major problem with some of the ^{13}C relaxation studies, especially for small oligosaccharides, is that internal motion may be on a time scale that is close to that of the overall molecular tumbling, making impossible their separation on the basis of time scales.

A possible solution to this problem is proposed by Poppe et al. (1994), who report ^{13}C T_1, $T_{1\rho}$, and NOE for the complex ganglioside GD1a, inserted in a micelle. The slow tumbling of the micelle provides the distinct time scale, revealing the relatively faster internal motion of a flexible glycosidic linkage in the glycolipid. The micelle plays a role analogous to that of the protein in the glycoprotein data discussed above, and the results are comparable. There are many technical difficulties in working with a complicated assembly such as a glycolipid in a micelle, but careful studies of the type reported by Poppe et al. (1994) could instruct us not only about oligosaccharide flexibility but also about how glycolipids move in membranes.

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How ATP Regulates the CFT Regulator

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For more than ten years we have known that cystic fibrous (CF) involves a defect in Cl conductance. Numerous articles have appeared that elucidate the mechanisms of this defect. Yet the obvious question remains: how does CFTR normally work. Michal Winter et al. (1994, this issue) make a significant step toward understanding that question by studying the ATP regulation of CFTR Cl channels. The cystic fibrous transmembrane conductance regulator (CFTR) has a curious name because the gene, cloned five years ago, was neither clearly a channel nor a channel regulator. We now know that the CFTR is a moderately low-conductance ohmic channel that selects Cl over other ions and responds to cAMP-dependent protein kinase A (PKA) and ATP. Viewed as a channel, CFTR seems fairly ordinary. What elevates it is that CFTR malfunctions in patients with CF. If the CFTR protein (~1500 residues) lacks one phenylalanine at position 508, it fails to reach the apical membrane of the airway epithelium. This most common naturally occurring mutation is responsible for 70% of all CF cases (~35,000 in the U.S. alone). In a rarer form of the disease, CFTR mutants get to the right place but fail to function. Thus, either the absence of these Cl channels or the collapse of Cl pathway can cause CF. The strategic location of the CFTR in the pulmonary epithelium and its probable role in the co-transport of water are the primary reasons. The airways of CF patients have abnormally thick mucus, and dehydration is the major problem. Because the movement of water requires the movement of Cl, problems with CFTR manifest as debilitating congestion.

Welsh and Anderson were among the first to show that low temperatures allow the F508 mutants to reach the cell surface. This discovery resolved several conflicting reports. Once they were in place, the F508 mutants can conduce Cl. This result helped reduce CF to a problem in protein trafficking—now under intense investigation. However, a substantial fraction of CF cases involves CFTRs that express normally do not transport Cl. Perhaps we could understand why the channel does not conduct Cl if we clearly understood normal conduction. The breakdown of function may reduce to the question: what regulates the CFT regulator?

We already know that CFTR must bind ATP. However, it remains unclear whether CFTR must also hydrolyze ATP. In one recent model, ATP activation occurs in two steps. ATP binds to two membrane domains, and then PKA phosphorylates a cytoplasmic loop called the R domain (Anderson et al., 1991). Despite numerous efforts, we still have very little understanding of how ATP interacts with these nucleotide-binding regions. Presumably, the R domain plugs the pore that conducts Cl. Perhaps phosphorylation adds negative charge to the R domain, leading to conformational rearrangements that open the channel (Rich et al., 1993). Numerous questions remain. If R is the plug that closes the channel, what exactly does phosphorylation do?

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Can we liken the R domain to the ball and chain of the Shaker K channel? Is activated PKA a prerequisite to ATP regulation? For example, in PKA-activated channels in the presence of ATP the channel might stay open longer. Alternatively, the channel conductance could increase or the channel closed times could decrease. Mutations that produce mild forms of CF create Cl channels with abnormally low conductance (Sheppard et al., 1993). A priori, the normal action of ATP could involve some combination of these effects.

In a first-rate biophysical study of single CFTR channels, Winter et al. provide a mechanistic answer to how ATP works. ATP increases the probability that the channel will open. However, the open time is independent of ATP concentration. Thus, a rare opening at 0 mM ATP and a frequent opening at 1 mM ATP both last about 50 ms. Furthermore, at least on the time scale of patch-clamp experiments, there is no rundown of the CFTRs in an excised patch. These facts imply that the CFTR does not require phosphorylation to reside in the membrane, but it does require both activated PKA and ATP to open.

Some researchers find the CFTR interesting in its own right. More than one anion can occupy the CFTR pore. Curiously, the conductance of the CFTR is lower with both types present. In 154 mM Cl, the conductance of the channel is 7 pS, and in 150 mM thiocyanate (SCN) and 4 mM Cl it is 10 pS. However, in 144 mM SCN and 10 mM Cl, the CFTR conductance is only 2 pS. SCN on the cytoplasmic side causes outward rectification that is consistent with voltage-dependent block (Tabcharani et al., 1993). Thus, CFTR becomes an ideal construct to study multi-ion pore behavior and mole-fraction effects—the sorts of things that biophysicists dream about.

Will biophysical studies offer any directions for ion-channel intervention to help CF patients? That eventually seems far down the road. This paper tells how ATP opens PKA-activated channels in the normal CFTR, and it should help us to understand the closed-channel mutants. Many questions remain. What is the minimal level of phosphorylation required to elevate Cl conductance? Does this relate to mild forms of the disease? Detailed biophysical studies could yield specific answers to clinically relevant questions. To find a successful drug therapy, the mechanisms underlying regulation of conduction need clarification. Strategies are being developed based on just such studies. For example, one of the side effects of CFTR abnormality is an increase in the uptake of Na ions. Na co-transport seems to contribute to the dehydration of the airways, and blocking Na channels has been suggested as a possible therapy. Learning how to open the nonconducting mutants is another obvious direction. The specific contribution of studies like the Winter et al. article is that it provides a testable model of the CFTR kinetics. Ultimately, this should allow a rational approach to intervention. This paper may also stimulate the application of ion-channel biophysics to other electrogenic molecules, like P-glycoprotein and neurotransmitter transporters. Researchers have begun to draw parallels between voltage-gated ion channels and other proteins that govern the movement of charged particles. But, unlike CFTR, the biophysics of transporters has remained nearly untouched by patch-clamp technology. Many of these membrane proteins belong to a group called the ATP Binding Cassette (ABC) superfamily. They are connected not by the usual amino acid homology but rather by their structural similarities within the membrane (Jan and Jan, 1992). A common feature of certain ABC proteins is the critical role of Cl ions. Cl channels are relatively late-comers to the voltage-gated ion channel field, but they are a major charge carrier for a wide range of membrane proteins (Ackerman and Clapham, 1993). The proposed basic structure of the ABC superfamily has six membrane-spanning domains, and the usual construction consists of two of these units linked together. This view point has allowed comparisons between molecules as different as P-glycoprotein and the L-type Ca channel (Greenberger and Ishikawa, 1994).

In these general terms, one challenge raised by Winter et al. is not only to extend the biophysics of the CFTR, but to make comparable advances in more recalcitrant molecules like P-glycoproteins and neurotransmitter transporters.

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P22 Phage Capsids Under Pressure

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Upon completion of the assembly process, virus and phage capsids have a dual role: first, the capsid protects the enclosed nucleic acid during the uncertain voyage to the receptor or interior of the next susceptible cell; then the capsid alters its structure and releases the enclosed nucleic acid upon binding to the targeted receptor or upon penetration of

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