ION CHANNELS

Enter the 'swinging gate'

Sanford Simon

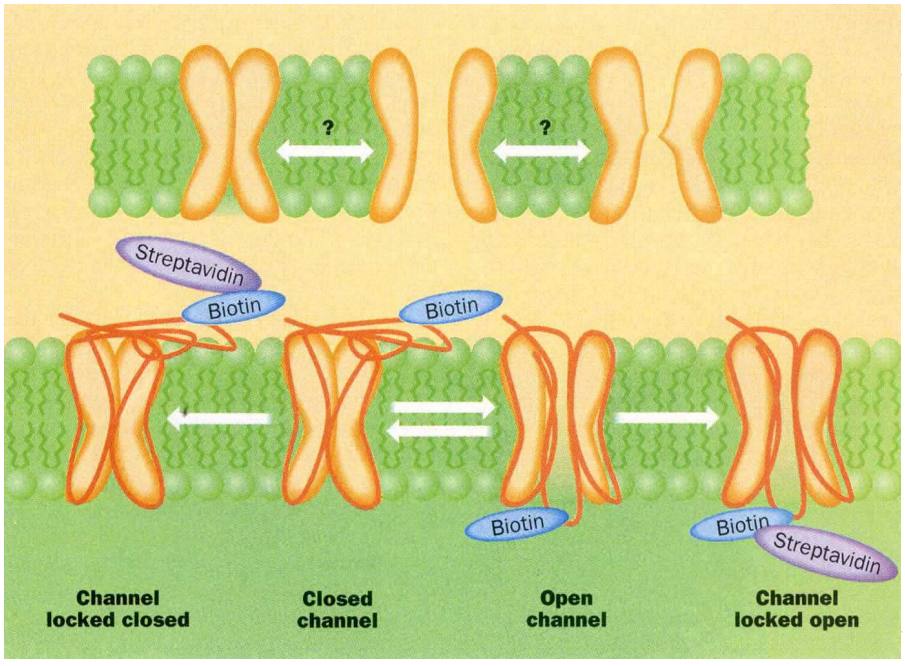
FORTY years ago Hodgkin and Huxley revolutionized biology with a quantitative description of neuronal excitation and a postulate of individual membrane-bound channels for ion transport. Ion channels are now known to be responsible for a plethora of physiologies, such as learning, and pathologies, such as cystic fibrosis. Channel proteins have been purified, their DNA has been sequenced, and the kinetics of ions flowing through individual channels have been analysed. But few tools exist for the structural analysis of membrane proteins and little is known of the conformational changes responsible for channel activity. Now a report on page 158 of this issue by S tin *et al.*[1] demonstrates that one ion channel, colicin Ia, radically reconfigures itself while opening and closing — and even has a domain that pops in and out of the membrane. The authors show that binding molecules to this 'swinging gate' keeps it from re-entering the membrane, raising the exciting possibility that other ion channels and membrane proteins may have similar gates. Regulation of these channels could ultimately prove to result from different molecules modifying such gates.

Slatin *et al.* investigate whether opening the colicin channel affects those parts of the protein exposed on the surface of the membrane. They achieved this by mutating unique amino acids to cysteine and conjugating them with biotin. After the insertion of colicin into the lipid bilayer, the channels were opened or closed by varying the electric field across the membrane. The water-soluble protein streptavidin, which binds biotin, was then added to the solutions bathing the membrane. Streptavidin on one side of the membrane bound a stretch of amino acids running from residues 511 to 547 when the channel was closed, but not when it was open. Further, binding of streptavidin to these amino acids prevented the channel from opening. Streptavidin added on the opposite side of the membrane did not bind to any of the stretch of amino acids if the channel was closed, but bound a subset of them (511–541) when it was open. Binding streptavidin to these amino acids prevented the channel from fully closing.

These results are the strongest evidence yet for a major conformational change during the opening and closing of colicin channels. It had been inferred previously from experiments creating new disulphide bonds in colicin to restrict molecular movement[2] and using chemical labels or protease sensitivity to probe the channel structure[3]. But what is surprising about the latest results is not only the magnitude of the conformational change but also that large domains of the protein can reversibly flip across the membrane.

Could other ion channels undergo similar radical changes? Such a molecular model was proposed twenty years ago for the channels of alamethicin and monazomycin[4]. But in the absence of physical evidence for major structural changes or, even more dramatically, domains reversibly dunking through the membrane, it has been generally accepted that ion channels undergo only a subtle shift of structure. Most pores are small and a displacement of only a few angstroms should be sufficient to block the conductive pathway. Yet evidence is now accumulating for major structural changes. Chemical reagents can differentially label channels in their open and closed states[5,6]. Further, the lumens of potassium and mitochondrial porin channels substantially shrink when they close[7], so each time a channel re-opens it has to extract water from the surrounding medium. Raising the concentration of solutes that cannot permeate the channel increases the energy barrier for channel opening, allowing the internal volume change to be calibrated. Cartoons of channel activity had to be redrawn to show channels closing along their entire length after these elegant 'osmotic stress' experiments.

Slatin *et al.*'s most intriguing observation is that at least 31 amino acids can reversibly flip across the membrane. Transmembrane proteins are usually permanently inserted across the membrane during synthesis by the same machinery that is used for secretory proteins[8]. For example, each of opsin's seven transmembrane domains requires the translocation machinery of the endoplasmic reticulum (ER) to be inserted in the membrane[9]. Colicin does not fit this pattern. True, it is a professional membrane perturbant, but its behaviour may be applicable to other membrane proteins. For example, the hepatitis-B viral protein is inserted into the ER membrane in a classic signal-sequence-dependent manner, but during subsequent viral assembly a large domain of the protein crosses the bilayer[10,11]. Although probably all transmembrane proteins require a signal sequence for targeting to and initial translocation into a particular membrane, they may have some domains that can be reversibly embedded in the bilayer. Indeed, for some proteins, like the colicins, certain domains may be continuously flipping in and out of the membrane by thermal energy. This may be particularly relevant for amphipathic domains of ion channels. How this domain crosses the membrane is



The upper part of the figure shows two models for channel gating. The channel could undergo a subtle shift of structure to block flow through its lumen (right) or it could close down along its entire length (left)[7]. The lower part illustrates the principles used by Slatin *et al.*[1] to explore colicin's conformational changes upon channel opening and closing. Unique amino acids on the colicin molecule (solid red line) are mutated to cysteines and then covalently linked to a biotin molecule. Streptavidin added to the upper chamber can only bind to biotin on a stretch of 31 amino acids when the membrane-bound colicin channel is closed. Binding of streptavidin locks the channel in this closed state. Streptavidin in the lower chamber can only bind this biotin-modified stretch when the channel is open, so keeping the channel from closing.

unresolved. Is it some property inherent to the swinging gate or to the rest of the colicin molecule? It will be interesting to see whether other domains, even large proteins, can be engineered into this region and flipped across the membrane.

It is not understood how channels such as the potassium channel or cystic fibrosis transmembrane regulator are opened or closed by binding of small molecules or by covalent modification. Let's assume that the physical properties of some domains of the ion channel allow them to bob in and out of the membrane reversibly. Modification of this domain, like the binding of streptavidin to colicin, would then trap the channel in an open or closed configuration. The modification does not induce a conformational change but instead stabilizes changes driven by brownian motion. Thirty years ago it was proposed that brownian motion could drive a machine if given a gradient of thermal (chemical) energy[12]. This model may be applicable to ion channels, where thermal motions drive their opening and closing and the chemical energy of modification locks them into one state or another. Such brownian ratchets[13] have been used to describe protein translocation across membranes[14], filapodial extension and the movement of bacteria and motor molecules[15].

The results of Slatin *et al.*[1] will certainly spur a rethinking among colicin toxicologists and channel physiologists. Together with demonstrations that other hydrophilic molecules such as proteins[16] and nucleotides[17] can also cross membranes through aqueous channels, this discovery should intrigue everyone interested in the functioning of membrane proteins. □

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HUMAN GENETICS

From the simple to the complex

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THE genes that cause Duchenne muscular dystrophy, Huntington's chorea, adult polycystic kidney disease and cystic fibrosis, to name a few, have all been identified by a combination of classical genetics and molecular biology. These diseases are genetically simple and relatively rare, whereas diseases such as diabetes that impose the largest burdens on Western societies are very common and often genetically complex. Unravelling the aetiology of complex genetic diseases used to be an intractable problem, but recent advances in high-throughput genotyping and high-resolution genetic mapping should lift the gloomy prognosis. As described on pages 130[1] and 161[2] of this issue, two groups have searched the entire genome to identify new genetic components of a complex genetic form of diabetes known as type 1 insulin-dependent diabetes mellitus (IDDM; see box).

A simple genetic disease can be described by a slight modification of Beadle and Tatum's famous dictum: one gene, one polypeptide, one disease; the inheritance of such diseases follows the laws of mendelian genetics. A complex genetic disease shows no discernible inheritance pattern but has a tendency to cluster in families. Family members share most of their genes but also their environment: both factors can cause familial clustering of a disease — an issue endlessly debated as nature versus nurture. For each disease, the problem can be reduced to how many genes are involved and what, if any, the environmental factors are. The amount of familial clustering of a disease can be quantified by comparing the increased disease risk in a sibling of a patient with the risk in the population as a whole. Comparing the difference in risk between identical twins and dizygotic twins can give an indication of the genetic contribution to this increased disease risk, leaving the problem of identifying the genes involved and measuring the relative contributions of each gene. For inbred animals and plants, in which both the starting genotypes and phenotypes are well defined, several methods have been successful[3,4].

But human genetics demands a different approach. Affected members of a family will share the genes causing the disease and this sharing will manifest itself as an excess of sharing over the 50% expected at random in siblings[5]. Disease genes can be identified by co-segregation with linked genetic markers which will show identity by descent in affected individuals. A genome scan combined with such an analysis makes no *a priori* assumptions about the number of genes involved in a disease, nor the types of interaction between them. The markers used must be close enough that recombination with the disease locus is unlikely and sharing among individuals can only be tested if the markers are polymorphic. The microsatellite markers developed at Généthon and elsewhere are ideal for this purpose: they are highly polymorphic and sufficient numbers of them have been positioned to provide a good coverage of the complete genome[6].

Nevertheless, the experiments are far from trivial. Hundreds of families containing more than one affected offspring must be found. The more families tested, the smaller is the genetic contribution to the disease that can be revealed. All individuals must be clinically evaluated, their DNA isolated and then tested. A collection of one hundred families requires over 120,000 typings to complete a gene scan of

A brief history of diabetes

DIABETES mellitus was recognized long ago in ancient Egypt but it was not until the last century that investigation began into the biochemical features of the disease, eventually revealing the association between insulin and blood glucose levels that is now well known. There are two types of diabetes, insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM or type 1, and NIDDM or type 2, respectively). IDDM usually affects children and results from an autoimmune response against the pancreas which causes selective destruction of insulin-producing cells and failure of insulin secretion in response to increased blood glucose. As insulin controls the uptake of glucose into cells, this lack of hormone forces them to switch to alternative fuels, with life-threatening metabolic consequences. Lifelong administration of insulin is essential in this condition.

Evidence for the involvement of genetic factors came from animal models (the NOD mouse and BB rat) and the increased risk of developing IDDM for members of a family affected with the disease. Population studies have shown that there is an association of IDDM with certain genes (*DR3* and *DR4*) in the major histocompatibility complex, and other gene associations have now been identified. On the other hand, NIDDM affects adults and is usually due to a combination of impaired insulin secretion and insensitivity of the target tissue to the hormone. These two forms of diabetes affect between 2 and 4 per cent of the UK population. **P.N.G. & K.S.**