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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the next host cell. This abrupt change in overall capsid structure and function translates into time-dependent changes in the conformation and organization of the capsid subunits.

Many spherical capsids contain multiples of 60 identical subunits, and for such capsids the identical protein subunits are necessarily located in nonidentical environments. So, depending on their spatial location in the capsid lattice, otherwise identical subunits assume different conformations.

Virus and phage capsid protein subunits, therefore, can be said to exhibit both spatial and temporal conformational changes that are essential for assembly and function. In other words, virus and phage capsids are not static structures, but rather are complex, dynamic machines that carry out the transport and delivery of their enclosed nucleic acid cargoes. Understanding the subunit conformational changes and the forces that underlie the functioning of these complex machines is a challenging problem for molecular biophysics.

The *Salmonella* bacteriophage P22 capsid, which contains 420 identical subunit proteins distributed in seven distinct structural environments, has special advantages for investigating these protein conformational changes. In particular, biologically active capsid subunit protein monomers, a precursor procapsid containing scaffolding protein, an empty procapsid form devoid of scaffolding protein, and a DNA-filled capsid can all be prepared to high purity in amounts suitable for biophysical characterization.

Recent three-dimensional reconstructions of the empty procapsid and the DNA-filled capsid show that incorporation of DNA is accompanied an overall expansion, the closure of holes, the formation of new subunit contacts and an overall subunit conformational change (Prasad et al., 1993) that is probably due more to domain movement than to an alteration in secondary structure. Release of the nucleic acid upon infection is also likely to involve a capsid protein conformational change.

The article by Prevelige et al. in this issue of *Biophysical Journal* reports a continuation of the investigations of the conformational changes of the P22 capsid and its protein subunits, herein describing pressure-induced dissociation of the capsid and pressure-induced unfolding of the coat protein monomer. As we are reminded by scenes from the recent Winter Olympics in picturesque Lillehammer, Norway, the events of which were made possible in large measure due to pressure-melted ice, changes in pressure can shift the equilibrium of a chemical system, with increases in pressure favoring the component with the smaller volume.

The remarkable finding of the article by Prevelige et al. is that the capsid subunit monomer of P22 is unusually sensitive to pressure-induced unfolding, but becomes stabilized against pressure when incorporated into the polymerized procapsid shell. Furthermore, because the procapsid shell is found to be cold labile under applied pressure, the stabilization of the protein subunit in the capsid must result from increased entropy, probably from the burying of hydrophobic groups during polymerization.

The volume decrease that accompanies pressure-induced depolymerization of protein aggregates and the volume decrease that accompanies pressure-induced unfolding of protein monomers are thought to have similar origins (Silva and Weber, 1993) involving the following factors: loss of free volume or voids in the native or polymerized state; electrostriction as buried polar groups in the native or polymerized state become exposed; and the squeezing of nonpolar moieties into the spaces within the structured water as these buried hydrophobic groups in the native or polymerized state become exposed. Currently, it is not simple to evaluate the relative weights of these various contributions; indeed, the weights of these factors might vary from system to system.

Partially unfolded protein forms induced by solvent perturbation (Dolgikh et al., 1981), forms now known as molten globules, evidently share some properties with partially unfolded forms induced by pressure (Silva and Weber, 1993), including the increased accessibility of hydrophobic groups. However, several recent papers show convincingly that the fluctuating, or molten, interiors of molten globule forms arise from an overall particle expansion, which allows the side-chain packing to become nonrigid. Because pressure-induced unfolding is incommensurate with an overall particle expansion, it is doubtful that the pressure-induced forms are true molten globules with nonrigidly packed interiors. Perhaps it will turn out that molten globules, with their slightly separated and nonrigidly packed hydrophobic contacts, are less unfolded than pressure-induced forms, which might be unfolded sufficiently to allow more of the hydrophobic surfaces to be exposed to solvent.

As noted above, an especially intriguing result in the article by Prevelige et al. is the discovery that the P22 capsid protein monomer undergoes pressure-induced unfolding at comparatively low pressures, near 0.5 kbar (1 kbar = 10^9 dyne/cm^2 $\approx$ 987 atmospheres). Except for Staphylococcal nuclease (Royer et al., 1993), the vast majority of native proteins studied so far require greater than 5 kbar for pressure-induced unfolding, and some do not begin to unfold before 11 kbar is reached (Silva and Weber, 1993). Thus, it is interesting to speculate that the intrinsic lack of stability of the P22 capsid subunit to increased pressure might be a manifestation of its need to display both spatial and temporal flexibility: spatial flexibility during procapsid assembly, in order to fit into the nonidentical environments of the capsid, and temporal flexibility during assembly or infection, in order to accommodate nucleic acid incorporation or release.

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Action at a Distance: Another Lesson from the Red Cell

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Secrets initially revealed by studies of the human red cell membrane have repeatedly proven to be of general biological significance. Membrane phospholipid composition and asymmetry, the biochemistry and functional importance of the membrane skeleton, and the physiology of membrane transport proteins are all examples of this progression in experimental membrane biology. In the field of signal transduction across biological membranes, many of the molecular events that mediate signaling upon ligand binding to the exofacial domain of a transmembrane receptor protein remain obscure. One reason for this lack of understanding is the structural and functional complexity of most biological membranes, making it difficult to tease out relationships among changes in membrane biochemistry and consequent alterations in membrane structure and function. Again the red cell may have come to the rescue.

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Although the membrane of the enucleated red cell is not amenable to study by the generation of site-specific mutant molecules, the wealth of available clinical material provides "natural" mutants that can be exploited with great effectiveness. In the present issue of the *Biophysical Journal*, Knowles et al. (1994) use such mutants to describe the effects of a monoclonal antibody directed against the major membrane sialoglycoprotein, glycophorin A, on membrane rigidity and transmembrane protein lateral mobility. The anti-glycophorin antibody is found to induce a marked increase in the rigidity of normal red cells as well as lateral immobilization of both glycophorin A and the major transmembrane protein, band 3. None of these effects are induced by the anti-glycophorin antibody in mutant red cells expressing a form of glycophorin A that lacks the cytoplasmic domain and, therefore, is incapable of interaction with the red cell membrane skeleton. These findings are remarkable and important for several reasons.

The first set of implications concerns the molecular mechanism by which the anti-glycophorin antibody induces transmembrane protein immobilization and membrane rigidification. The antibody presumably induces a conformational change in glycophorin A that affects the interaction between this integral protein and the red cell membrane skeleton. The conformational change, therefore, could effect transmembrane protein immobilization and membrane rigidification by: 1) inducing increased interactions between band 3 and the membrane skeleton; or 2) inducing increased interactions between glycophorin A and the membrane skeleton, thereby pulling the skeleton closer to the membrane lipid bilayer. In the first mechanism, band 3 immobilization would result from increased direct binding interactions between band 3 and the membrane skeleton, whereas in the second mechanism, increased steric interactions would be responsible for band 3 immobilization. Although lateral mobility data cannot distinguish between direct binding and steric mechanisms as causes of lateral immobilization, rotational mobility measurements can shed light on this question. Rotational mobility is sensitive to the molecular environment (nanometer scale) of the rotating species, whereas lateral mobility is measured over micrometer scale distances. Band 3 rotation has been measured in normal red cells incubated with the anti-glycophorin antibody; the results show that the antibody causes rotational immobilization of band 3 (Nigg et al., 1980). These data suggest strongly that the anti-glycophorin antibody induces increased direct binding interactions between band 3 and the membrane skeleton, although concomitant increases in steric interactions cannot be ruled out.

Are glycophorin A and band 3 linked in a molecular complex? This question was first raised almost thirty years ago, and it remains unresolved to the present time. Data in favor of a glycophorin A-band 3 complex include the finding that the anti-glycophorin antibody induces rotational immobilization of band 3 (see above), and the observation that the Wr^b blood group antigen is made up of determinants from the exofacial domains of both band 3 and glycophorin A (Telen and Chasis, 1990). Data against such a complex include band 3 and glycophorin mobility measurements in a number of red cell states that show discordant values for the rates of band 3 and glycophorin translation. (If the two transmembrane proteins were tightly linked in a long-lived complex, they should manifest identical rates of translational diffusion.) Examples include: 1) normal red cells, in which glycophorin lateral diffusion rates are about 50% greater than band 3 diffusion rates (reviewed by Golan, 1989); 2) Southeast Asian ovalocytes (Liu et al., 1990; Mohandas et al., 1992) and Band 3^{PRAGUE} red cells (Jarolim et al., 1994), in which band 3 is laterally immobile, but glycophorin diffuses at control rates; and 3) anti-glycophorin antibody-treated Miltenberger V (mutant) red cells, in which lateral diffusion rates for band 3 and glycophorin A differ by a factor of about 3 (see Figs. 1 and 3 of Knowles et al., 1994). The work of Knowles et al. (1994) would appear to add to existing data favoring