectivity filter (Zhou et al., 2001; Noskov et al., 2004). A K^+ ion can be surrounded by a cage of up to 8 of these oxygens, effectively replacing the hydration shell that stabilizes the ions in bulk solution. For Na^+ ions, which are smaller but which form larger hydration spheres in water, this arrangement of water-replacing contacts is not as effective and the ions are excluded from the filter on energetic grounds.

In addition to the narrow selectivity filter at the outer mouth of the pore and a relatively large cavity spanning the lipid bilayer, K_{ir} channels feature an extension of the pore deep into the cytoplasm. This "cytoplasmic pore" (Kuo et al., 2003) contains negatively charged amino acids that are important for binding impermeant cations such as polyamines and which help to confer the property of inward rectification. This pore is fairly narrow in places may also comprise a significant fraction of the total electrical resistance of the channel (Zhang et al., 2004).

Although Kir1 channels spend most of their time in the open state, they briefly visit a closed state that has a mean lifetime of around 1 ms (Choe et al., 1999). The physical basis of these transitions is unknown. However, they may represent fluctuations in the protein components surrounding the permeation pathway, as they are influenced both by the nature of the conducted ion and by the rate at which ions are passing through the pore. In addition to these transitions, the channels can undergo closures to a state that is relatively long-lived (lasting for many seconds or minutes). Such closures can be elicited, for example, by lowering the pH of the cytoplasm below 7.0. The physiological importance of this pH-dependent gating remains obscure, although it probably underlies the well-described phenomenon of K^+ retention by the kidneys during acidosis. However, it has parallels with other inward-rectifier K^+ channels. In Kir6.2 channels, for example, channels are shut down by high concentrations of ATP (or a high ATP/ADP ratio) (Enkvetchakul et al., 2000). This regulation ultimately couples the secretion of insulin by pancreatic beta cells in response to high levels of plasma glucose. Kir3 or GIRK channels are constitutively in a long-lived closed state but can be activated by the binding of the beta/gamma subunits of heterotrimeric G proteins (Sui et al., 1999). This regulation also has clear physiological significance in the process of slowing the heart rate by the parasympathetic nervous system.

12.3.3 CFTR

CFTR also forms pores with a low single-channel conductance—5 to 10 pS at room temperature with Cl^- as the conducted ion [see (Dawson et al., 1999; Sheppard and Welsh, 1999) for more comprehensive reviews]. Like the Na^+ channels, currents through open CFTR channels are approximately linear under symmetrical ion conditions, and in the presence of ion gradients across the membrane the I–V relationships show Goldman-type rectification. In addition, the open probability of the channels is not markedly dependent on voltage. Therefore, like the ENaC channels, the major effect of transmembrane voltage is to establish the driving force for ion movement. In the case of CFTR, this is an important property since, as discussed above, the direction of ion movement can be either inward or outward depending on the cell type and the physiological conditions.

CFTR is highly selective for negatively charged ions, but does permit the permeation of a wide variety of anions. Analysis of a wide range of anions showed that permeabilities followed the lyotropic series, suggesting that the basis for discrimination among them depends on the relative energies of dehydration and binding to sites within the conduction pathway (Smith et al., 1999). The size of the ion is much less important in these channels, at least up to a point; anions with diameters of 5.5 Å or even higher can permeate. Positively charged residues on the transmembrane domains of CFTR, particularly the sixth domain, are important in the formation of the conduction pathway and the establishment of ion selectivity and binding affinity. The detailed structure of the pore has not been determined.

As with the other channels, CFTR makes abrupt transitions between open and closed states. These events involve processes that operate on at least three different structural levels and time scales. First, the channels must be phosphorylated to have any activity at all (Sheppard and Welsh, 1999). This process will be discussed in the next section. Second, the opening of the phosphorylated channels is coupled to the binding and hydrolysis of ATP (Vergani et al., 2003). Opening of the channels requires binding of ATP to both nucleotide-binding domains. The nucleotide bound to the second (C terminal) NBD can be hydrolyzed to ADP and Pi, and this results in channel closure. This ATP-driven cycling between open and closed configuration may reflect the evolution of the channel protein from energy-dependent pumps that can move solutes against a concentration gradient. In this case, conformational changes determine whether the channels are open or not, with the direction of movement of ions determined by differences in their electrochemical activities. Finally, channels that have been opened by phosphorylation and ATP binding flicker back and forth between relatively short-lived open and closed states, similar to the Kir channels described above. The physical basis for these transitions is unknown.

12.4 Regulation of Epithelial Ion Channels

All of the epithelial ion channels discussed in this chapter are highly regulated. These regulatory processes are not nearly as fast as those that govern the activities of voltage-gated channels, which can be turned on and off in a matter of milliseconds through rapid changes in protein conformation. In contrast, the activities of epithelial channels are usually modulated over time scales of minutes or hours. The regulatory events include both covalent modification of the channel protein (e.g., phosphorylation) and translocation of channels to and from the membrane.

12.4.1 ENaC

Epithelial Na channels are strongly regulated by hormones. The most important of these is aldosterone, a steroid secreted by the adrenal gland in response to a deficit in the amount of Na or in the volume of the extracellular fluids in the body. Like that of other steroid hormones, the actions of aldosterone require alterations in gene expression. As a consequence the effects depend on the transcription of new mRNA and the synthesis of new proteins, processes which usually take an hour or more before the eventually physiological effects can be effected. In the continued presence of hormone, the effects persist indefinitely. The short-term effects (hours) and the long-term effects (days) may be different (Verrey et al., 2000).

Aldosterone appears to stimulate channel activity at least in part by increasing the number of channel proteins in the membrane. The mechanisms involved are different in different tissues. In the colon, αENaC subunits are expressed constitutively, but the amounts of β and γ subunits depend on the hormonal status. When aldosterone levels are low as the result of a high salt intake or of adrenalectomy, β and

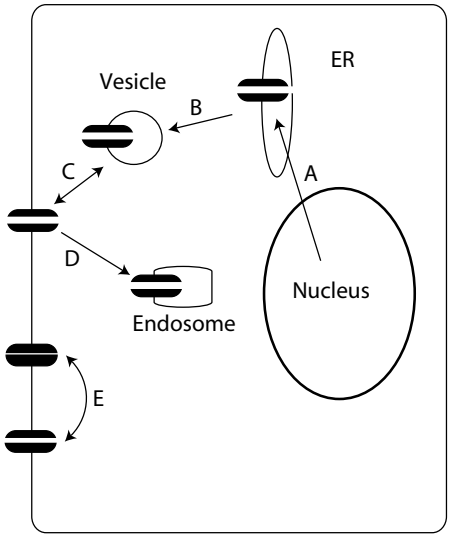

Fig. 12.3 Regulation of epithelial ion channels. (A) Regulation of the synthesis of the channels can occur by altering the rates of mRNA production (gene transcription) and/or protein translation. (B) Channel protein export from the endoplasmic reticulum may be regulated. (C) Channels may reside in submembrane vesicles and shuttled to and from the membrane by regulated exocytosis and endocytosis. (D) Channels in the membrane may be removed and degraded in endosomes or proteosomes through regulated processes. (E) Channels in the membrane may be activated by phosphorylation, membrane tension, or other chemical or mechanical processes.

γENaC are virtually undetectable. Aldosterone induces the transcription of mRNA encoding these subunits which in turn promotes the synthesis of new protein (Asher et al., 1996) (see Fig. 12.3, path A). These new subunits presumably combine with preexisting αENaC to form conducting channels in the apical plasma membrane. In the kidney, the same hormone (aldosterone) has the same ultimate effect (increase in ENaC protein in the apical membrane) but, remarkably, seems to act through different mechanisms. All three ENaC subunits are expressed constitutively in the kidney, but with low levels of hormone the protein appears to reside primarily in intracellular membranes (Masilamani et al., 1999; Loffing et al., 2000). These membrane sites have not been precisely identified, but they are distributed throughout the cytoplasm reminiscent of the distribution of the endoplasmic reticulum. A major effect of aldosterone is to induce the movement of the channels from these cytoplasmic stores to the apical membrane (Fig. 12.3, path B).

The turnover rate of ENaC in the plasma membrane is rapid; estimates of the half-life of channels at the surface of cultured epithelial cells range from around 20 minutes to about 2 hours (Weisz et al., 2000; Alvarez de la Rosa et al., 2002). Thus, an increase in the number of channels at the surface could result from either an increase in the rate of insertion into or a decrease in the rate of retrieval from the apical membrane. According to one hypothesis aldosterone induces the synthesis of a

protein kinase (SGK) which phosphorylates a ubiquitin ligase (Nedd4-2), reducing its ability to bind to and eventually internalize ENaC (Debonneville et al., 2001) (Fig. 12.3, path D). Evidence exists for all of these events, but the model has not been fully tested in the in vivo situation. No such detailed schemes have been proposed for what pathway is involved in trafficking of channels to the apical membrane, or whether the hormone might increase the rates of this movement. In addition, there is evidence that in some epithelia the hormone may also act on channels already residing in the membrane (Garty and Edelman, 1983; Kemendy and Eaton, 1990). This would result in an increase in the open probability of the channels. Such a mechanism, which may occur during short-term stimulation, could be combined with that of channel translocation to increase the range of channel activities that can be achieved.

Liddle's Syndrome is a rare form of hypertension that is transmitted as a dominant Mendelian genetic trait (Lifton et al., 2001). Its cause is a mutation in the C-terminal end of either the β or γ ENaC subunit which eliminates a putative internalization signal sequence (PPPxY). This sequence is thought to interact with Nedd4-2 (see above) which ubiquitinates the channel proteins and facilitates their removal from the membrane, possibly through the proteosome. Defects in this internalization process are expected to increase the residence time on the membrane and to promote the excess reabsorption of Na^+ (Fig. 12.3, path D). This accounts for the hypertension observed in these patients and further illustrates the importance of membrane trafficking events in the regulation of these channels.

In addition to aldosterone, Na^+ channels in some epithelia are activated by antidiuretic hormone, a peptide from the posterior pituitary gland that acts through increases in the intracellular concentration of cAMP. The physiological significance of this regulation is unclear, as the best-known function of ADH is to control water reabsorption by the kidney. ADH/cAMP likely also acts by stimulating the translocation of ENaC to the cell surface (Garty and Edelman, 1983; Morris and Schafer, 2002) (Fig. 12.3, path C) although again the mechanism is not understood. It is different from that of aldosterone as it does not require protein synthesis.

12.4.2 Regulation of Kir1.1

Epithelial K^+ channels are also influenced by dietary factors, particularly by the intake of K^+ (Palmer, 1999). In rats fed a diet very rich in K, the number of K^+ channels that can be detected by electrophysiological means on the apical membrane increases several-fold. This process can be observed after several hours but takes a few days to be complete. The precise signal involved in this upregulation has not been determined. It could be mediated by a hormone, although none has been identified (aldosterone appears to act in a permissive fashion). Alternatively, the kidney could respond to increases in plasma K^+ levels. Finally, it is possible that K sensors in the GI tract may detect increases in dietary K intake directly and signal the kidney through the nervous system (Rabinowitz, 1996).

The cellular mechanisms involved in regulation of these channels have been better studied in the opposite case of a decrease in dietary intake leading to K deprivation (Wang et al., 2000). The events here may or may not be the converse of those occurring with an increased dietary K load. Prolonged (1 week) K deprivation upregulates the activity of the protein tyrosine kinase *src* in the kidney and stimulates the phosphorylation of tyrosine residues on ROMK (Lin et al., 2002). This probably does not affect channel activity directly, but leads eventually to their internalization into endosomes (Lin et al., 2004).

The rate of insertion of channels into the membrane may also be regulated. In cultured cells, ROMK protein appears to be retained in the ER unless an N-terminal serine (S44) is phosphorylated, in which case the channels are translocated to the plasma membrane (O'Connell et al., 2005; Yoo et al., 2005). It is not clear whether or how this process might be linked to regulation by dietary K. As in the case of ENaC, apical K^+ channels are also regulated by ADH through cAMP-dependent mechanisms. Phosphorylation of the critical S44 PKA provides a possible connection to the actions of this hormone, although such a linkage remains to be established.

While the sections above have discussed the major events regulating Na^+ and K^+ channels as if they were separate and independent, this is clearly not the case. For example, acute increases in plasma K^+ increases aldosterone secretion by the adrenals, which will impact Na^+ channel activity. In addition, chronic increases in K intake stimulate Na^+ channels by a mechanism that appears to be independent of aldosterone levels. Superimposed on all of these interactions is the simple coupling of Na^+ and K^+ transport through changes in the apical membrane voltage described above. The kidney does not control Na^+ and K^+ excretion by keeping track of these ions separately. Rather, it seems to regulate the levels of these ions through complex and overlapping control systems.

12.4.3 Regulation of CFTR

Epithelial Cl^- secretion is in general regulated on a somewhat faster time scale, mediating fluid movements over a matter of minutes (Welsh, 1996). The secretion is controlled by hormones and neurotransmitters that act on membrane receptors which are coupled to adenylate cyclase. Increases in cAMP activate the protein kinase type A (PKA). In contrast to the case of ENaC and perhaps also to that of ROMK, the major regulation of CFTR involves the activation of channels residing in the membrane (Fig. 12.3, path E). This takes place primarily through the direct PKA-dependent phosphorylation of several serine and threonine residues on the regulatory domain of the channel protein. This process is faster than that which controls apical Na and K channels, presumably because it does not entail protein synthesis or protein translocation. It is analogous to these synthetic and trafficking events in that it is necessary but not sufficient for channel activity. In the case of CFTR the phosphorylated channel must still be opened by the binding of ATP to the NBD, as described above. The structural events entailed in phosphorylation-dependent channel activation are unknown. It is possible, for instance, that the R-domain acts

as a pore-occluding plug in the nonphosphorylated state, and that phosphorylation permits the plug to move away from the inner mouth of the channel. There is no direct structural evidence for this or for any other mechanism.

While translocation of CFTR may be of secondary importance in the overall regulation of Cl conductance and transport, there are indications that some of the channels in epithelial cells are present in submembrane vesicles, and that these channels can recycle to the cell surface (Prince et al., 1993; Bradbury et al., 1994). In addition, the most common form of CFTR giving rise to cystic fibrosis is the ΔF508 deletion. The defect in Cl^- transport associated with this mutation results from a failure of the channel to translocate properly between the ER and the plasma membrane (Welsh, 1996). Thus, membrane trafficking issues are important at least in the pathophysiology of this channel.

12.5 Summary

Ion channels form important routes for the transepithelial movement of salt. Often, this movement of salt is accompanied by a parallel movement of water. These movements mediate a number of important physiological functions including maintenance of Na and K levels in the body fluids (homeostasis) and the formation of fluid for the delivery of digestive enzymes and mucous to the gut and the airways, as well as for sweat, saliva, and tears. These channels share basic principles of ion permeation with those of excitable cells. However, their regulation is quite different. In some cases, like CFTR, regulation depends on the generation of cAMP and the phosphorylation of the channel protein. In other cases, such as ENaC and possibly ROMK, translocation of channels between intracellular compartments and the plasma membrane underlies the modulation of channel activity. Defects in these processes lead to important diseases including cystic fibrosis and hypertension.

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