Regulation of Ion Channels by ABC Transporters That Secrete ATP

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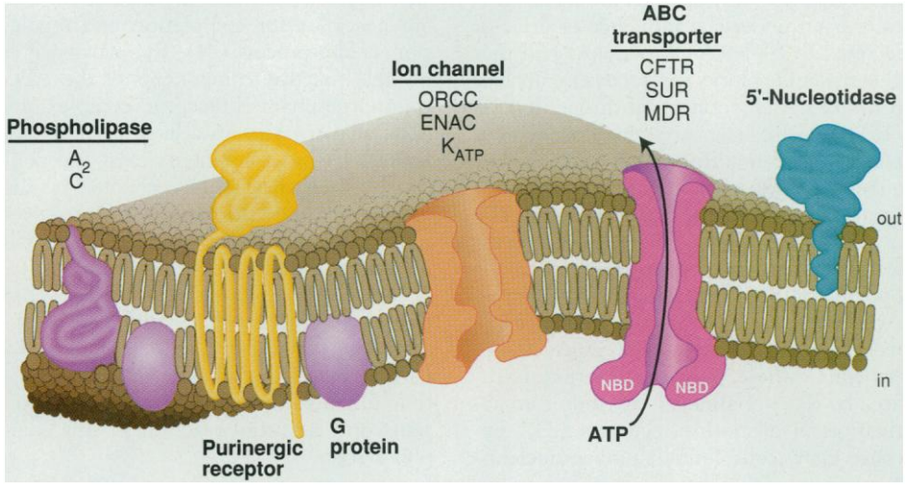
When epithelial cells of patients with cystic fibrosis (CF) were found to have a low permeability to chloride (*1*), there was much excited anticipation that the molecule responsible would be rapidly identified, resulting in a deeper understanding of the pathogenesis and even treatment of the disease. This well-founded optimism was due to the success of the patch clamp in analyzing the details of ion channel regulation in many cells, including secretory epithelia, the site of the CF defect. Indeed, the apical membrane of CF epithelia was found to contain a Cl^- channel that, unlike those in normal epithelia, could not be opened by cyclic AMP–dependent protein kinase (PKA) (*2*) (it had been known that sweat secretion induced by cyclic AMP was defective in CF). The single-channel conductance of the misregulated Cl^- channel was ~40 pS, and its conductance rectified in the outward direction, that is, the current per volt was larger when Cl^- was moving into the cell.

In 1989 the CF gene was identified, to much acclaim. The gene product was a large, integral membrane protein with two adenosine triphosphate (ATP)–binding domains and, like the product of the multidrug resistance gene, MDR, belonged to the ATP binding cassette (ABC) family of proteins. Was it a regulator or was it the Cl^- channel itself? Riordan named it CFTR, for cystic fibrosis transmembrane conductance regulator—an awkward name, but one that seemed to cover all the bases (*3*). When CFTR was expressed in heterologous cells, it produced not the expected 40-pS channel but a smaller channel (–10 pS) with a conductance that did not vary with the membrane potential, that is, it had no rectification (*4*). Reexamination of epithelial cells showed the presence of this small channel in normal but not in CF cells. Two studies conclusively showed that CFTR is a channel. First, a mutation in the transmembrane domain of CFTR altered one of its fundamental properties, that of reversing its preference for Cl^- over I^- (*5*). And second, incorporation of purified CFTR into planar bilayers produced small channels with a constant conductance (*6*). The channel was activated by hydrolyzable ATP and was resistant to most Cl^- channel inhibitors; surprisingly, only glibenclamide [an inhibitor of ATP-regulated K^+ channels (K_{ATP})] seemed to be an effective inhibitor (*7*). The absence of the outward-rectifying Cl^- channel (ORCC) in these new studies was at first embarrassing, but Guggino *et al.* resolved this apparent inconsistency when they found that, indeed, ORCC was present in CF epithelia and that this channel could not be opened by PKA. Transfection with CFTR restored the stimulatory effect of the kinase (*8*). Because ORCC was present in the CFTR knockout mice, the larger channel must be another protein (*9*). So CFTR was, after all, a transmembrane conductance protein and a regulator, living up to Riordan's admirably prescient name. Guggino's group recently identified the mechanism of interaction of the two proteins (*10*). They first confirmed Cantiello's important observation that ATP can permeate through CFTR (*11*) (some have doubted that finding). The secreted ATP activates a purinergic P_{2U} type receptor, which in turn opens the ORCC, as had been previously observed (*12*). Only nanomolar concentrations of extracellular ATP were needed to open the ORCC, and enzymatic removal of extracellular ATP created the CF phenotype (that is, misregulation of ORCC) in CFTR-expressing cells. The whole regulatory system—ORCC, CFTR, the ATP receptor, and presumably the coupling heterotrimeric GTP-binding protein (G protein)—was present in single patches of no more than 1 μm^2, and (as far as one could tell) these proteins were always present together. Activation of ORCC by ATP or uridine 5′-triphosphate has obvious therapeutic implications for CF, and it will be important to develop more specific agonists for these receptors.

In addition to the defect in Cl^- channel regulation, the open probability of the amiloride-sensitive Na^+ channel was higher in CF epithelia than in normal cells (*13*). In this issue of *Science*, this phenomenon is directly addressed; heterologous cells were made to express the complementary DNA (cDNA) for the three subunits of this Na^+ channel. Expression of CFTR in these cells suppressed the activity of the Na^+ channel, and cyclic AMP reduced it further (*14*).

Components of the ABC Transporter:Channel:Receptor Complex



The mechanism of coupling has yet to be identified, but by analogy with the mechanism of ORCC regulation, the activation of a purinergic receptor by ATP or its degradation product, adenosine, might be involved. The receptor and channel could be directly coupled by a G protein, in the same way that the muscarinic receptor activates a K^+ channel in the heart (*15*). It has been shown that $G\alpha_{i3}$ is present in the amiloride-sensitive Na^+ channel complex (*16*). But purinergic receptor activation might also generate inhibitory products from phospholipase activation, such as inositol lipids and arachidonic acid. Finally, CFTR itself might secrete some other inhibitory factor.

CFTR is not the only ABC protein through which ATP can permeate; MDR also transports ATP (*17*). Further, some (though not all) studies have shown that cells overexpressing MDR have a distinct volume-activated Cl^- channel (*18*). Is it possible that ATP transported by MDR

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could activate other proteins, for example, a volume-activated Cl^- channel—or even drug transport by a different protein? MDR should be purified in a functional form to demonstrate whether, by itself, it can transport drugs or Cl^-.

The recent identification of the sulfonylurea receptor (SUR) as a member of the ABC gene family homologous to CFTR and MDR (*19*) promises to clarify the regulation of another important class of ion channels, K^+ channels that are blocked by cytoplasmic ATP (K_{ATP}). The K_{ATP} channels are involved in insulin secretion and in the control of muscle excitability (*20*). Some sulfonylurea drugs, such as glibenclamide, close the K_{ATP} channels in pancreatic islet cells and muscle (hence their utility in the treatment of diabetes mellitus and shock), whereas others, such as diazoxide, open the K_{ATP} channels, causing hyperglycemia and vasodilatation. It is likely that the K_{ATP} channel protein is tonically open under the influence of a factor secreted by SUR (ATP, perhaps) and that glibenclamide blocks this secretion much as it inhibits ATP permeation through CFTR (*10*). Indeed, shear stress in blood vessels releases ATP, and this release is blocked by glibenclamide (*21*). Further, diazoxide opens K_{ATP} channels, but only when ATP or ADP are present in the cell (*22*), an effect that can be explained by diazoxide opening SUR and allowing ATP secretion. Activation of K_{ATP} channels by purinergic receptors is well described, especially in ischemia, where muscle relaxation mediated by K_{ATP} channels is attributed to activation of adenosine receptors (*23*). Because many cells contain surface nucleotidases, secreted ATP could rapidly be hydrolyzed to adenosine. Obviously, direct measurement of ATP (or adenosine) transport by heterologous cells expressing SUR will be required before this idea can be taken seriously.

That these ion channels are individual players in a regulatory ensemble raises the question of the nature of the association between the ABC transporters, the channels, receptors, G proteins, phospholipases, nucleotidases, and other proteins. Classical biochemistry has shown us that some ensembles exist as large particles of enzymes that convert a substrate to a product in a stepwise manner. These particles also contain other proteins that speed up or slow down each partial reaction. The particles responsible for protein translocation across the endoplasmic reticulum include a protein-conducting channel, as well as factors located at the cytoplasmic surface and the extracellular surface of the membrane that are necessary for completion and regulation of the process (*24*). By analogy, it is possible that the components of the ABC transporter:channel:receptor complex are associated together in a large particle (see figure). If the components exist independently, then they will be coupled by the diffusion of the critical intermediates over long distances, and some, like ATP, will be dumped in the essentially infinite reservoir of the extracellular space. That the whole process of regulation can be observed in a small patch makes the idea of a particle plausible. What seems apparent is that ion flux through the channel represents only a partial reaction in this complex system.

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