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Membrane channels

Introduction

The Nobel Prize in Chemistry for 2003 is shared between two scientists who have made fundamental discoveries concerning how water and ions move through cell membranes. Peter Agre discovered and characterized the first water channel protein and Roderick MacKinnon has elucidated the structural and mechanistic basis for ion channel function.

Living cells are enclosed by a lipid bilayer membrane that separates them from other cells and the extracellular medium. Cells also contain membrane-enclosed organelles such as the nucleus, mitochondria and chloroplasts.

Lipid bilayer membranes are generally impermeable to water, ions, and other polar molecules; yet, in many instances, such entities need to be rapidly and selectively transported across a membrane, often in response to an extra- or intracellular signal. Transport along a concentration gradient is mediated by membrane channel proteins, whereas transport against a concentration gradient is mediated by membrane pumps such as the Na^+/K^+ ATPase (a protein discovered in 1957 by Jens Skou, who received the Nobel Prize in chemistry in 1997).

Water channels allow the cell to regulate its volume and internal osmotic pressure, and are needed when water must be retrieved from a body fluid such as when urine is concentrated in the kidney. In plants, water channels are critical for water absorption in the root and for maintaining the water balance throughout the plant. Water channels are crucial for life and are found in all organisms, from bacteria to man.

Ion channels make it possible for cells to generate and transmit electrical signals, and are the basic molecular building blocks in the nervous system. Ion channels can be made to open and close in response to different stimuli (ligand binding, transmembrane voltage, temperature, mechanical stress), i.e., they are gated. Many ion channels are highly selective for a particular ion (Na^+, K^+, Ca^{2+}, Cl^-), and can reach very high transport rates (~10^8 ions per

second). In man, ion channels are involved in a whole range of diseases in organs such as the brain, the heart, and the muscles.

Water channels

The existence of channels mediating the flow of water and small solutes through biological tissues such as the wall of the urinary bladder or even across the membrane of individual cells was postulated as early as in the mid-nineteenth century (Brücke, 1843; Ostwald, 1890; Pfeffer, 1877). In the late 1950s, it was found that water is rapidly transported through the red blood cell membrane via water-selective channels that exclude ions and other solutes (Sidel and Solomon, 1957). Studies of water transport in various organisms and tissues over the next 30 years suggested that water channels have a narrow selectivity filter that prevents proton (H_3O^+) flow while maintaining a very high permeation rate for H_2O (up to 10^9 molecules per second); but even as late as 1987 nobody had been able to identify a water channel protein (Finkelstein, 1987) and the very concept of water-specific channels was still controversial.

The elusive water channels were finally discovered by Peter Agre. In the mid 1980s, Agre was studying Rh blood group antigens from the red cell membrane. In 1988, he isolated a new 28 kDa membrane protein of unknown function, CHIP28, from both red cells and renal tubules (Denker et al., 1988). After obtaining an N-terminal peptide sequence (Smith and Agre, 1991) and then the whole cDNA sequence (Preston and Agre, 1991) of CHIP28, he realized that this might be the long-sought-after water channel. Shortly thereafter, Agre proved this conclusively by demonstrating that expression of CHIP28 in *Xenopus* oocytes made the cells swell rapidly when placed in a hypo-osmotic medium (Preston et al., 1992), Fig. 1. The same phenomenon was observed when purified CHIP28 was reconstituted into liposomes (Zeidel et al., 1992). In both cases, swelling was inhibited by Hg^{2+}, a treatment known to block water transport across the red cell membrane.

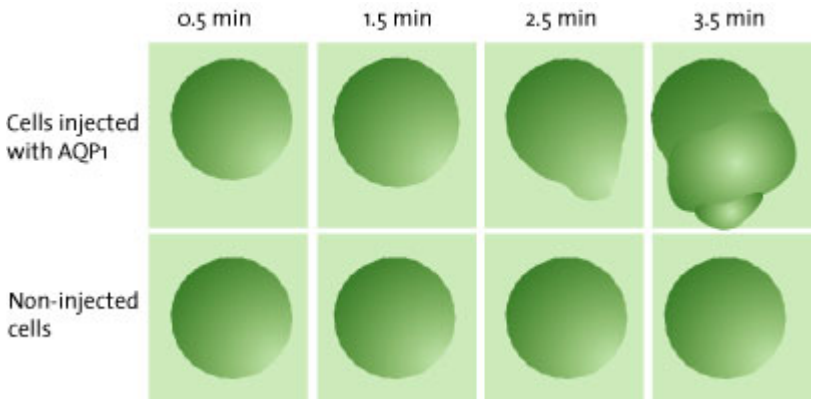

Fig.1. *Xenopus* oocytes microinjected with AQP1 mRNA swell rapidly when placed in a hypo-osmotic medium, in contrast to noninjected oocytes.

The discovery of CHIP28 (now called aquaporin 1 or AQP1) was a decisive moment in the study of cell water channels. Aquaporin-like proteins have since been found throughout the living world; in humans alone, there are at least 11 different aquaporin-like proteins, many of which have been linked to various diseases (Agre et al., 2002; Schrier and Cadnapaphornchai, 2003). Plants have an even higher number of aquaporins, with no less than 35 different versions found in the model plant *Arabidopsis thaliana* (Javot and Maurel, 2002).

The physiological importance of the aquaporins is perhaps most conspicuous in the kidney, where some 150-200 liters of water need to be resorbed from the primary urine each day. This is made possible mainly by the AQP1 and AQP2 aquaporins. AQP1 is expressed in the proximal tubules and the descending vasa recta, while AQP2 is expressed in the collecting duct. The expression of AQP2 at the plasma membrane is regulated by vasopressin, and decreased or increased AQP2 levels have been associated with nephrogenic diabetes insipidus as well as with several conditions associated with fluid retention such as congestive heart failure (King and Yasui, 2002).

In 2000 and 2001, the first high-resolution 3D structures of AQP1 and a related glycerol-selective bacterial channel protein (GlpF) were reported (Fu et al., 2000; Murata et al., 2000; Ren et al., 2001; Sui et al., 2001). Based on these structures, detailed models have been put forward to explain the high permeation rate, the strict water selectivity, and the ability of AQP1 to prevent proton leakage (de Groot and Grubmüller, 2001; Tajkhorshid et al., 2002). In essence, the architecture of the channel allows water molecules to pass only in single file, and positively charged residues in the channel repel H_3O^+. Furthermore, the local electrostatic field generated by the protein switches polarity in the middle of the channel, forcing the passing water molecules to rotate in such a way that their dipole moments are oriented in opposite directions in the upper and the lower halves of the channel. This reorientation prevents the formation of a continuous network of hydrogen-bonded water molecules across the channel, and thus blocks the passage of protons via "proton hopping" (also called the Grotthuss mechanism after (Grotthuss, 1805)).

In the short span of just over ten years, an almost complete atomic-level understanding of water channel function has been reached, the physiological roles of water channels in both eukaryotic and prokaryotic organisms have been elucidated, and their role in health and disease are becoming increasingly well documented. Agre's unexpected discovery of the aquaporins revolutionized the study of water transport, and laid a firm biochemical foundation for a very important area of physiology and medicine.

Ion channels

As early as 1890, Wilhelm Ostwald (Nobel laureate in chemistry 1909) suggested, based on experiments with artificially prepared colloidal membranes, that electrical currents in living tissues might be caused by ions moving across cellular membranes (Ostwald, 1890). Work in the early 1900s (Bernstein, 1902; Loeb and Beutner, 1912) established that membrane potentials are electrochemical in nature, and in 1925 the existence of narrow ion channels was proposed (Michaelis, 1925).

Work by Hodgkin and Huxley in the early 1950s on ion transport across the membrane of the squid giant axon (for which they were awarded the Nobel Prize in physiology or medicine in 1963) ushered in the modern era of neurophysiology, and rapidly led to a very detailed model for the action potential in nerve cells based on the idea that separate, voltage-gated ion channels for Na^+ and K^+ (and sometimes Ca^{2+}) are present in the membrane (Hodgkin, 1970; Huxley, 1970). It was also demonstrated that potassium ions move through the membrane in single file (Hodgkin and Keynes, 1955), further substantiating the idea of membrane-embedded channel structures. The central concepts of rapid transport, ion selectivity, channel gating, and channel inactivation were clearly identified already at this early stage, but the underlying molecular mechanisms were totally unclear.

Biochemical work in a number of laboratories during the 1960s and 1970s on the ligand-gated acetylcholine receptor (a member of the Cys-loop ion channel family) from the electric ray *Torpedo californica* led to the first biochemical identification of an ion channel protein, and low-resolution structural studies of the acetylcholine receptor showed a large extracellular funnel leading to a narrow membrane channel (Kistler et al., 1982; Ross et al., 1977).

By the early 1970s, the dimensions of the 'selectivity filter' in neuronal voltage-gated Na^+ and K^+ channels (members of the P-loop ion channel family) had been measured using biophysical techniques, and the notion that the gate and the selectivity filter are separate structural elements was established (Armstrong, 1975). Very detailed studies of ion permeation were made possible by the technique of single-channel recordings introduced by Neher and Sakmann (Nobel laureates in physiology or medicine in 1991), and when this technique was combined with the possibility to clone, mutagenize, and express ion channel proteins in cells such as *Xenopus* oocytes (Noda et al. 1982, 1983), rapid progress in mapping different functional regions of various ion channels ensued.

By the mid 1990s, it was clear that the P-loop ion channels must have a narrow selectivity filter near their extracellular end and a separate gate near their intracellular end. It was proposed that selectivity was achieved by the proper placement of oxygen atoms in the selectivity filter in a way that ions of

the correct radius could be preferentially desolvated when entering the narrow filter. The segment of the protein that forms the selectivity filter – the P-loop – had thus been identified. The detailed molecular design of the selectivity filter and the mechanisms responsible for gating were unknown, however, and it was clear that little further progress would be possible unless high-resolution structural data could be obtained (Armstrong and Hille, 1998).

It is very difficult to determine high-resolution 3D structures for membrane proteins, and ion channels are no exception. In particular, eukaryotic membrane proteins seem to be more difficult to handle than prokaryotic ones, and the cloning and overexpression of a bacterial K^+ channel with high homology to eukaryotic K^+ channels (Schrempf et al., 1995) suggested to some workers that prokaryotic channels might finally provide the missing key to structural studies of ion channels.

The breakthrough came in 1998, when Roderick MacKinnon succeeded in determining the first high-resolution structure of an ion channel, the KcsA K^+ channel from *Streptomyces lividans*, (Doyle et al., 1998). The design of the selectivity filter was seen to be perfectly adapted to the job of desolvating potassium ions while keeping smaller sodium ions out, Fig. 2, thus explaining the high K^+ selectivity and the high transport rate. At higher resolution, hydrated potassium ions could even be seen in "hold position" on both sides of the selectivity filter (Zhou et al., 2001b), and it became clear that the selectivity filter is composed of a succession of K^+ binding sites that each almost exactly mimics the hydration shell normally present around a potassium ion.

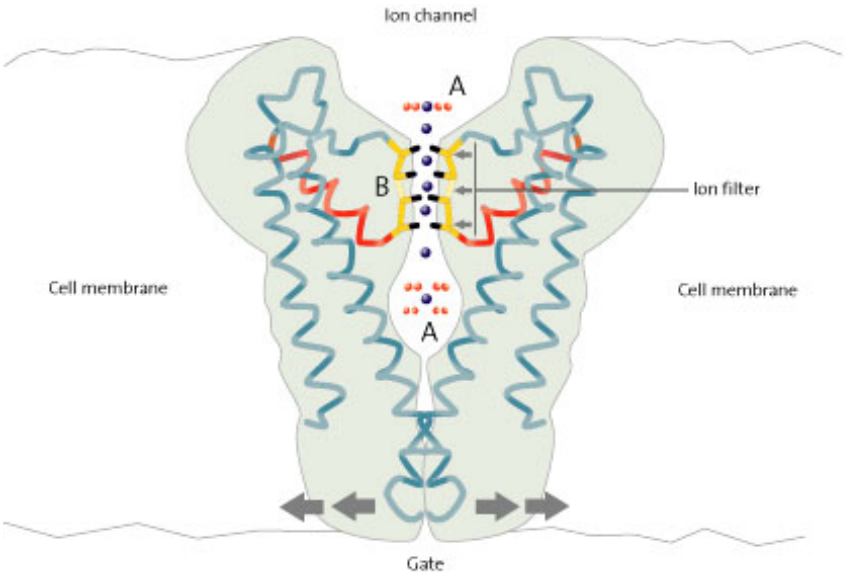

Fig. 2. The KcsA K^+ channel. Fully or partially hydrated potassium ions (blue) are seen just below and above the selectivity filter. In the selectivity filter, potassium ions are coordinated by oxygen atoms (black) in the protein backbone. The channel is closed by a gate in the lower part(arrows). The gate can be opened by sensor domains (not shown) that pull the gate open.

The KcsA structure showed the channel in a closed conformation. The structure of the Ca^{2+}-activated bacterial K^+ channel MthK, again solved by MacKinnon (Jiang et al., 2002a), captured the channel in an open conformation. A comparison of the KcsA and MthK structures suggested a general mechanism for channel gating, in which a conformational change in the sensor domain pulls the transmembrane helices apart near the intracellular end of the channel (Jiang et al., 2002b), Fig. 2.

Some K^+ channels conduct ions in only one direction, serving as "molecular diodes". Such inward rectifying channels are blocked by Mg^{2+} and polyamines that penetrate into the channel from its cytosolic end when the membrane is depolarized. The first structure of a domain responsible for inward rectification was presented by MacKinnon in 2002 (Nishida and MacKinnon, 2002), showing a cytoplasmic extension to the basic pore structure lined with acidic and hydrophobic residues that lengthens the ion channel to almost 60 Å and provides internal biding sites for polyamines. The molecular basis for another important kind of channel inactivation process - ball-and-chain inactivation - was clarified by mutation analysis of a eukaryotic K^+ channel homologous to KcsA (Zhou et al., 2001a).

As already shown by Hodgkin and Huxley, in excitable cells such as nerve, muscle, and endocrine cells, voltage-induced gating of ion channels is the central principle of activation. Very recently, MacKinnon solved the structure of the archaeal voltage-gated K^+ channel KvaP in a complex with antibody fragments directed against the voltage sensor domain (Jiang et al., 2003). Interestingly, the antibody fragments appear to have pulled the sensor domains away from the ion channel itself. The precise structure of a non-perturbed voltage gated channel is thus still unknown, but the work nevertheless provides a first insight into the structural details of the voltage-sensing mechanism.

During the past few years, structural work has also begun to shed light on the molecular function of mechanosensitive (Bass et al., 2002; Chang et al., 1998) and Cl^- selective ion channels (Dutzler et al., 2002; Dutzler et al., 2003). In parallel, X-ray and electron crystallography studies have led to successively better structural models of the acetylcholine receptor (Brejc et al., 2001; Miyazawa et al., 2003; Toyoshima and Unwin, 1988; Unwin, 1993; Unwin, 1995).

MacKinnon's structural and mechanistic work on K^+ channels has unraveled the molecular underpinnings of ion selectivity, gating, and inactivation; and has opened up entirely new possibilities for very detailed biochemical, biophysical and theoretical studies of ion channel function. His discoveries also provide a firm basis for a molecular understanding of many neurological,

muscular, and cardiac diseases (Cooper and Jan, 1999; Hatta et al., 2002), opening up new possibilites for drug design.

Final remarks

Membrane channels allow rapid, selective, and regulated transport of water, ions and small solutes across biological membranes. They are found in all living cells, and underlie critical cellular functions such as neuronal signalling, muscle contraction, cardiac function, water resorption in the kidney, water uptake in plant roots, and the response to osmotic stress in microorganisms.

The rapid progress in our understanding of membrane channel function over the past decade is in large part due to fundamental discoveries concerning water and ion channels. Peter Agre's discovery of the aquaporin water channels and Roderick MacKinnon's detailed structural and mechanistic studies of K^+ channels are singular achievements that have made it possible for us to see these exquisitely designed molecular machines in action at the atomic level.

References

Agre, P., King, L. S., Yasui, M., Guggino, W. B., Ottersen, O. P., Fujiyoshi, Y., Engel, A., and Nielsen, S. (2002). Aquaporin water channels--from atomic structure to clinical medicine, J Physiol *542*, 3-16.

Armstrong, C. M. (1975). Ionic pores, gates, and gating currents, Quart Rev Biophys *7*, 179-210.

Armstrong, C. M., and Hille, B. (1998). Voltage-gated ion channels and electrical excitability, Neuron *20*, 371-380.

Bass, R. B., Strop, P., Barclay, M., and Rees, D. C. (2002). Crystal structure of *Escherichia coli* MscS, a voltage-modulated and mechanosensitive channel, Science *298*, 1582-7.

Bernstein, J. (1902). Untersuchungen zur Thermodynamik der biologischen Ströme, Pflügers Arch *92*, 521-562.

Brejc, K., van Dijk, W. J., Klaassen, R. V., Schuurmans, M., van Der Oost, J., Smit, A. B., and Sixma, T. K. (2001). Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, Nature *411*, 269-276.

Brücke, E. (1843). Beiträge zur Lehre von der Diffusion tropfbarer Flüssigkeiten durch poröse Scheidewände, Am Phys Chem *58*, 77-94.

Chang, G., Spencer, R., Lee, A., Barclay, M., and Rees, D. (1998). Structure of the MscL homolog from *Mycobacterium tuberculosis*: A gated mechanosensitive ion channel, Science *282*, 2220-2226.

Cooper, E. C., and Jan, L. Y. (1999). Ion channel genes and human neurological disease: Recent progress, prospects, and challenges, Proc Natl Acad Sci USA *96*, 4759-4766.

de Groot, B. L., and Grubmüller, H. (2001). Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF, Science *294*, 2353-2357.

Denker, B. M., Smith, B. L., Kuhajda, F. P., and Agre, P. (1988). Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules, J Biol Chem *263*, 15634-15642.

Doyle, D., Cabral, J., Pfuetzner, R., Kuo, A., Gulbis, J., Cohen, S., Chait, B., and MacKinnon, R. (1998). The structure of the potassium channel: Molecular basis of K^+ conduction and sensitivity, Science *280*, 69-77.

Dutzler, R., Campbell, E. B., Cadene, M., Chait, B. T., and MacKinnon, R. (2002). X-ray structure of a ClC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity, Nature *415*, 287-94.

Dutzler, R., Campbell, E. B., and MacKinnon, R. (2003). Gating the selectivity filter in ClC chloride channels, Science *300*, 108-112.

Finkelstein, A. (1987). Water movement through lipid bilayers, pores, and plasma membranes (New York, Wiley Interscience).

Fu, D., Libson, A., Miercke, L. J., Weitzman, C., Nollert, P., Krucinski, J., and Stroud, R. M. (2000). Structure of a glycerol-conducting channel and the basis for its selectivity, Science *290*, 481-6.

Grotthuss, C. J. T. (1805). Mémoire sur la décomposition de l'eau et des corps qu'elle tient en dissolution à l'aide de l'électricité galvanique (Rome).

Hatta, S., Sakamoto, J., and Horio, Y. (2002). Ion channels and diseases, Med Electron Microsc *35*, 117-126.

Hodgkin, A. L. (1970). The ionic basis of nerve conduction. In Nobel Lectures in Physiology or Medicine 1963-1970 (Amsterdam, Elsevier Publishing Company).

Hodgkin, A. L., and Keynes, R. D. (1955). The potassium permeability of a giant nerve fibre, J Physiol *128*, 61-88.

Huxley, A. F. (1970). The quantitative analysis of excitation and conduction in nerve. In Nobel Lectures in Physiology or Medicine 1963-1970 (Amsterdam, Elsevier Publishing Company).

Javot, M., and Maurel, C. (2002). The role of aquaporins in root water uptake, Annal Bot *90*, 301-313.

Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R. (2002a). Crystal structure and mechanism of a calcium-gated potassium channel, Nature *417*, 515-522.

Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B. T., and MacKinnon, R. (2002b). The open pore conformation of potassium channels, Nature *417*, 523-526.

Jiang, Y., Lee, A., Chen, J., Ruta, V., Cadene, M., Chait, B. T., and MacKinnon, R. (2003). X-ray structure of a voltage-dependent K^+ channel, Nature *423*, 33-41.

King, L. S., and Yasui, M. (2002). Aquaporins and disease: lessons from mice to humans, Trends Endocrinol Metab *13*, 355-360.

Kistler, J., Stroud, R. M., Klymkowsky, M. W., Lalancette, R. A., and Fairclough, R. H. (1982). Structure and function of an acetylcholine receptor, Biophys J *37*, 371-383.

Loeb, J., and Beutner, R. (1912). Über die Potentialdifferenzen an der unversehrten und verletzten Oberfläche pflanzlicher und tierische Organe, Biochem Z *41*, 1-26.

Michaelis, L. (1925). Contribution to the theory of permeability of membranes for electrolytes, J Gen Physiol *8*, 33-59.

Miyazawa, A., Fujiyoshi, Y., and Unwin, N. (2003). Structure and gating mechanism of the acetylcholine receptor pore, Nature *424*, 949-955.

Murata, K., Mitsuoka, K., Hirai, T., Walz, T., Agre, P., Heymann, J. B., Engel, A., and Fujiyoshi, Y. (2000). Structural determinants of water permeation through aquaporin-1, Nature *407*, 599-605.

Nishida, M., and MacKinnon, R. (2002). Structural basis of inward rectification: cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 Å resolution, Cell *111*, 957-965.

Ostwald, W. (1890). Elektrische Eigenschaften halbdurchlässiger Scheidewände, Z Phys Chem *6*, 71-82.

Pfeffer, W. (1877). Osmotische Untersuchungen. Studien zur Zellmechanik. (Leipzig, Verlag von Wilhelm Engelmann).

Preston, G. M., and Agre, P. (1991). Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family, Proc Natl Acad Sci U S A *88*, 11110-11114.

Preston, G. M., Carroll, T. P., Guggino, W. B., and Agre, P. (1992). Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein, Science *256*, 385-387.

Ren, G., Reddy, V. S., Cheng, A., Melnyk, P., and Mitra, A. K. (2001). Visualization of a water-selective pore by electron crystallography in vitreous ice, Proc Natl Acad Sci U S A *98*, 1398-1403.

Ross, M. J., Klymkowsky, M. W., Agard, D. A., and Stroud, R. M. (1977). Structural studies of a membrane-bound acetylcholine receptor from *Torpedo californica*, J Mol Biol *116*, 635-659.

Schrempf, H., Schmidt, O., Kümmerlen, R., Hinnah, S., Müller, D., Betzler, M., Steinkamp, T., and Wagner, R. (1995). A prokaryotic potassium ion channel with two predicted transmembrane segments from *Streptomyces lividans*, EMBO J *14*, 5170-5178.

Schrier, R. W., and Cadnapaphornchai, M. A. (2003). Renal aquaporin water channels: from molecules to human disease, Prog Biophys Mol Biol *81*, 117-131.

Sidel, V. W., and Solomon, A. K. (1957). Entrance of water into human red cells under an osmotic pressure gradient, J Gen Physiol *41*, 243-257.

Smith, B. L., and Agre, P. (1991). Erythrocyte Mr 28,000 transmembrane protein exists as a multisubunit oligomer similar to channel proteins, J Biol Chem *266*, 6407-6415.

Sui, H., Han, B. G., Lee, J. K., Walian, P., and Jap, B. K. (2001). Structural basis of water-specific transport through the AQP1 water channel, Nature *414*, 872-8.

Tajkhorshid, E., Nollert, P., Jensen, M. O., Miercke, L. J., O'Connell, J., Stroud, R. M., and Schulten, K. (2002). Control of the selectivity of the aquaporin water channel family by global orientational tuning, Science *296*, 525-530.

Toyoshima, C., and Unwin, N. (1988). Ion channel of acetylcholine receptor reconstructed from images of postsynaptic membranes, Nature *336*, 247-250.

Unwin, N. (1993). Nicotinic acetylcholine receptor at 9 Å resolution, J Mol Biol *229*, 1101-1124.

Unwin, N. (1995). Acetylcholine receptor channel imaged in the open state, Nature *373*, 37-43.

Zeidel, M. L., Ambudkar, S. V., Smith, B. L., and Agre, P. (1992). Reconstitution of functional water channels in liposomes containing purified red cell CHIP28 protein, Biochemistry *31*, 7436-7440.

Zhou, M., Morais-Cabral, J. H., Mann, S., and MacKinnon, R. (2001a). Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors, Nature *411*, 657-661.

Zhou, Y., Morais-Cabral, J. H., Kaufman, A., and MacKinnon, R. (2001b). Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution, Nature *414*, 43-48.

Further reading

Agre, P. and Konozo, D. (2003). Aquaporin water channels: molecular mechanisms for human diseases. FEBS Letters (in press). http://www.elsevier.nl/febs/14/241/article.pdf

MacKinnon, R. (2003). Potassium channels. FEBS Letters (in press). http://www.elsevier.nl/febs/14/show/toc.htt

Yellen, G. (2002). The voltage-gated potassium channels and their relatives, Nature *419*, 35-42.

Fujiyoshi, Y., Mitsuoka, K., de Groot, B. L., Philippsen, A., Grubmüller, H., Agre, P., and Engel, A. (2002). Structure and function of water channels, Curr Opin Struct Biol *12*, 509-515.

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