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THE MOLECULAR BASIS OF CHLORIDE TRANSPORT IN SHARK RECTAL GLAND

JOHN R. RIORDAN*

The Hospital for Sick Children, Toronto, Ontario, Canada

BLISS FORBUSH, III

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA

AND JOHN W. HANRAHAN

Department of Physiology, McGill University, Montreal, Quebec, Canada

Summary

Transepithelial Cl^- secretion in vertebrates is accomplished by a secondary active transport process brought about by the coordinated activity of apical and basolateral transport proteins. The principal basolateral components are the Na^+/K^+-ATPase pump, the $Na^+/K^+/2Cl^-$ cotransporter (symporter) and a K^+ channel. The rate-limiting apical component is a cyclic-AMP-stimulated Cl^- channel. As postulated nearly two decades ago, the net Cl^- movement from the blood to the lumen involves entry into the epithelial cells with Na^+ and K^+, followed by active Na^+ extrusion *via* the pump and passive K^+ exit *via* a channel. Intracellular [Cl^-] is raised above electrochemical equilibrium and exits into the lumen when the apical Cl^- channel opens. Cl^- secretion is accompanied by a passive paracellular flow of Na^+. The tubules of the rectal glands of elasmobranchs are highly specialized for secreting concentrated NaCl by this mechanism and hence have served as an excellent experimental model in which to characterize the individual steps by electrophysiological and ion flux measurements. The recent molecular cloning and heterologous expression of the apical Cl^- channel and basolateral cotransporter have enabled more detailed analyses of the mechanisms and their regulation. Not surprisingly, since hormones acting through kinases control secretion, both the Cl^- channel, which is the shark counterpart of the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator), and the cotransporter are regulated by phosphorylation and dephosphorylation. The primary stimulation of secretion by hormones employing cyclic AMP as second messenger activates CFTR *via* the direct action of protein kinase A (PKA), which phosphorylates multiple sites on the R domain. In contrast, phosphorylation of the cotransporter by as yet unidentified kinases is apparently secondary to the decrease in intracellular chloride concentration caused by anion exit through CFTR.

*Present address: S. C. Johnson Medical Research Center, Mayo Clinic Scottsdale, Scottsdale, AZ 85259, USA.

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Introduction

With respect to the movement of NaCl, the tubular epithelial cell of the shark rectal gland can be considered '*un porteur par excellence*'. This is because it employs a secondary active transport system involving the coupled translocations of at least three different ions at several transporters and channels to accomplish the secretion of salt from the blood to sea water. Although the specific porters participating in NaCl secretion or absorption in different epithelial tissues vary, the basic paradigm remains the same (Nellans *et al.* 1973). The shark rectal gland has served as an extremely useful model for the characterization of such transcellular porter systems primarily because of the high degree of specialization of the tissue (Burger and Hess, 1960; Epstein and Silva, 1985); its utility has been analogous to that of the electric tissues of rays and eels as overexpressors of sodium channels and acetylcholine receptors. Its ready availability to seaside laboratories may also have contributed to the attention this organ has received. As will be discussed explicitly at the end of this article, tissues in other organisms have apparently evolved similar mechanisms of transepithelial NaCl movement (Haas, 1989).

The experimental work describing the salt transport process at the level of the whole organism, the tissue, the isolated tubules, subcellular fractions in the form of membrane vesicles and more recently cultured cells has been extensively documented during the past two decades (Siegel *et al.* 1976; Stoff *et al.* 1979; Epstein *et al.* 1983; Forbush *et al.* 1992; La *et al.* 1991; Greger and Schlatter, 1984*a*,*b*; Greger *et al* 1984). This brief treatise will not attempt to be comprehensive in outlining all the important contributions to our present understanding of the system. Rather, initial glimpses at the underlying molecular events will be emphasized. This has become possible because of the molecular cloning of three of the four main transporters and channels that combine to accomplish NaCl secretion. Because much of the regulation of the overall process appears to occur at the levels of the basolateral symporter, which moves Cl^- (as well as Na^+ and K^+) from the blood into the cells, and the apical Cl^- channel, which allows the anion to move out of the cell and into the duct lumen, these molecules will receive most attention.

'The model' of rectal gland NaCl secretion

The secretory tubules of the gland all empty into a single collecting duct, just as the arterial and venous capillaries with which they interdigitate connect to a single artery and vein. The tubular walls are composed of a highly uniform population of epithelial cells, one of which is illustrated in Fig. 1. The steps in the NaCl secretion process as they are presently conceived and the involvement of each of the transport and permeation pathways will be outlined, followed by the evidence supporting this interpretation. The stimulus for secretion is mediated by cyclic AMP. Elevation in the intracellular level of this cyclic nucleotide activates the apical CFTR chloride channel. This activation allows Cl^- to exit the luminal side of the cell down its electrochemical gradient. The resulting transient reduction in the intracellular Cl^- concentration can be sensed by the basolateral $Na^+/K^+/Cl^-$ cotransporter (NKCC). This activation of the symporter results in the inward flux of all three ions from the blood side on the driving force provided by Na^+ movements down its large electrochemical gradient. The immediate energy supply for the overall

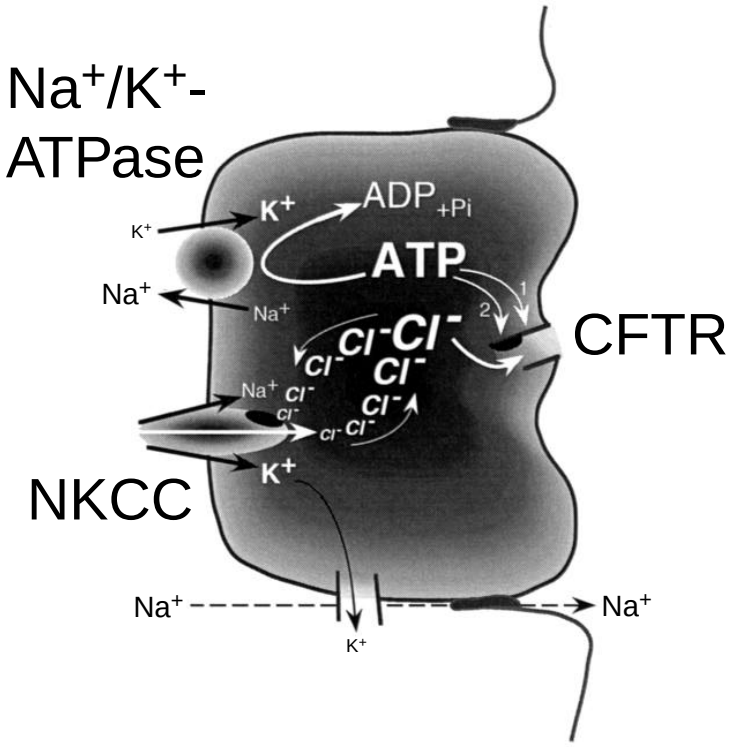

Fig. 1. Schematic representation of an epithelial cell in the wall of the secretory tubule of the shark rectal gland. The principal molecules responsible for transcellular Cl^- secretion are indicated: the sodium pump (Na^+/K^+-ATPase), $Na^+/K^+/Cl^-$ cotransporter (NKCC) and a K^+ channel on the basolateral side and the CFTR chloride channel on the apical side. ATP utilization by the Na^+ pump and its interaction with CFTR are indicated. The latter occurs at two levels: (1) binding to nucleotide binding folds and (2) as substrate for protein kinases. This opening of the CFTR chloride channel results in a transient diminution in intracellular [Cl^-], which stimulates basolateral NKCC to replenish the anion so that its movement across the cell can continue. The Na^+ that also enters *via* NKCC is removed by the Na^+ pump and the K^+ exits through the K^+ channel. The lumen-negative potential generated by the net Cl^- translocation contributes to the passive paracellular Na^+ movement indicated by the broken arrow. Pi, inorganic phosphate.

process derives from the active transport of Na^+ back out across the basolateral membrane by the Na^+/K^+-ATPase.

The other cation which has entered *via* NKCC also exits on the basolateral side through a K^+ channel. The Cl^- which has been transported in by NKCC restores its level to above electrochemical equilibrium with respect to the luminal cell exterior so that exit through the CFTR channel can continue if its activation persists. This transcellular chloride movement contributes substantially to the lumen-negative transepithelial potential that provides the driving force to pull Na^+ through a paracellular permeation pathway from blood to lumen (Silva *et al.* 1983*b*). The net effect is Cl^- transport through the cell *via* the integrated action of transporters and channels and passive Na^+ diffusion around the cell.

The apparent paradox is that NaCl is actively moved in a vectorial fashion across the epithelium without primary active transport of either Na^+ or Cl^- in that direction. In fact, the active Na^+ transport step is in the opposite direction across the basolateral membrane and Cl^- moves by secondary active transport across the basolateral membrane, coupled to the Na^+ gradient by the cotransporter (NKCC) and by passive transport at the apical membrane Cl^- channel (CFTR). The rate-limiting and primary regulatory step resides at the CFTR Cl^- channel, which is optimally designed to respond to hormones employing cyclic AMP as second messenger. However, the means by which the regulation of each of the translocation steps is coordinated are of equal importance and provide an interesting prototype for the dissection of multicomponent transport systems. The hormonal control involves distinct up-regulation and down-regulation arms. The most physiologically relevant secretory agonist may be vasointestinal peptide (VIP), a neurotransmitter released by nerves supplying the rectal gland, which activates adenylate cyclase (Stoff *et al.* 1979). Adenosine is an especially interesting regulator which also activates through a stimulatory A_2 receptor in the micromolar concentration range but inhibits through an A_1 receptor in the low nanomolar range (Kelley *et al.* 1990, 1991). The former effect is mediated through cyclic AMP and probably reflects CFTR activation; however, the down-regulation, which may be a form of feedback control of the hormonal activation, occurs mainly downstream of cyclic AMP although the specific site has not yet been identified.

Individual molecular constituents

The ubiquitous Na^+/K^+-ATPase maintains the basic monovalent cation homeostasis in virtually all vertebrate cells and is basolaterally located in epithelia. It has been extensively studied over nearly the past 40 years and hence is one of the best understood P-type transport ATPases (Shull *et al.* 1985), although detailed structure–function relationships remain to be established. In contrast, the K^+ channel, which is also essential to rectal gland NaCl secretion, has not been characterized beyond its sensitivity to inhibition by Ba^{2+}, which also blocks secretion (Silva *et al.* 1981). The key molecules that are directly responsible for the passage of Cl^- across the basolateral and apical membranes are NKCC (Xu *et al.* 1994) and sCFTR (Hanrahan *et al.* 1993*a*,*b*), respectively. The latter was discovered 5 years ago and, because it is the site of the defect in cystic fibrosis, has been extensively studied since then (Riordan *et al.* 1989; Riordan, 1993). NKCC, however, has been described at the molecular level only this year (Xu *et al.* 1994) and as yet is less well understood.

sCFTR

Shortly after cloning of the human CFTR gene (Riordan *et al.* 1989), cDNAs with very high sequence similarity were isolated from libraries constructed using RNA isolated from rectal glands of *Squalus acanthias* (Grzelczak *et al.* 1990; Marshall *et al.* 1991). Overall amino acid sequence identity approached 80 % and was greater than 90 % for the putative transmembrane and cytoplasmic domains (excepting the R domain). At that time, evidence that CFTR was itself a Cl^- channel had not yet been obtained and its

presence in such a highly conserved form in a tissue devoted to Cl^- secretion supported the idea that the molecule was directly involved in this process (Riordan *et al.* 1991). Extensive heterologous expression and *in vitro* mutagenesis studies (Welsh and Smith, 1993), in addition to the purification and reconstitution of human CFTR (Bear *et al.* 1992), subsequently provided convincing evidence that the protein constitutes a finely regulated low-conductance Cl^- channel. Hence, in many epithelial tissues that perform cyclic-AMP-stimulated Cl^- secretion, such as mammalian intestine, CFTR is the major apical conduit as well as the main regulatory element. In the case of sCFTR, it has more recently been possible to assemble a stable full-length cDNA and to express it in heterologous systems including mammalian and insect somatic cells and *Xenopus* oocytes (Hanrahan *et al.* 1993*a*). As illustrated in Fig. 2, sCFTR forms a channel with very similar properties to human CFTR. The macroscopic currents produced in *Xenopus* oocytes expressing cloned sCFTR had properties that were identical to those obtained by Sullivan *et al.* (1991) after injection of poly(A+) mRNA from shark rectal glands. Thus, conservation of function over this rather large evolutionary span is apparently as great as that of the primary structure. These data, however, do not prove that CFTR is the secretory Cl^- channel. In fact, earlier studies of chloride channels in cells of rectal gland tubules had shown the presence of two different channels, a smaller one with a conductance of 11 pS, which is close to that of human CFTR, and a larger one with a conductance of approximately 40 pS (Greger *et al.* 1985, 1987). Detection of the latter channel was enhanced by secretory stimuli (i.e. cyclic AMP), suggesting that it might carry the secretory current. However, in a more recent study carried out in conjunction with measurement of Cl^- channel activity associated with heterologously expressed sCFTR, it was found that a still smaller channel of approximately 4 pS appeared more frequently in stimulated tubules (Hanrahan *et al.* 1993*a*). The similarities between the endogenous channel and the recombinant one are shown in Fig. 3. We are unsure of the basis for the apparent discrepancy in the two studies of endogenous chloride channels. The role of the larger channel, if indeed it is not involved in stimulated Cl^- secretion, remains to be determined. However, present evidence favours sCFTR as the secretory channel and its properties seemed to be aptly suited to this role.

CFTR structure and function

Although sCFTR has not yet been extensively studied, human CFTR has been well characterized and present indications are that the molecules are extremely similar. Fig. 4 shows a schematic representation of the molecule. This two-dimensional depiction was originally based primarily on hydropathy analyses and sequence similarities to members of a superfamily of ATP-binding integral membrane proteins (Riordan *et al.* 1989). The unit structure, which is the hallmark of this class of molecules, is a polytopic membrane-associated segment followed by a nucleotide binding fold (NBF). Two such units separated by a large hydrophilic central domain (R domain) and moderately sized N- and C-terminal extensions make up the molecule. This topological disposition with respect to the lipid bilayer has recently been verified experimentally (Chang *et al.* 1994); there is increasing evidence that several of the twelve transmembrane sequences contribute to the ion pore (Anderson *et al.* 1991; Tabcharani *et al.* 1993). Regulation of the channel is

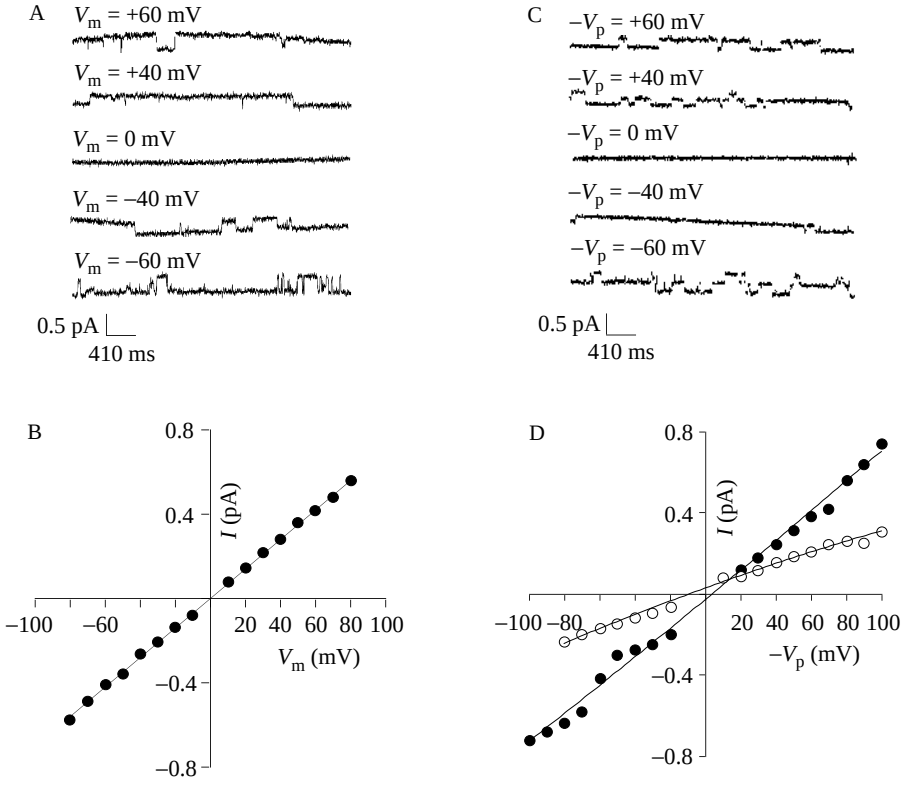

Fig. 2. Comparison of channels produced by heterologous expression of human and shark CFTR. (A) Traces from human CFTR channels recorded using patches excised from stably transfected CHO cells. The cytoplasmic side was bathed with 1 mmol l^{-1} ATP and 178 nmol l^{-1} PKA catalytic subunit, as described previously (Tabcharani *et al.* 1991). (B) Current–voltage (*I–V*) relationship of human CFTR channel shown in A. (C) Traces from shark CFTR (sCFTR) recorded on Sf9 insect cells (2 days after infection with a recombinant baculovirus containing the sCFTR cDNA). (D) Mean current–voltage relationships for single human (●) and shark (○) CFTR channels expressed on Sf9 cells. Channels resembling CFTR were not observed on Sf9 cells infected with a control baculovirus containing the *β*-galactosidase gene. V_m, membrane potential; $-V_p$, imposed patch potential.

extremely complex (Tabcharani *et al.* 1991), involving the action of ATP as substrate for several protein kinases that phosphorylate CFTR as well as binding to the NBFs (Baukrowitz *et al.* 1994). From the point of view of cyclic-AMP-mediated hormonal activation of Cl^- secretion, such as that occurring in the rectal gland, phosphorylation of the R domain by PKA and dephosphorylation are most relevant (Cheng *et al.* 1991; Chang *et al.* 1993). Some insight is beginning to be gained into the mechanism whereby phosphorylation of multiple R domain sites activates the channel (Dulhanty and Riordan, 1994*a*,*b*). The additional layer of regulation stemming from NBF interactions with ATP becomes most obvious in CFTR variants created by *in vitro* mutagenesis in which the R domain is either removed (Rich *et al.* 1991) or its PKA phosphorylation sites converted to residues which are already negatively charged (aspartates or glutamates; Riordan *et al.*

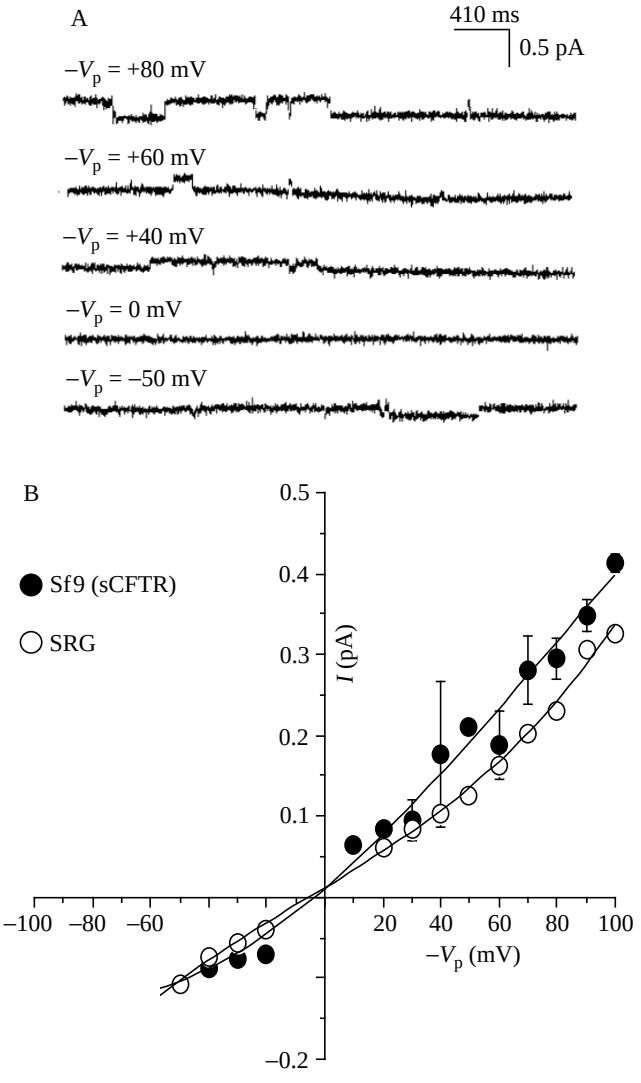

Fig. 3. sCFTR channels recorded on shark rectal gland (SRG) cells and on Sf9 insect cells expressing shark CFTR. (A) Cell-attached recording from the luminal surface of a microdissected SRG tubule. (B) Comparison of current–voltage relationships for sCFTR channels on Sf9 cells and for channels endogenously expressed on native SRG tubule apical membrane. Values are means ± s.e.m., N=4.

1993) without addition of phosphoryl groups. However, the physiological basis of regulation by ATP action at the NBFs is not yet clear, although it has been suggested that it is simply a means of turning off secretion in cells with a suboptimal energy charge so that remaining ATP would not be further dissipated (Quinton and Reddy, 1992). Nevertheless, even without this capability, phosphorylation of the R domain of sCFTR by PKA on hormonal stimulation would suffice to initiate the series of steps outlined above, including activation of NKCC.

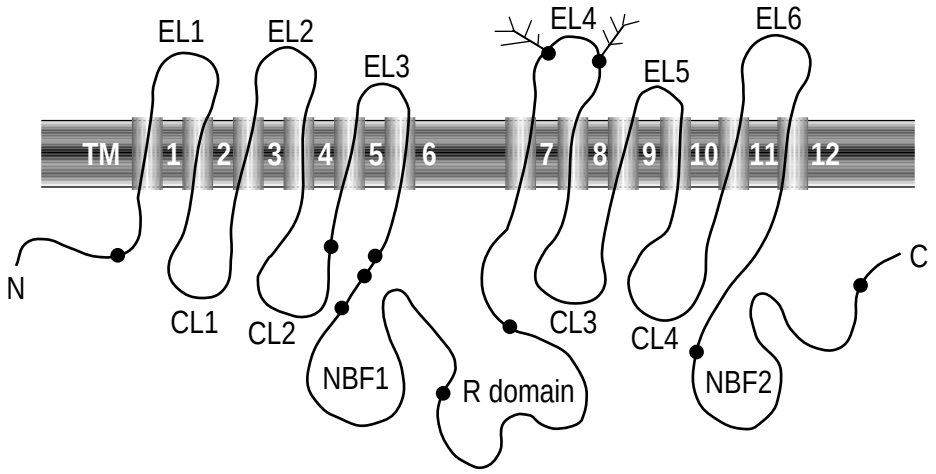

Fig. 4. Topological model of CFTR indicating twelve putative transmembrane helices (TM), six extracytoplasmic loops (EL), four cytoplasmic loops (CL), two nucleotide binding folding (NBF1 and NBF2) and the R domain. Filled circles indicate positions of consensus N-glycosylation sites and the branched twigs are the two that are used.

Shark $Na^+/K^+/Cl^-$ cotransporter

The non-electrogenic coupled transport of Na^+, K^+ and Cl^- has been extensively characterized in many different cell types, in both ordinary symmetrical cells and bipolar epithelial cells and in plasma membrane vesicles derived from them (Kinne *et al.* 1985). Hallmarks of the process include the absolute interdependency of the movement of four ions (1 Na^+, 1 K^+ and 2 Cl^-) in the inward direction across the plasma membrane and sensitivity to inhibition by drugs such as furosemide and bumetamide (Haas, 1989). Activation of the cotransporter is brought about by treatments which elevate cyclic AMP levels, such as VIP, and by manipulations which decrease cellular Cl^- concentration. The binding of some members of this class of compounds, such as benzmetamide or 4-benzoyl-5-sulphamoyl-3-(3-thenyloxy)benzoic acid, to cells or membranes provided a means of identifying and measuring NKCC (Forbush *et al.* 1992). The latter photoactive compound bound specifically to a protein in rectal gland membranes with an apparent M_r of approximately 195 (Lytle *et al.* 1992*a*). This binding occurred only when the transporter was in an active state. This specific labelling provided a means of following the protein during purification from detergent-solubilized rectal gland membranes. Use of this highly specialized tissue was especially advantageous for this purpose because of its elevated level of expression of the transporter protein (approximately 1% of total protein). This enabled the acquisition of sufficient protein for the immunization of mice for monoclonal antibody production and the generation of proteolytic fragments for amino acid sequencing (Lytle *et al.* 1992*b*). It also ensured abundant representation in cDNA libraries constructed using the RNA isolated from the tissue (Xu *et al.* 1994). This is in contrast to CFTR, which is also dedicated to the specialized function of the tissue

but, being a channel, need not be present in very high copy number to provide adequate Cl^- conductance.

A panel of monoclonal antibodies (mAbs) was obtained and successfully employed as probes for the isolation of cDNAs for NKCC by conventional bacterial expression cloning techniques (Xu *et al.* 1994) as well as in immunoprecipitations of the protein from detergent lysates of activated tubules (Lytle and Forbush, 1992). The latter methodology was used to detect phosphorylation of the polypeptide, which correlated with activation by either cyclic AMP agonists or osmotic manipulations (Lytle and Forbush, 1992). The data obtained supported the idea that phosphorylation is directly involved in cotransporter activation. This interpretation is based primarily on the observations that broad protein kinase inhibitors blocked the stimulated binding of benzmetamide, which parallels cotransporter activity, and the fact that the kinetics of phosphorylation and dephosphorylation were synchronized with NKCC activation and inactivation. Both phosphoserine and phosphothreonine were identified by phosphoamino acid analysis. Proteolysis and fractionation of phosphopeptides that could be sequenced accounted for much of the phosphorylation at two sites near the N and C termini of the protein (see below). As yet, the protein kinases responsible for phosphorylation at these sites have not been identified. Lack of correspondence of the polypeptide sequences around these two sites with the consensus sequences for phosphorylation by PKA (Xu *et al.* 1994) suggests that activation does not result from the direct action of PKA (Lytle and Forbush, 1992). Because the rectal gland cotransport process is known not to be strongly influenced by either phorbol esters or calcium ionophores, it is unlikely that PKC or calcium/calmodulin-dependent protein kinases are responsible. Hence, in contrast to CFTR, in which at least a major part of the cyclic AMP activation can be accounted for by the direct action of PKA on the R domain, there must be a more indirect route from both cyclic AMP and osmotic stimuli to the phosphorylation and activation of NKCC. It appears that a decrease in intracellular $[Cl^-]$ is an important part of this pathway (Lytle *et al.* 1992*a*). Elucidation of these pathways should now be possible with the availability of both the purified protein and expressible cDNAs.

Proof that the cDNA isolated with the mAb probes contained authentic NKCC sequence came from the correspondence between the amino acid sequences of several peptides generated from the purified 195 kDa polypeptide and regions of the translated cDNA sequence as well as the functional effects of the heterologous expression of the full-length cDNA in mammalian cells (Xu *et al.* 1994). This resulted in the production of a polypeptide which, after removal of oligosaccharide chains, had the same apparent molecular size as the deglycosylated protein endogenous to the rectal gland. Reduction of intracellular $[Cl^-]$ was necessary for cotransporter activity of the recombinant protein, which is consistent with evidence that reduction in $[Cl^-]_i$ may provide the signal for NKCC activation when the CFTR Cl^- channel is initially activated by secretory stimuli. When function was assessed by the uptake of $^{86}Rb^+$ (as a K^+ surrogate) into the heterologous cells, bumetamide-sensitive flux was elevated 10-fold and was absolutely dependent on the presence of extracellular Na^+ and Cl^-.

Properties of the NKCC protein discerned largely from its primary structure are

illustrated schematically in Fig. 5. The predicted 1191-residue polypeptide has features consistent with its identity as an integral membrane glycoprotein. Analysis of the sequence using hydropathy algorithms suggests that there may be 12 membrane-spanning sequences as in CFTR. These are bracketed by long hydrophilic sequences at the N and C termini which, on the basis of the presence of verified phosphorylation sites and intracellular epitopes for antibodies (Xu *et al.* 1994), should be cytoplasmically oriented. This topological disposition would provide five cytoplasmic and six extracytoplasmic loops separating the transmembrane segments. Of the total of nine consensus sequences for N-glycosylation, one is present in the first and three in the fourth extracytoplasmic loop. The diagram suggests that the latter three are employed, although this has not yet been demonstrated experimentally. If it is confirmed, it would mean that the same relative location is glycosylated in the CFTR and NKCC molecules. The two positions which are phosphorylated in the secretory tubules *in situ* at residues T189 and T1114 in the N- and C-terminal hydrophilic domains, respectively, are indicated in Fig. 5.

When the NKCC sequence was compared with others in protein databases, homology was found with several genes coding for proteins of unknown function as well as with a Na^+/Cl^- cotransporter from flounder urinary bladder (Gamba *et al.* 1993). Overall sequence identify with the latter was 47 %, with regions of much greater identity indicated by the thickened lines along the polypeptide in Fig. 5. They include the third, sixth, eight and tenth putative transmembrane sequences, the first cytoplasmic loop and a short peptide about 180 residues from the C terminus. Because these two molecules share the ability to cotransport Na^+ and Cl^-, it can be speculated that these regions may participate in the handling of these two ions. These and other structure–function relationships are now amenable to investigation by *in vitro* mutagenesis. The construction of hybrid molecules in which portions of the two can be interchanged, as well as with other related molecules that have been detected by cross-hybridization, should provide important clues to domain functions and guide the design of site-specific mutagenesis. The ability to co-express shark NKCC and CFTR should also facilitate the elucidation of the regulatory interrelationsips between these two molecules, which are the primary determinants of the Cl^- concentrative and Cl^- dissipative arms of this transepithelial transport mechanism.

Intermolecular regulation

The complete description of this overall cellular symport system that accomplishes NaCl secretion requires not only a characterization of each of the transporters and channels involved, but also of how their activities are coordinately regulated. Although there were some earlier suggestions that the Na^+/K^+-ATPase might be directly activated during rectal gland stimulation *via* cyclic AMP (Silva *et al.* 1983*a*), these effects were small. A more recent study indicated an inhibition of Na^+/K^+-ATPase by kinase-mediated phosphorylation (Bertorello *et al.* 1991). However, the pump probably works primarily to maintain cellular Na^+ and K^+ homeostasis as it does in most cells. What is clear is that activity of the sodium pump is essential to the secretory process, since ouabain blocks rectal gland secretion completely (Silva *et al.* 1977). Control of the K^+ exit channel has

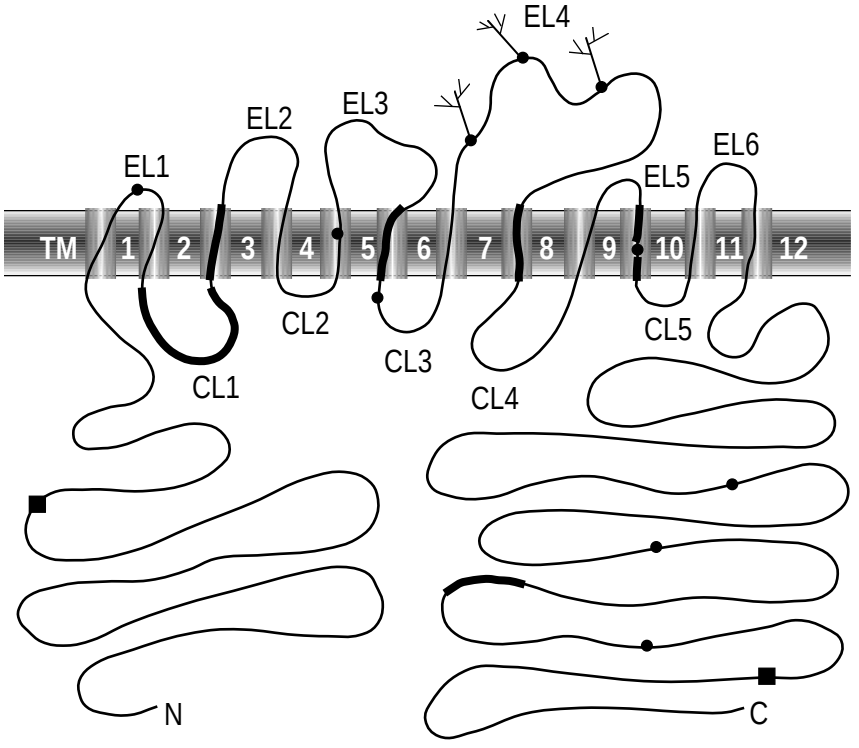

Fig. 5. Topological model of NKCC indicating twelve putative transmembrane helices (TM), six extracytoplasmic loops (EL), five cytoplasmic loops (CL) and large N- and C-terminal cytoplasmic domains. Thickened lines indicate regions of increased sequence similarity with a Na^+/K^+ cotransporter (Gamba *et al.* 1993). Filled circles indicate consensus N-glycosylation sites. Filled squares indicate biochemically confirmed phosphorylation sites.

not been extensively examined and its molecular make-up is entirely unknown. Greger *et al.* (1984) showed that changes in K^+ conductance on stimulation of secretion were modest compared with the large increase in Cl^- conductance. Rapid advances in understanding of CFTR as a highly apical Cl^- channel that is activated and inactivated by PKA-mediated phosphorylation and dephosphorylation, respectively, has provided insight into the initial regulatory event in Cl^- secretion. Even more recent molecular studies of NKCC clearly indicate that it is not an analogous PKA substrate. However, phosphorylation by other kinase(s) probably does contribute to activation, possibly as a consequence of the reduction in $[Cl^-]_i$ resulting from CFTR channel activity (Lytle *et al.* 1992*a*).

Relevance to Cl^- transport in other epithelia

This multimolecular mechanism of Cl^- secretion that is so magnified in shark rectal gland is widely employed in other secretory epithelia. Avian salt gland is another very specialized example which functions very similarly (Simon, 1982). However, not all of

the constituents are identical in each case. For example, additional or alternative Cl^- channels may be employed in some tissues, such as tracheal epithelium (reviewed by Hanrahan *et al.* 1993*b*; see also Boucher, 1993).

Some reabsorptive epithelia use most of the same transporters and channels to move chloride in the opposite direction (Epstein and Silva, 1985). The thick ascending limb of the loop of Henle in the kidney is among the best characterized examples (Haas, 1989). The sidedness, with respect to the apical and basolateral surfaces, of the Cl^- channels, the cotransporter and the K^+ channel is reversed from that in the secretory tissues so that salt is moved from the luminal to the blood side. The cotransporter from reabsorptive kidney tubules in particular has many of the same properties as the rectal gland NKCC, and isolation of the homologous cDNA has recently confirmed this relatedness (Payne and Forbush, 1994).

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