

Aquaporins. A Molecular Entry into Plant Water Relations

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Water relations are obviously crucial to the physiology of terrestrial plants, but 25 years ago most plant biologists viewed this area of research as somewhat of an oddity. Dominated by equations and unusual physical theories about liquids flowing in pipes, water relations seemed to be a field with little seeming need for the burgeoning concepts and approaches offered by molecular and cellular biology. The discovery of aquaporins united these two "cultures" of biophysicists and molecular geneticists and piqued the interest of cell and molecular biologists in plant water relations. Early (and present day) plant biophysicists, exploiting sophisticated physical theories, formulated the general and unifying concept that water flow through plant cells and tissues can be understood as the product of motive force and conductance (3, 16). In plants, under natural conditions, water uptake by the roots and loss from the leaves are driven by ever changing forces, and plants keep a proper water balance by continuously adjusting the water conductance of their tissues. The concept of water potential unifies the description of these forces, whether hydrostatic, osmotic, matrix-derived, or gravitational in nature (3, 16). However, the in situ measurement of these forces is subject to pitfalls, as exemplified by current controversies over the mechanisms of the ascent of xylem sap. Water potential gradients can also be experimentally manipulated and in these studies the conductance of plant cells and tissues has long been viewed as a black box (16). How do cells regulate their conductance in this black box?

WATER CHANNELS: A REVOLUTIONARY DISCOVERY?

Vascular tissues and guard cells play a central role in conducting water and controlling the transpiration stream, but to get in and out of vascular tissues water has to flow through living cells. When water flows across living tissues, it can follow different routes: across cell walls (apoplastic path), from cell to cell across either the plasmodesmata (symplastic path), or traversing the cell membranes (transcellular path). Biophysical criteria have been developed to distinguish between the apoplastic and cell-to-

cell paths (16). These studies will now benefit from recent biochemical and genetic descriptions of critical cell barriers, such as the root endodermis with its Casparian bands, and from new insights into the molecular mechanisms of plasmodesmatal gating. The most significant breakthrough to date in understanding conductance of living cells has come from the discovery of a class of water channel proteins named aquaporins (1). Aquaporins have now been found in nearly all living organisms. They belong to a larger family of membrane proteins homologous to bovine major intrinsic protein (MIP) and exhibit a typical structure with six membrane-spanning domains and an internal symmetry showing two highly conserved Asn-Pro-Ala motifs (Fig. 1). Because of their abundance, plant MIP homologs were identified in the late 1980s (4), but several years went by before it was recognized that some of them can function as highly efficient water channels and facilitate the diffusion of enormous amounts of water along transmembrane water potential gradients (10). When this discovery was presented at the Plant Membrane Conference in Monterey, CA in 1992, audience reactions clearly showed that the discovery was out of step with the current thinking of many. At that time, and in contrast to what had been established in erythrocyte and kidney membranes, it was commonly assumed that water flow across the lipid moiety of plant membranes would be sufficient to take care of all cellular needs. However, aqueous channels in plant membranes had been discussed more than 25 years before (3), and functional evidence had been provided by a laboratory (20). It has been argued that the discovery of aquaporins did not revolutionize the biophysical basis of plant water relations (17), and this may well be true. Yet, we believe that the identification of aquaporins has greatly changed our thinking about how plants might regulate water flow, well beyond the simple representation of water-filled pores in a model membrane. The discovery of aquaporins constitutes a conceptual advance in plant physiology.

NEW PERSPECTIVES ON THE MOLECULAR AND CELLULAR BIOLOGY OF MEMBRANES

In contrast to what was initially assumed, specific membrane lipid compositions can hardly account for

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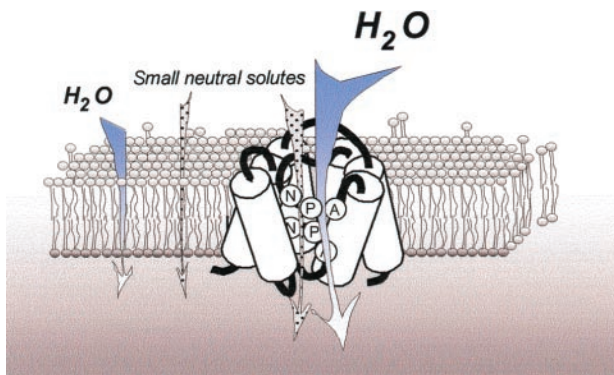


Figure 1. Aquaporins facilitate the diffusion of water and small neutral solutes across plant cell membranes. The residual permeability of the lipid membrane to these molecules is also indicated. The capacity of some aquaporins to transport solutes was demonstrated using test molecules such as glycerol and urea, and the physiological significance of this property is not yet clearly understood (see text). Plant aquaporins similar to their animal counterparts are thought to form tetramers, each monomer being functionally independent. The putative structure of an aquaporin monomer with six tilted membrane-spanning domains is shown. Studies on the structure of human AQP1 suggest that the two connecting loops that contain the highly conserved Asn-Pro-Ala motifs dip into the membrane and form part of the aqueous pore.

some of the high water permeability values ($>100 \mu\text{m s}^{-1}$) that were initially reported for plant membranes. Thus, the discovery of aquaporins gave strong support to the idea that such high water permeability properties might be required for certain physiological processes. Most importantly, aquaporins immediately provided a solid molecular basis for the fast and reversible regulation of transmembrane water transport. Once a protein is involved, the cell has the ability to regulate its abundance (transcriptional or post-transcriptional regulation) or to modulate its activity. Gene expression of aquaporins is regulated developmentally in a cell-specific manner, via hormones, and by environmental signals as diverse as nematode infection or drought (reviewed in 5 and 15). Reversible phosphorylation controls the activity of some plant aquaporins (9). In spinach leaves, for instance, the phosphorylation level of a plasma membrane aquaporin is dependent on the apoplastic water potential, thus establishing an intriguing link between driving force and conductance (reviewed in 5). Recent work done in animals points to other mechanisms for aquaporin regulation, through pH or free calcium change or controlled protein targeting (reviewed in 19). Exquisite gating control by aquaporins as has been revealed for ion channels can thus be expected. With these and new regulatory mechanisms to be discovered in plants, the water transport properties of membranes now appear as a new and important focus in modern plant cell physiology. Tools, such as the cell pressure probe, which were developed earlier to measure water transport at the cellular level (16), are thus gaining a renewed interest.

After the discovery of the first aquaporins, a second surprising finding has been their amazing diversity in plants (21). More than 30 genes encode aquaporin homologs in *Arabidopsis*. Based on sequence homology, plant aquaporins can be classified into three plant-specific subfamilies (5, 15), two of which appear to correspond to distinct subcellular locations. Tonoplast intrinsic proteins (TIPs) and plasma membrane intrinsic proteins (PIPs) reside mainly in the vacuolar and plasma membrane, respectively. Nodulin 26-like MIPs occur in the peribacteroid membrane of nitrogen-fixing nodules (4), at least in legumes, but their location in other plants remains to be discovered (21). Although all proteins in the PIP clade may not be in the plasma membrane (7) and all proteins in the TIP clade in the tonoplast, it is clear that distinct aquaporin isoforms are targeted to distinct subcellular compartments and aquaporins have emerged as important markers of plant cell membrane differentiation. For instance, antibodies raised against various TIP homologs have been used to distinguish distinct vacuolar subtypes or morphologies within the same plant cell (13), and PIP antibodies have revealed invaginated plasma membrane subdomains called plasmalemmasomes (15).

Early studies on plant cell water relations focused on the hydraulic conductivity of whole cells and assumed that it is determined by the plasma membrane. However, in contrast to animal cells, many plant aquaporins are located intracellularly. Several groups recently investigated the water transport properties of vesicles obtained from different cellular membranes. Vesicles derived from the tonoplast of root or suspension cells or from the peribacteroid membrane of nitrogen-fixing nodules exhibited water permeability values (11, 12, 14) that exceeded by severalfold that of the red blood cell membrane, which has long been considered the prototype of the water channel-containing membrane. These findings made us rethink the osmoregulation of plant cells in relation to their high degree of compartmentation. The cytoplasm, which represents only a minor fraction of the overall cell volume, must be critically sensitive to any differential flow of water occurring across its limiting membranes. Efficient osmoregulation of this compartment can be achieved by rapid water transport across intracellular membranes, to provide quick volume equilibration and dissipation of water potential gradients within the cell (11, 12, 18).

A third surprising finding is the discovery that some aquaporins are multifunctional proteins. Homologs in microorganisms have a well-established role in the transport of osmocompatible solutes such as glycerol (1). Is it a biophysical curiosity that some plant aquaporins can be permeated by such molecules (14, 18) (Fig. 1)? If the relevance of this permeation can be proven in planta, the solute transporting properties of plant aquaporins may establish one more connection between driving force and conduc-

tance. The transport by plant aquaporins of gaseous substances, such as CO_2 or NH_3 , is an even more provocative idea that might be highly relevant in leaves or in symbiotic root nodules (18).

MEMBRANES, WATER TRANSPORT THROUGHOUT THE PLANT, AND DEVELOPMENTAL PLASTICITY

Whether at the cell or tissue level, an intrinsic difficulty in studying water transport is to distinguish between the parallel paths mentioned above. At the cell level, the regulatory properties and the high transport capacity of aquaporins have established a predominant role for these proteins with respect to the lipid membrane itself. At the tissue level, aquaporins point to the importance of transcellular water transport as an alternative to apoplastic and symplastic transport. The ubiquitous and highly regulated expression of aquaporins in plants also suggests that transmembrane water transport may be relevant to many processes other than those related to transpiration. For instance, regulation of aquaporin expression in flowers or during seed maturation and germination has been described (reviewed in 5, 15). More generally, aquaporins provide valuable spatial markers to explore the intricate flows of water and solutes that play a critical role throughout all plant development or in the response of plants to various biotic or abiotic stresses (Fig. 2).

After only a few years, the search for aquaporin function at the whole plant level has already given rise to new experimental approaches, mostly in the study of water uptake by roots. Roots can finely adjust their hydraulic conductivity in response to environmental changes, such as an increase in soil salinity, nutrient deprivation, day and night cycles, or anaerobiosis. Aquaporins could perfectly account for such regulation (18) and their function in roots is being investigated in several laboratories.

A critical issue that remains to be addressed is the collection of unambiguous evidence for aquaporin activity at the tissue or organ level. The pharmacology of aquaporins remains poor, and mercury derivatives are the only general blockers of aquaporins known to date. These compounds target Cys residues in aquaporins but, unfortunately, in other cellular proteins as well. Some laboratories have discounted these obvious side effects and characterized the effects of mercury on root water transport (2, 8). Although each individual study should be interpreted with caution, the consistency of the data provides compelling evidence that a significant part of root water transport (20%–80%) is under rapid metabolic control, and involves the activity of cellular proteins, most likely aquaporins.

Reverse genetics allows a more rigorous approach and may reveal unexpected functions of aquaporins. Transgenic *Arabidopsis* plants expressing an antisense copy of the *pip1b* gene showed reduced expres-

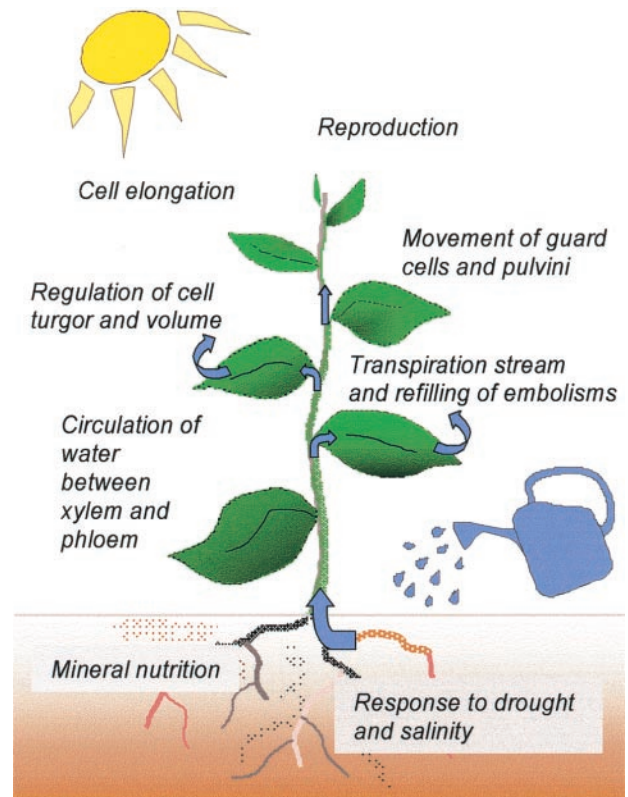


Figure 2. Aquaporins may be involved in a large number of physiological functions in plants. These functions were inferred from the expression patterns of specific aquaporin isoforms or from the blocking effects of mercury on water transport through plant tissues.

sion of several PIP1 homologs and provided definitive evidence for the contribution of aquaporins to plasma membrane water transport (6). Surprisingly, these antisense plants showed an increased root mass, whereas the development of the shoot was unchanged. Even though this phenotype might be related to the old observation that the root/shoot ratio of plants adjusts in response to their water status, it directly emphasizes how membrane transport can influence the developmental plasticity of plants. In the near future, analysis of single knockout aquaporin mutants will hopefully provide evidence for the multiple functions of aquaporins in the growth and development of plants and in their adaptive response to stresses.

It has been fascinating to observe during the last few years, how the discovery of aquaporins has challenged general concepts about the role of membranes in plant water relations. At one time it was assumed by most plant biologists that the residual water permeability of plant membrane lipids was sufficient for water flow in plants. Enthusiasm about the discovery of aquaporins led to the unrealistic proposition that transmembrane water flow must necessarily be mediated by these proteins. The truth must lie somewhere in between and we still have a long way to go

to fully understand the significance of these proteins. Nevertheless, aquaporins provide a unique molecular entry point into the water relations of plants and establish fascinating connections between water transport, plant development and the adaptive responses of plants to their ever-changing environment.

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