MARTIN STEWARD

Water Channels in the Cell Membrane

Water is a vital constituent of all living organisms, but surprisingly little was known, until recently, about how water molecules pass through membranes. Martin Steward describes the discovery of a family of membrane proteins which form highly selective water channels, whose functions range from a role in urine production in animals to transpiration in plants.

Without water, life as we know it could never have evolved. From the outset, the unique physical properties of water provided an ideal environment for simple organic molecules and chemical reactions to develop gradually into living organisms. From their first appearance in the primordial soup, cells had to find ways of maintaining an aqueous internal medium, the cytoplasm, that was protected from changes in the external environment. A crucial step was the development of the plasma membrane.

The plasma membrane that surrounds each cell consists mainly of phospholipid molecules and proteins (see BIOLOGICAL SCIENCES REVIEW, Vol. 6, No. 4, pp. 16–20). The phospholipid molecules are arranged tail-to-tail in two layers so that their water-loving (hydrophilic) head groups point outwards into the aqueous medium, and their long, fatty, water-hating (hydrophobic) tails mingle in the middle (see Figure 1). Interspersed among the phospholipid molecules are various proteins, some of which span the whole thickness of the phospholipid bilayer. One of the major functions of the plasma membrane is to act as a barrier — it prevents undesirable foreign material from entering the cell, and it ensures that vital macromolecules and organelles are not lost from the cell. Because the membrane is such an effective barrier, the cell faces a problem — how to take up nutrients and get rid of waste products. For lipid-soluble molecules such as fatty acids or cholesterol this is no problem — they can diffuse through the cell membrane. Oxygen and carbon dioxide enter and leave the cell quite freely because they are small, uncharged molecules which can also diffuse through the phospholipid bilayer. More polar molecules and electrolytes, for example salt (sodium chloride, Na^+Cl^-) have to be selectively transported through the membrane by transport proteins. What about water?

Because water is such a small molecule, it is able to diffuse through the phospholipid bilayer to a limited extent. So all cell membranes have a small but finite water permeability. But some cells have membrane water permeabilities that are 100 to 1000 times higher than this basal level. These include red blood cells, the endothelial cells that line blood capillaries, and the epithelial cells that line parts of the kidney tubule.

DISCOVERY OF CHIP28

Although scientists had suspected the existence of pores or channels which specifically allow water to pass through some of these highly permeable membranes, it was quite by chance that the first water channel protein was isolated in 1988. At that time, a group of scientists in the USA was attempting to purify a protein from red blood cell membranes. The protein they were interested in was the one responsible for the Rhesus antigen — one of the blood group antigens, which make blood matching important before transfusions are given. A second protein, of about the same molecular mass (approximately 28 000 daltons), was found to be contaminating the preparations. It did not correspond to any known protein and it was present in surprisingly large amounts — about 150 000 molecules per red blood cell (2.4% of the total red blood cell membrane protein). Out of curiosity, the scientists purified the protein and determined the amino acid sequence of one end of the polypeptide chain. This information enabled them to use one of the tricks of molecular biology — the polymerase chain reaction (PCR) — to generate millions of copies of

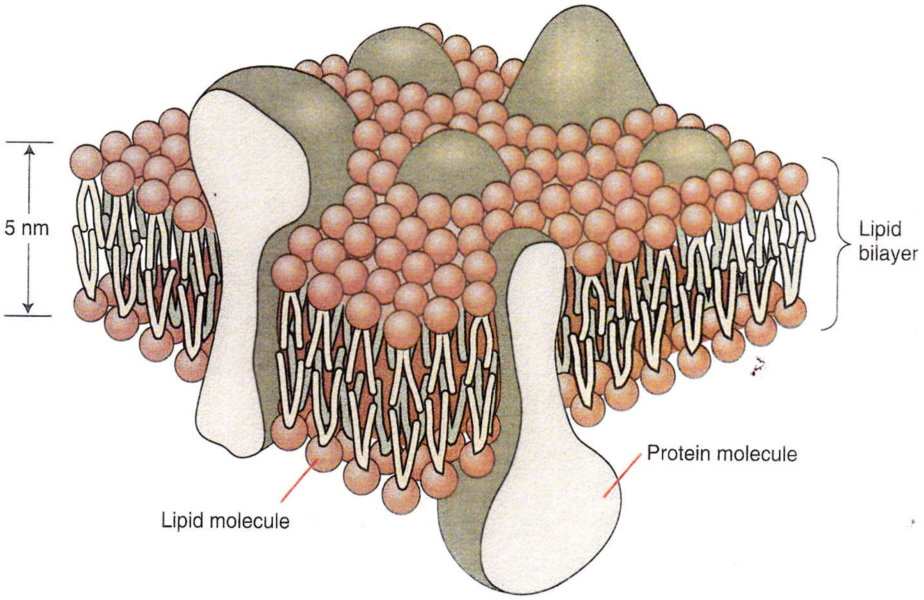


Figure 1 The plasma membrane consists of a bilayer of phospholipid molecules interspersed with membrane proteins, some of which span the whole thickness of the membrane. Each protein molecule, shown here as an amorphous blob, consists of a long polypeptide chain which may zig-zag back and forth across the bilayer several times.

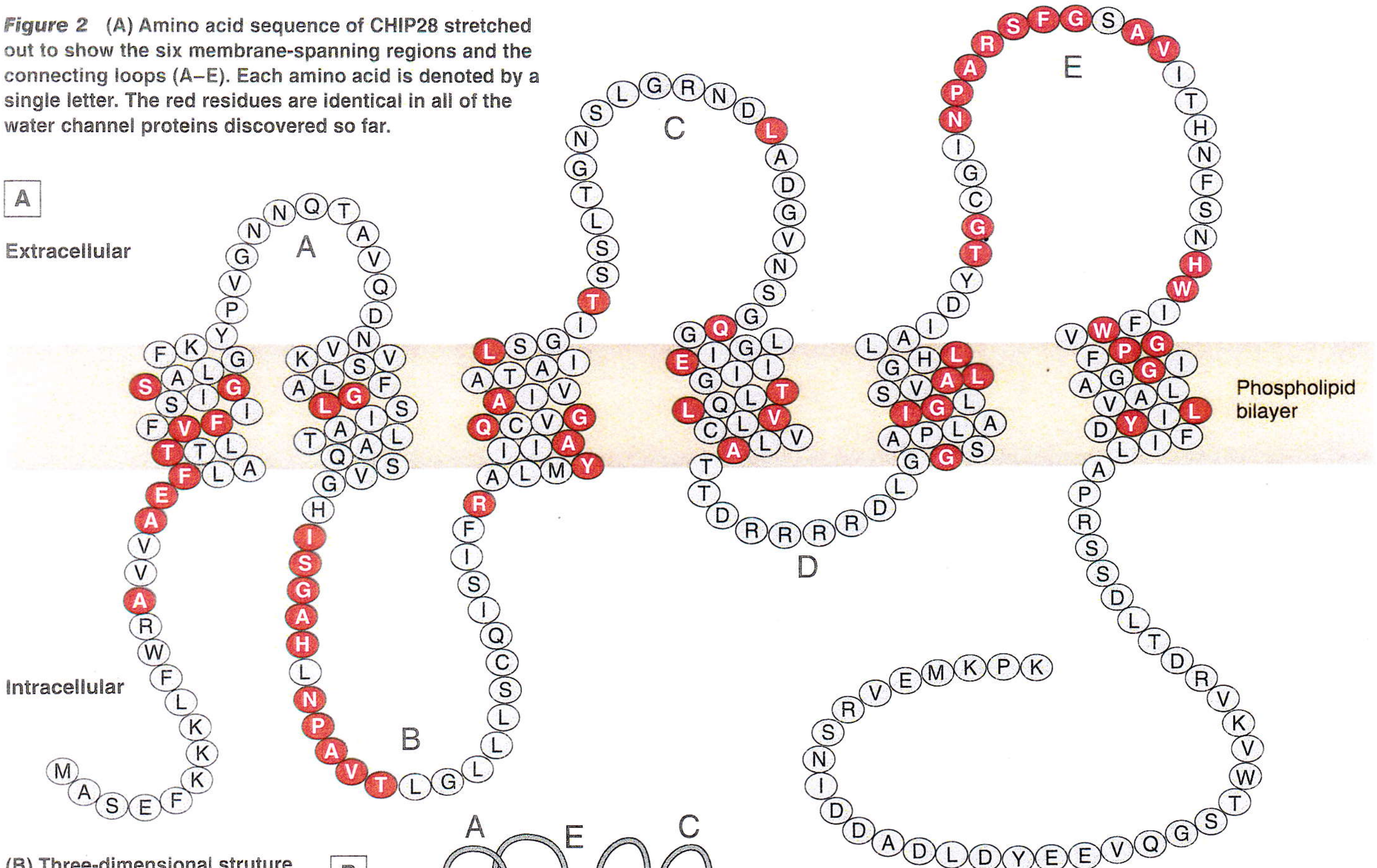
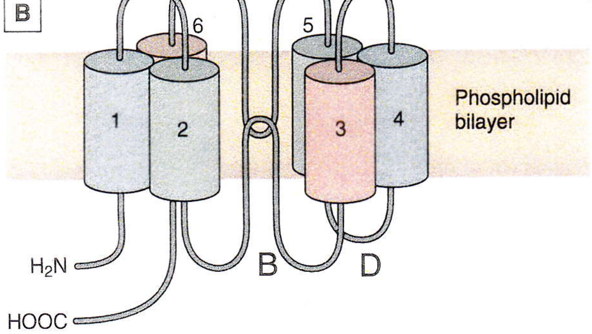


Figure 2 (A) Amino acid sequence of CHIP28 stretched out to show the six membrane-spanning regions and the connecting loops (A–E). Each amino acid is denoted by a single letter. The red residues are identical in all of the water channel proteins discovered so far.



(B) Three-dimensional struture of CHIP28. The six membrane-spanning regions shown in A are represented here by cylinders (1–6) clustered around a central pore formed by the B and E loops.

the full DNA sequence coding for the protein (see Biological Sciences Review, Vol. 4, No. 3, pp. 23–25). From the DNA sequence they were able to predict the complete amino acid sequence of the protein (see Figure 2A). They gave it the name CHIP28, which stood for '**CH**annel-forming **I**ntegral **P**rotein of molecular mass 28 000'. At that stage, however, it was not clear what it did.

STRUCTURE OF CHIP28

Given the amino acid sequence of a protein, molecular biologists can make predictions about what sort of protein it is. For example, the CHIP28 sequence has six regions containing large numbers of hydrophobic amino acids. Work on other membrane proteins has shown that these regions are common features of ion channels and transport proteins that span the phospholipid bilayer. The hydrophobic parts of these proteins are believed to form spiral structures within the bilayer which associate happily with the hydrophobic tails of the phospholipid molecules. CHIP28 appears to have six of these membrane-spanning regions (domains), so the polypeptide chain probably weaves its way back and forth across the membrane, as shown in Figure 2. The two ends of the polypeptide chain hang freely on the cytoplasmic side of the membrane, and the six membrane-spanning domains are connected by five short loops (A–E). Loops B and E are particularly important. Both contain several hydrophobic amino acids and are believed to dip into the phospholipid bilayer — one from the inside and one from the outside — thereby creating a pore through the membrane (see Figure 2B).

CHIP28 IS A WATER CHANNEL

Proof that CHIP28 is a water channel came in 1991 from experiments in which a cell with a very low water permeability was given the 'instructions' for synthesising CHIP28 (see Figure 3). Briefly, DNA coding for the CHIP28 protein was used to make messenger RNA (mRNA) and this was then injected into immature egg cells (oocytes) taken from a frog (*Xenopus laevis*). Over a period of 3 or 4 days, each oocyte synthesised large quantities of CHIP28, following the instructions in the mRNA as if it were mRNA coding for one of its own proteins. (This clever trick has been used to study various types of membrane proteins including ion channels from nerve cells and receptor proteins from muscle cells.) Having injected some of the oocytes with mRNA coding for CHIP28, and others with just a small volume of water (as an experimental control), the researchers then measured the water permeability of the plasma membrane using a very simple test. They transferred the oocytes from their normal saline bathing solution (similar in composition to the plasma of the frog) into saline diluted with water. Oocytes that had been injected with the CHIP28 mRNA swelled rapidly and soon burst, while the control oocytes swelled extremely slowly. The swelling was due to water flowing into the oocytes by osmosis (from the higher concentration of water molecules in the diluted bathing solution to the lower concentration of water molecules in the cytoplasm) and clearly the presence of CHIP28 made the plasma membrane much more permeable to water. Other experiments have shown that the CHIP28 water channel is remarkably selective. It allows water molecules, which are about 0.3 nm in diameter, to pass through the membrane, but it excludes charge-bearing ions of a similar size and uncharged molecules, such as urea (0.4 nm), that are only slightly larger.

THE MIP FAMILY

Quite early on it was noticed that CHIP28 has an amino acid sequence very similar to another membrane protein which had been discovered a few years earlier. This is the major intrinsic protein (MIP), found in the lens fibre cells of the eye. There are also similarities between CHIP28 and other MIP-like proteins including a membrane protein found in soybean root nodules, a glycerol transporter in bacteria, and a brain protein in the fruit fly. The MIP 'family' is clearly an extremely ancient one on the evolutionary time-scale. Some of the amino acid residues in the important B and E loops are always the same (conserved) in all these proteins, even though they are found in such very different organisms. This shows that these regions of the proteins are very important and any change in their amino acid sequence (such as might occur from random mutations) would be likely to destroy the function of the protein.

These 'sequence homologies' between the known members of the MIP family have enabled molecular biologists to use the PCR technique to search for related proteins in other organs and tissues — from plants as well as animals. In this way, four more water channel proteins have been identified in mammals — several of them, not surprisingly, are involved in kidney function. Others have been identified in amphibia and invertebrates, and many more have been found in plants. Those members of the MIP family that function as water channels are now known collectively as the 'aquaporins' — and CHIP28 has consequently been renamed **aquaporin-1 (AQP1)**.

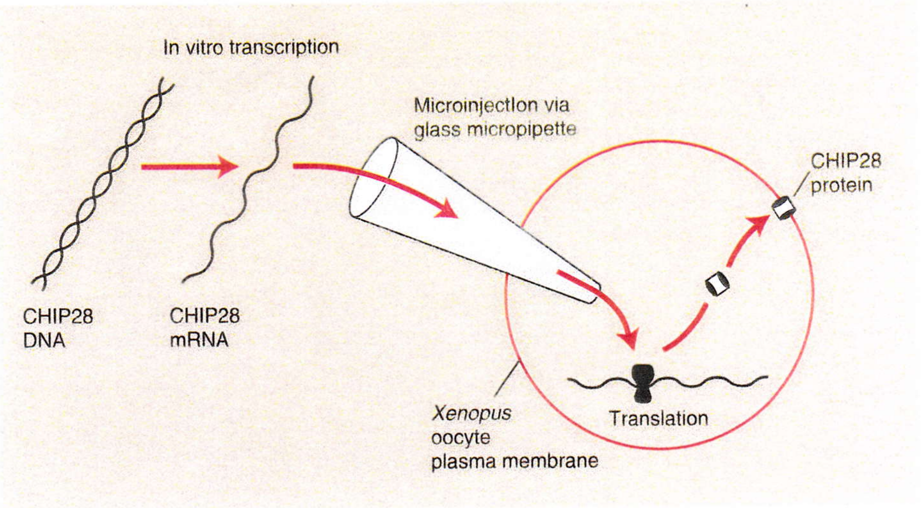
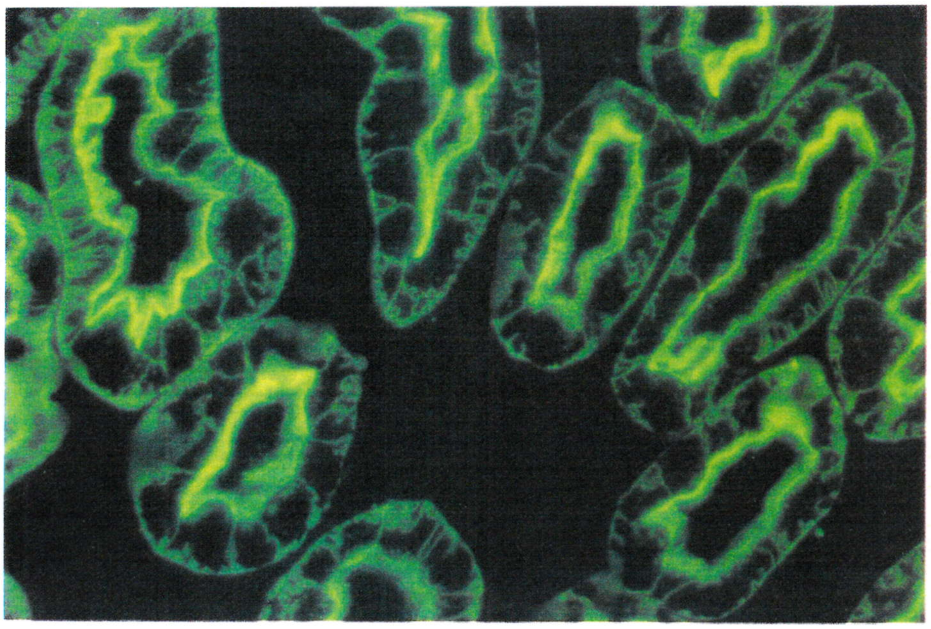


Figure 3 CHIP28 is synthesised by ribosomes in *Xenopus* oocytes that have been injected with mRNA made from the CHIP28 DNA sequence.

AQUAPORINS IN THE KIDNEY

The main function of the kidney is to rid the body of waste products. But at the same time it has to regulate water balance so that the body does not become dehydrated or over-expanded. The principle on which the kidney operates is rather clever. First of all, thousands of glomeruli — tiny bunches of leaky blood capillaries — use the blood pressure to filter the blood through the glomerular basement membrane, which acts as a very fine protein mesh (see BIOLOGICAL SCIENCES REVIEW, Vol. 9, No. 1, p. 5). The red and white cells and the plasma proteins are retained in the blood, but much of the water and the smaller molecules pass into the kidney tubules that lead away from the glomeruli (see Figure 4A). These solutes include both the waste products and valuable nutrients and minerals. As the filtrate travels down the tubule, the cells that line it selectively reabsorb the 'goodies' (e.g. glucose, NaCl) back into the bloodstream and allow the 'nasties' (e.g. urea) to carry on to the bladder. In addition, the kidney carefully regulates how much water is reabsorbed. About 85% of the filtered water is reabsorbed automatically by osmosis in the earlier parts of the tubule (the proximal tubule and the descending limb of the loop of Henle — see Figure 4A). If you are dehydrated, much of the remaining 15% is reabsorbed in the final part of the kidney tubule (the collecting duct) and only a small amount of very concentrated urine is excreted (antidiuresis). If you have been drinking a lot of water, only a little of the 15% is reabsorbed and you produce a lot of very dilute urine (diuresis).

At least four different aquaporins are involved in this process. Figure 4B shows the water permeabilities of the different parts of the tubule and where the aquaporins are located. Aquaporin-1 (CHIP28) is present in the proximal tubule and descending limb cells, and this enables them to reabsorb the 85% of the filtered water that is reabsorbed, regardless of the body's overall water balance. In the brain, the presence of aquaporin-4 in the membranes of specialised nerve cells in the hypothalamus enables them to monitor the osmotic pressure of the blood. These 'osmoreceptors' shrink when you are dehydrated and this triggers the release of a hormone — antidiuretic hormone (ADH) — from the nearby pituitary gland into the bloodstream. When ADH reaches the kidney, it instructs the collecting duct cells to make more aquaporin-2 and insert these water channel proteins into the plasma membrane where it faces the lumen of the duct. Both aquaporin-3 and



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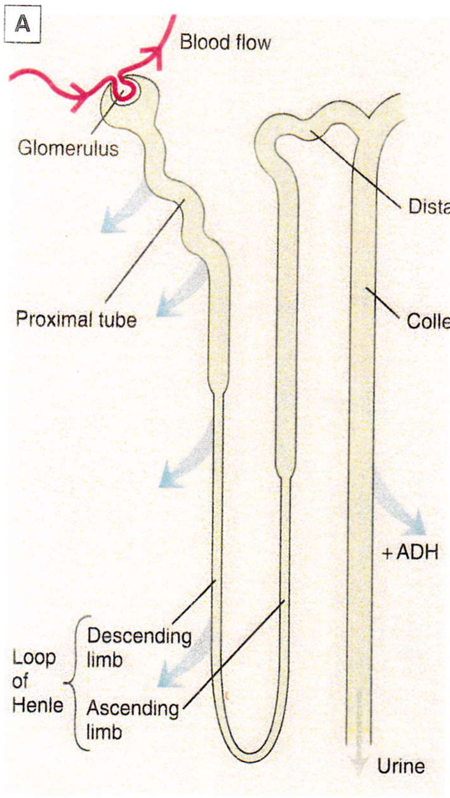
Fluorescence micrograph showing the distribution of aquaporin-1 water channels in the cortex of a rat kidney. Green = fluorescent antibodies bound specifically to the water channels in the section. Several proximal tubules are visible — each is about 60 µm in diameter and both the inner (luminal) and outer (peritubular) membranes are labelled with the antibodies.



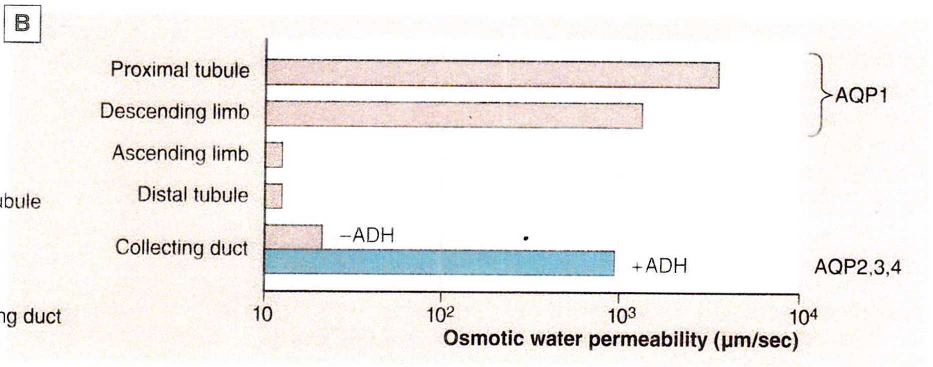


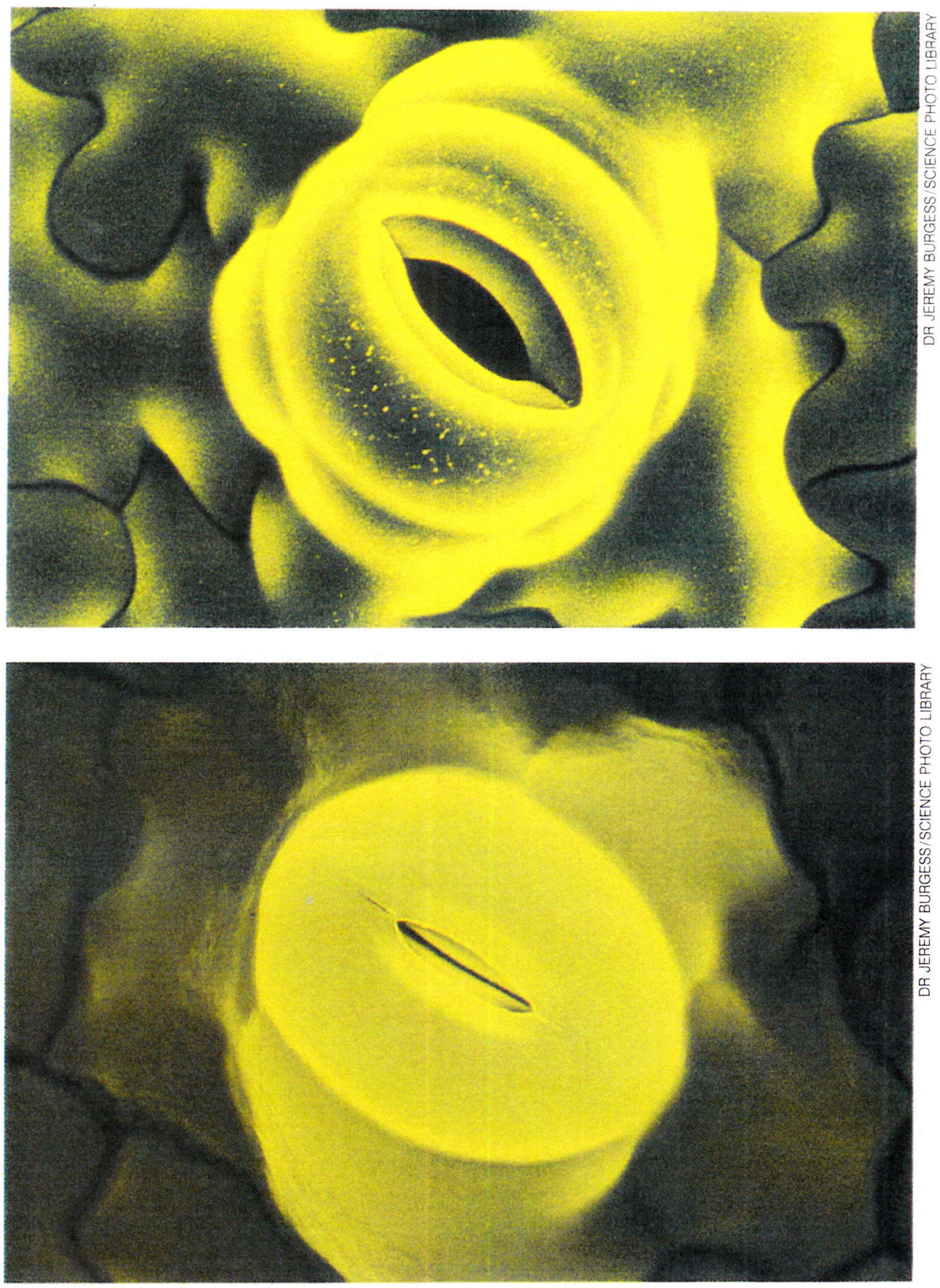
Figure 4 **(A) Water filtered at the glomerulus is reabsorbed (blue arrows) as it flows along the kidney tubule. (B) Water permeabilities of the different segments of the tubule, and location of aquaporins (AQP) 1–4. In the presence of ADH (blue bar), the water permeability of the collecting duct increases nearly 100 times as a result of AQP2 insertion into the membrane.**

aquaporin-4 are present in the plasma membrane on the blood side of the cells. Together they give the collecting duct a high water permeability, and much of the remaining 15% of the filtrate is drawn back into the blood by the very high salt and urea concentrations that are maintained in the central part (medulla) of the kidney. When the circulating ADH concentration falls, the aquaporin-2 molecules are removed from the plasma membrane, the permeability of the collecting duct decreases and the 15% of the filtrate is mostly excreted.

Aquaporins are not exclusively involved in kidney function. Aquaporin-1 is also found in blood capillaries and lymphatics, in the choroid plexus that secretes the fluid bathing the brain, and in various cells in the eye, liver and lungs. The newest member of the family — aquaporin-5 — is specifically expressed in the salivary glands and lacrimal glands and is probably involved in the secretion of saliva and tear fluid. Researchers in this field are convinced that many other mammalian aquaporins remain to be discovered.

AQUAPORINS IN PLANTS

The mammalian aquaporin list looks rather short compared with that of plants. For example, in one common species of weed (*Arabidopsis thaliana*) scientists have already identified 22 different aquaporins! Water movement through the plant occurs primarily during daylight hours when the pores (stomata) in the leaves are open. The path of water is from the soil through the root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False-colour scanning electron micrographs showing (top) the daylight condition for most stomata — open — and (bottom) the night-time or water-stressed condition of a stoma — closed. (× 1300)

cortex into the xylem, a system of hollow tubes that conducts water all the way to the leaves and then through the mesophyll tissue (the cells that carry out photosynthesis), from where water molecules escape into the air through the open stomata. To pass through the living tissue (cortex and mesophyll), water can either flow between the cells (the apoplastic route) or through them (the symplastic route), as shown in Figure 5. Water entering or leaving the symplast has to cross the plasma membrane; it is here that aquaporins known as **PIP**s (plasma membrane intrinsic proteins) are located.

Many plant cells are occupied by large, fluid-filled vacuoles, so water flows more easily through the symplast if it is able to pass through the vacuole — in on one side and out of the other. It is therefore in the vacuolar membrane (tonoplast) that we find another group of aquaporins — the **TIP**s (tonoplast intrinsic proteins).

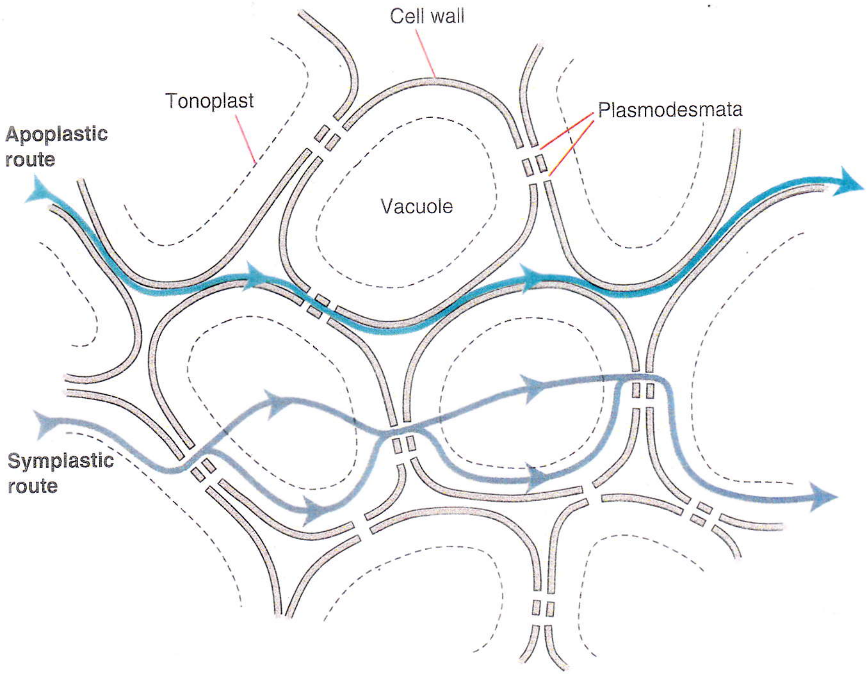
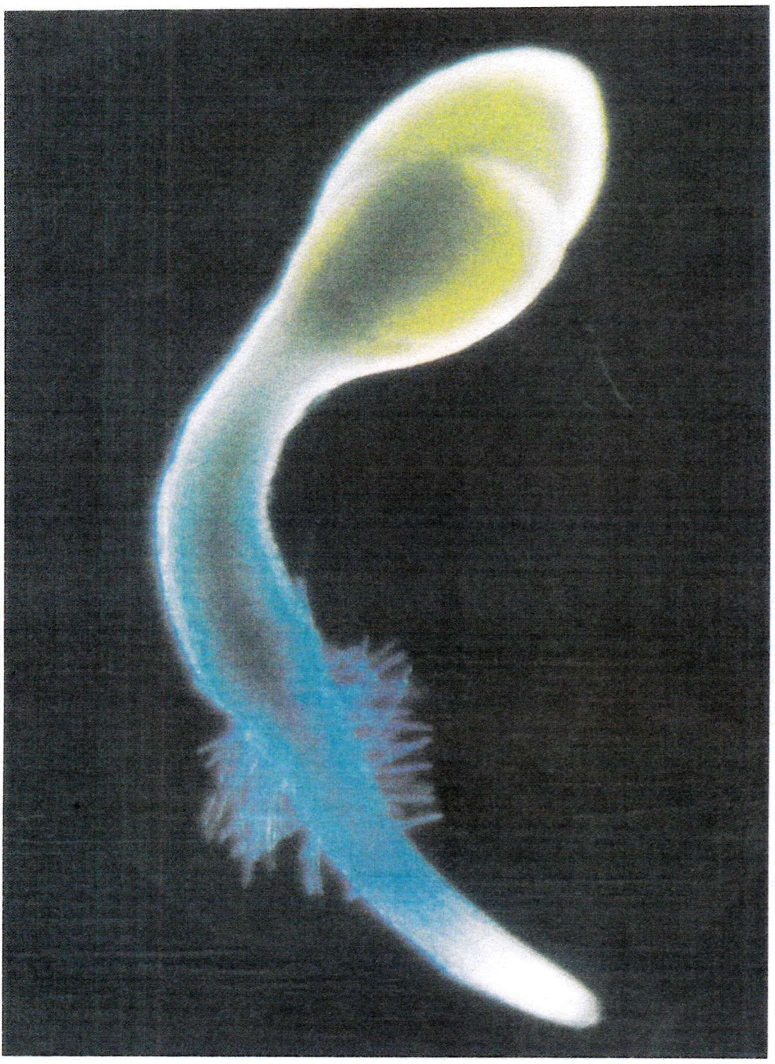


Figure 5 **Apoplastic and symplastic routes for water flow through plant tissue.**

Different TIPs and PIPs are expressed, in varying amounts, in all of the plant organ systems — roots, leaves, fruits and stems. For example, γ-TIP is particularly plentiful in the elongation zone of growing roots, where it helps water flow into the cells as they push their way through the soil. α-TIP is specific to seeds and may be involved in the earliest stages of germination. Another of the TIPs, RD28, is expressed specifically during water stress (drought) and it probably helps the cells to maintain their turgor. Finally, there are root-knot nematodes which cause serious damage to crops by deliberately instructing the root cells from which they feed to produce greater numbers of PIP water channels.

FUTURE PROSPECTS

The aquaporin story is a good example of how modern molecular biology can give us fresh insights into familiar biological processes. For example, this new knowledge enables us to understand genetic diseases in man like nephrogenic diabetes insipidus, where patients excrete huge quantities of dilute urine because the collecting ducts in the kidneys are unable to respond to ADH. One form of this disease is now known to be due to a mutation in the gene coding for aquaporin-2, which prevents its insertion into the membranes of the collecting duct cells. Our knowledge of plant aquaporins may one day help us to develop better drought- and pest-resistant crops. These fascinating new discoveries also remind us of the common origins of all living organisms. And the fact that human kidneys and plant leaves use such similar proteins to control water excretion is a sure sign of the very special place that water has in the evolution of life on this planet.



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Distribution of the aquaporin γ-TIP in an *Arabidopsis thaliana* seedling. The blue colour is the product of a chemical reaction showing where the channels are — confined to the growing elongation zone behind the root tip.

FURTHER READING

Alberts, B., et al. (1994) *Molecular Biology of the Cell* (3rd edn.), Garland Publishing.

Laver, H. (1995) 'Osmosis and water retention in plants', *Biological Sciences Review*, Vol. 7, No. 3, pp. 14–16.

Lowe, A. (1996) 'Water', *Biological Sciences Review*, Vol. 8, No. 3, pp. 38–41.

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