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Review

Aquaporins are multifunctional water and solute transporters highly divergent in living organisms

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ABSTRACT

Aquaporins (AQPs) are ubiquitous membrane proteins whose identification, pioneered by Peter Agre's team in the early nineties, provided a molecular basis for transmembrane water transport, which was previously thought to occur only by free diffusion. AQPs are members of the Major Intrinsic Protein (MIP) family and often referred to as water channels. In mammals and plants they are present in almost all organs and tissues and their function is mostly associated to water molecule movement. However, recent studies have pointed out a wider range of substrates for these proteins as well as complex regulation levels and pathways. Although their relative abundance in plants and mammals makes it difficult to investigate the role of a particular AQP, the use of knock-out and mutagenesis techniques is now bringing important clues regarding the direct implication of specific AQPs in animal pathologies or plant deficiencies. The present paper gives an overview about AQP structure, function and regulation in a broad range of living organisms. Emphasis will be given on plant AQPs where the high number and diversity of these transport proteins, together with some emerging aspects of their functionalities, make them behave more like multifunctional, highly adapted channels rather than simple water pores.

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1. Introduction

Water is the main component of all living cells, and water exchange may be considered as a property of life [1]. At the extracellular level, water is the main component of biological fluids, allowing, for instance, the long distance trafficking of important solutes such as sugars and ions in human blood and plant phloem sap. At the extracellular/intracellular interface, water exchange through the plasma membrane maintains the osmolality of the cytoplasm and thus the integrity of the cell. At the molecular level, water is involved in the configuration of some important molecules (e.g. plant hydrophilins [2]). Indeed, water is a polar molecule and displays electrostatic properties, forming hydrogen bonds, that confer its capacity of interacting with solutes but also participates in the stability and specificity of protein–protein and protein–DNA interactions [3,4]. Water is also involved in metabolic processes such as cellular respiration and photosynthesis in plants [5].

The biological membrane surrounding living cells is not a pure lipidic bilayer. There is a simple diffusion of water through a lipidic membrane but it does not explain the high velocity of water exchange observed, for instance, in red blood cell membranes [6], nor the low-energy activation observed for such phenomena [7,8]. Furthermore, first measurements of water movement and its effective re-absorption through kidney proximal tubules comforted the hypothesis of some specific path for water molecules trafficking [9,10]. In accordance to previous data reported by Pfeffer [11], these observations strengthened the idea of the existence of selective water pores in biological membranes. Many clues of their presence were then provided in a broad variety of organs such as in salivary glands, eyes and brain (reviewed by [12]). However, the molecular basis for the water transport phenomenon was first identified by the team of Peter Agre. A 28 kDa protein, subsequently known as CHIP28, was purified from red blood cells and renal proximal tubule membranes [13,14], characterized as a water channel in *Xenopus* oocytes and re-named as AQP1 for aquaporin-1 [15,16]. The corresponding cDNA was cloned and the deduced amino acid sequence related to the ancient family of membrane channels, MIP for Major Intrinsic Protein [17]. Many MIP members were then identified in both prokaryotes and eukaryotes with a most remarkable variety in plants, in which they may exert some redundant functions. The family is now referred to as AQP family, and the increase of the identified members has supported their importance for life [18]. In October 2003, The Royal Swedish Academy of Sciences awarded the Nobel Prize in Chemistry “for discoveries concerning channels in cell membranes”, with one half of the prize to Peter Agre for the discovery of aquaporins (AQPs). Functional characterization has given new insights regarding the role of these proteins that for some members is not restricted or not linked to water movement but to the transport of non-ionic, small neutral solutes, as well as, for some isoforms, to the passage of ions, such as nitrate, chloride and ammonia. The determination of a physiological role for each AQP is impaired by their ubiquitous expression in organisms and tissues, and by the diversity of their regulation patterns, most of all in plants. However, the involvement of a specific AQP member in some diseases and the use of knock-out and mutagenesis techniques have shown the striking roles displayed by some of them.

Present review gives an overview about AQPs structure, function and regulation in some living organisms, particularly in plants where the high number and variety of these transport proteins, together with some emerging aspects of their functionalities, tend to consider them as multifunctional channels involved in many physiological processes, rather than simple water pores.

2. Structural features

The MIP family comprised around 800 members in 2004 ([19]; MIPDB data base, <http://genouest.org/Prot/>). They cluster in 3 major

subfamilies determined by functional data, although the continuous identification of new potential substrates tends to thinner the borders between them: i) the aquaporin (AQP) subfamily includes water and ions conducting proteins, ii) the glycerol-facilitators, which transport glycerol instead of water, and iii) the aquaglyceroporins, which allow the passage of water and small uncharged molecules like polyols, urea and arsenite, playing important roles in nutrient uptake, osmoregulation and probably other biological processes [20–22]. However, in literature, the term aquaglyceroporins is often used to define both glycerol-facilitator and aquaglyceroporins. The movement of water and other solutes through AQPs or aquaglyceroporins is a passive mechanism driven by the concentration gradient of the transported molecule.

Members of the MIP family have average molecular weights between 28 and 30 kDa, although the yeast Fps1 and *Drosophila* BIB proteins have higher molecular weights due to their longer hydrophobic N- and C-termini [23]. MIP members share relatively high homology and common general structure [24]. They comprise 6 transmembrane domains (TM) connected by five loops, the N- and C-termini being cytoplasmic (Fig. 1). The primary structure can be divided into 2 similar halves (TM1–3, hemipore-1 and TM4–6, hemipore-2) that probably arose by gene duplication during evolution [25,26]. Because of its specific structure, authors often compare the MIP protein with an hourglass, the two hemipores facing each other in reverse within the membrane bilayer (Fig. 2B and C) [27]. At the center of the pore, highly conserved NPA (Asn-Pro-Ala) motifs, located at the loops B and E that form short hydrophobic helices and dip halfway into the membrane from opposite sides, face each other and participate in substrate selectivity [28,29]. The structure of several MIPs, determined after crystallisation, is available in the Protein Data Bank (PDB: <http://www.pdb.org/>, [30]). Proteins such as AQP1 [31], AqpZ [32], AQP0 [33], GlpF [34], AqpM [35], and SoPIP2;1 [36] have enabled the conceptualization of a general structure for MIPs. In the biological membrane, MIPs are grouped as homotetramers embedded in the lipid bilayer (Fig. 2A). Each monomer functions independently as a single channel pore.

The molecular basis for MIP substrate specificity has been widely investigated and it was demonstrated that, in spite of a general highly conserved structure, differences at key areas of the MIP sequences are responsible for the differences in channel selectivity [37,38]. This variability at the primary structure defines two constriction points within the pore, referred to as the NPA constriction and the aromatic/arginine (ar/R) selectivity filter, respectively and the selection is done through mechanisms of charge, polar and size exclusions [39,40].

The NPA region is positively charged because of the dipole moments of the two half-membrane spanning loops B and E and provokes the reorientation of the water molecule within the constriction. Here also,

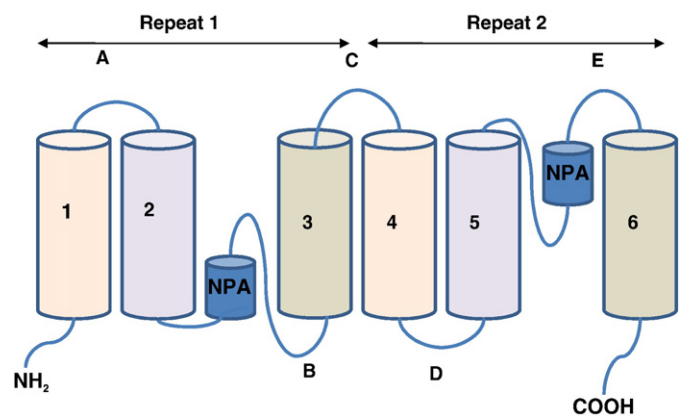


Fig. 1. MIP topology. The primary structure of MIPs comprises 6 transmembrane domains (1–6) connected by five loops (A–E), with cytoplasmic N- and C-termini. Highly conserved NPA (Asn-Pro-Ala) motifs are located at the loops B and E and form short hydrophobic helices that fold back into the membrane from opposite sides.

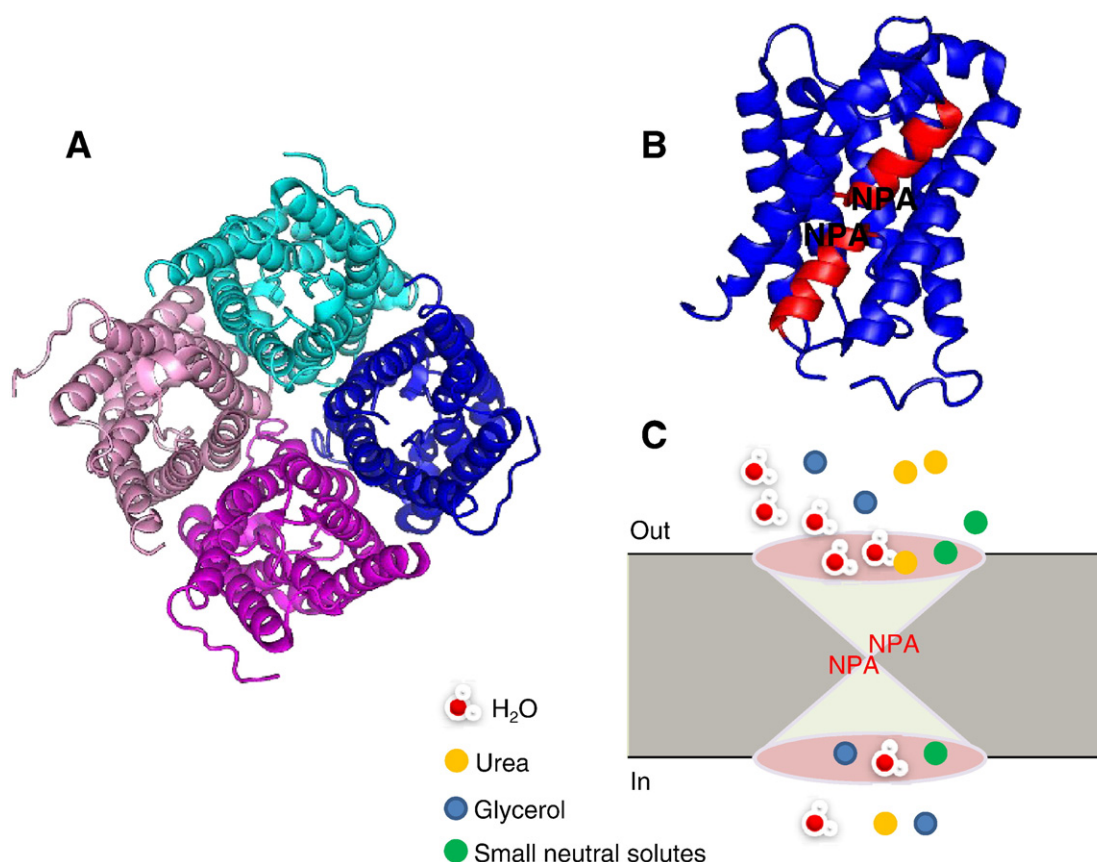


Fig. 2. Three-dimensional structure of spinach SoPIP2:1 MIPs are grouped as tetramers in biological membranes (top view, A). Each monomer (lateral view, B) functions as a single channel pore. PyMol deduced structures of the open SoPIP2 (PDB accession code 2B5F; PyMol simulation: <http://pymol.sourceforge.net> [261]). The intracellular loop B and the extracellular loop E (red) fold into the membrane and interact with each other through the NPA motifs, forming a central constriction and participating to the pore selectivity (C). In addition to water, several small molecules, such as glycerol and urea, and small neutral solutes and ions, are reported to permeate some aquaporins.

the presence of a P (Pro) (voluminous and hydrophobic) promotes the interaction of the water molecule with the N (Asn) residues. Moreover, the ar/R constriction, located 8 Å up the NPA, at the entrance of the pore, also impairs the entrance of high molecular weight substrates (~2.8 Å in AQPs, ~3.4 Å in aquaglyceroporins) but it is also a checkpoint site for uncharged molecules in both AQPs and aquaglyceroporins [40]. The ar/R constriction is formed by the interaction of 4 amino acids within the pore. In AQP1 these residues are: F-58 (Phe) and H-182 (His), from TM2 and TM5, respectively, and C-191 (Cys) and R-197 (Arg) from loop E, situated at the vicinity of NPA motif [31,41]. In AQP1, the presence of such amino acids provides hydrogen bonds that confer high selectivity to water [24,42]. Other residues, located in the side-chains at the neighbourhood of the ar/R constrictions can influence the polarity and diameter of the pore. The divergence of these amino acids among MIP isoforms constitutes the major difference between AQPs and aquaglyceroporins (Fig. 3) [32,39]. Within the pore, electrostatic effects provided by the NPA repeats and the ar/R constriction result in the exclusion of protons, a feature apparently common to all AQPs [43–46]. At these points, an interruption of the hydrogen-bonded water chain constitutes a strong barrier for protons to transit across the channel [45,47]. However, the amino acids of the ar/R region (H and R) act as a hydrophilic interface that offers hydrogen bonds to promote single-file transport of water molecules. The aromatic, hydrophobic residue F facilitates the interaction of water molecules with the H and R amino acids [48]. In aquaglyceroporins, some substitutions of these key residues at this selectivity filter allow the passage of wider compounds such as glycerol. For instance the large H present in the pore of AQP1, facing the R, is substituted in an equivalent position by a smaller G residue in the aquaglyceroporins GlpF, providing a hydrophobic corner

that interacts with bigger molecules [31,46]. Consequently, the pore of GlpF is approximately 1 Å wider than AQP1, and the presence of some hydrophobic amino acids favours the passage of glycerol [34,40,49].

Although NPA motifs are highly conserved in MIPs, some aquaglyceroporins present some divergence at that key sequence. For instance, in the yeast *Saccharomyces cerevisiae*, the traditional NPA sequences of the glycerol-facilitator Fsp1p were replaced during evolution by the unusual NPS and NLA in loops B and E, respectively, without these substitutions having affected the native glycerol-transport function of the protein. On the other hand, the substitution of conserved NPA motifs of GlpF, the bacterial closest homologue of Fps1p, abolishes the function of the protein and affects its correct expression at the plasma membrane. Additional observations point out the involvement of side chain sequences in loops and in TM, which participate in the formation of a functional pore [22,50]. Indeed, TM interactions stabilise the monomer and the overall sequence may participate to the pore conductance ability and regulation: Fps1p and GlpF, both glycerol-facilitators, differ from their substrate flexibility as indicated above. GlpF is more reluctant to substitutions at its NPA motifs, and this may be due to the side sequences of selectivity filters [39,50]. Eventually, some differences regarding the length and the sequences of the N- and C-termini have been linked with regulation mechanisms affecting gating, protein–protein interaction or osmosensitivity [22,51,52].

3. Aquaporins in microorganisms

Unlike glycerol-facilitators, AQPs are not widespread in bacteria and archaea, in which water fluxes must then occur through the membrane by passive diffusion or unspecific pores [38,53]. Indeed, the

release glycerol, adapting the cell turgor. Fsp1p can also mediate the entrance of glycerol into the cell; however fsp1-deleted cells are able to grow in media with glycerol as sole carbon source, suggesting a minor role of Fsp1p on glycerol uptake [72]. In fact, it has been demonstrated that the active glycerol transporters GUP1 and GUP2, play major roles in glycerol influx in yeasts [73,74]. Conserved amino acids in the long hydrophilic N- and C-termini are involved in the functionality and regulation of the Fsp1p protein: the N-terminal is essential for the response to external osmolarity (reviewed by [22]). Interestingly, Fsp1p has been shown to transport metalloids, and then to be involved in the resistance to these toxic compounds [58]. Yfi054-like aquaglyceroporins are common to yeast and filamentous fungi. Such a protein constitutes the unique aquaglyceroporin of the yeast *Schizosaccharomyces pombe*, a highly divergent yeast. Neither the subcellular location nor the physiological role of this protein is well established yet [22].

In the Protocista, AQPs have been isolated from *Plasmodium*, *Toxoplasma*, *Leishmania*, *Trypanosoma* and *Dictyostelium* (reviewed by [75]). As stated by the authors increasing attention is being directed to the promising potential of developing AQP inhibitors as therapeutics against some of these extremely important protozoan parasites.

4. Aquaporins in invertebrates and amphibians

In a recent paper of Campbell et al. [75] the current state of knowledge of invertebrate AQPs is extensively reviewed. They propose a classification system of insect AQPs, by phylogenetic analysis of the total AQP complement of several completed insect genomes, and discuss the physiological role of AQPs in invertebrates (insects, ticks and nematodes), including their function in common invertebrate phenomena such as high-volume liquid diets, cryoprotection and anhydrobiosis. However, although invertebrates represent about 97% of all Animalia, only 6 invertebrate species have had one or more AQPs, at least partially, functionally characterized. *Drosophila* species each contain seven AQP homologues while the mosquitoes *Aedes aegypti*, *Anopholes gambiae* and *Culex pipiens* have five homologues. The leafhopper (*Cicadella viridis*) AQP_{Cic}, the first insect AQP to be functionally expressed in oocytes, is specific to water transport. None of the additional insect AQPs expressed in oocytes up to now have shown the capacity of channeling glycerol or urea.

From these six invertebrate species, the model nematode *Caenorhabditis elegans*, is the sole non-insect species from which AQPs have been studied. Eleven genes encoding MIPs (from aqp-1 to aqp-11) have been identified in the *C. elegans* genome [76], 8 of which have been expressed in *Xenopus* oocyte transport assays [75]. Aqp1, aqp3 and aqp7 are aquaglyceroporins, whereas aqp-2, aqp-4 and aqp-6 are strict water channels. These proteins are expressed in several *C. elegans* cell types and osmoregulatory tissues, such as the intestine, excretory cells, and hypodermis. However, mutations experiments demonstrated that AQPs involvement in animal whole osmoregulation is not crucial [77].

In amphibians, over 17 MIPs have been identified. They are classified into 6 clusters, clusters 1, 2, 3 and 5 and clusters a1 and a2, the latter being specific to anurans. AQP_{a2}-like proteins may be involved in the terrestrial adaptation of anurans (for a review see [78]). *Xenopus laevis* oocytes are the favourite model for MIP heterologous expression and functional studies. Indeed, in spite of possessing MIPs, because of their size (1.0–1.3 mm diameter), robustness and natural low membrane permeability to water, they constitute a powerful expression system for animal, plants, fungi, and prokaryotes channel proteins [15]. Due to the experimental conditions selected to perform the heterologous expression studies, native AQPs do not interfere significantly on overall water uptake by oocytes. AQP_{xlo}, for instance, is an aquaglyceroporin permeable to water, glycerol and urea but impermeable to larger molecules and is expressed in the membrane of the oocyte [79]. AQP-x5 is homologue to the mammalian AQP5 and is

expressed in the skin, most particularly in the apical plasma membrane of secretory cells. A role of AQP-x5 in the maintenance of skin moistness and body temperature is reported [80].

5. Mammalian aquaporins

After the characterization of AQP1 and its subsequent association with MIP, 11 other mammalian homologues were cloned and studied. They are mostly associated with fluids formation [81]. The 13 AQP proteins, from AQP0 (the original member previously discovered in bovine lens cells [82]) to AQP12, are clustered into 3 functional groups according to their permeability characteristics [75,83]: i) water channels, ii) aquaglyceroporins, and iii) aquaporins with unknown substrate specificity. AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8 are considered water channels; however, AQP0, AQP1 and AQP6 also permeate nitrate and chloride ions, and AQP8 ammonia [24,84–86].

AQP3, AQP7, AQP9 and AQP10 are aquaglyceroporins, a subfamily usually permeable to water, glycerol, urea and to a limited number of small neutral solutes [24,87]. However, AQP10 is permeable to water, but not to glycerol and urea [88]. AQP9 is a neutral solute channel, permeable to water, glycerol, urea, purines, pyrimidines and monocarboxylates [89]. AQP7 and AQP9 are also able to transport arsenite [90].

AQP11 and AQP12 present only 20% of sequence homology with the MIP family and cluster together with plant SIPs (Fig. 3). Indeed, they have a different “NPA” box at the HB helix (NPC and NPT, respectively), and AQP11 has a C-terminal extremity with a putative ER-related retention signal (Table 1). Both unusual AQPs are intracellular proteins, even AQP12 that lacks this ER retention signal [91]. AQP11, which displays a crucial role in ER integrity maintenance [92], failed to display water permeability in oocytes, but transports water when reconstituted in liposomes, with efficiency comparable to AQP1 [93]. AQP12 substrate specificity is still unknown (for a review, see [24,93–95]). It is remarkable that some conventional AQPs, such as AQP6 and AQP10, have also been found in intracellular structures of the cell. AQP6 is almost exclusively intracellular and may be associated with the endosome [85]. Furthermore, although it does not belong to the group of intracellular aquaporins, AQP6 has a putative ER-related retention signal. Additionally, it has been demonstrated that the N-terminus confers its retention in intracellular membranes [96]. Water movements inside the cell are essential for its survival, as they are involved in cellular activity and signalling [95,97]. These particular locations, together with the broad specificity observed for AQP6 and predicted for SIPs, suggest a major role of these proteins in intracellular structures that deserves further investigation [95].

AQPs are relatively ubiquitous in mammalian organism and are usually not restricted to a sole tissue. However, their specific distribution in certain cell types of an organ often reflects a precise

Table 1

In silico comparative study of 12 putative members of intracellular aquaporins with Wolf PSORT (<http://wolfsort.org/>) and SosuiSignal (http://bp.nuap.nagoya-u.ac.jp/sosui/sosuisignal/sosuisignal_submit.html) programmes.

AQP	N-myristoylation	1 st “NPA” motif	ER retention signal (N-terminal)	2nd NPA motif	ER retention signal (C-terminal)
AtSIP1;1	–	NPT	–	NPA	KKQKKA
AtSIP1;2	–	NPC	–	NPA	KKQKKA
AtSIP2;1	–	NPL	GRIG	NPA	KAKSE
ZmSIP1;1	MAMGATVRA	NPC	–	NPA	KTKK
ZmSIP1;2	–	NPT	–	NPA	KAKK
ZmSIP2;1	–	NPL	–	NPA	KIKK
VvSIP1	MGVIKAA	NPT	–	NPA	KQKKA
VvSIP2	–	NPL	AKIR	NPA	KAKSE
OeSIP1;1	MRAIKVAIA	NPT	RAIK	NPA	KKQKKA
OeSIP1;2	MGAIKAA	NPT	–	NPA	KAKK
AQP11	–	NPC	–	NPA	INKK
AQP12	?	NPT	–	NPA	–

function. Because they increase water membrane permeability, AQP's are important actors of fluid homeostasis maintenance and secretion/re-absorption. In lungs and airways for instance, AQP's maintain airway humidification by driving water transport across the endothelial and epithelial barriers. AQP0 is present in the eye lens and its mutation provokes cataracts formation [98]. AQP1, the first characterized AQP, is mostly expressed in red blood cells, brain, lungs and kidneys and is associated with water re-absorption and fluid secretion [99]. AQP1-null mice and humans with AQP1 mutation have shown reduced urinary concentration (see below) [100,101]. Recently, it has been suggested that AQP1 is involved in cerebrospinal fluid secretion [102]. AQP2 is expressed in kidney collecting duct and is permeable to water [103]. AQP2 mutations can cause an autosomal form of hereditary nephrogenic diabetes insipidus in humans [104]. AQP3 is also expressed in different tissues including kidney collecting duct, skin, conjunctiva of the eye, oesophagus, colon, spleen, stomach, small intestine, and urinary bladder, and respiratory tract airway epithelium. It is permeable to water and glycerol and may be important for proper skin hydration and elasticity [103,105,106]. Mice lacking AQP3 manifest nephrogenic diabetes insipidus with polyuria, polydipsia, and urinary hypoosmolality [107]. AQP4 is permeable to water and expressed in astroglial cells at blood–brain barrier and spinal cord, kidney collecting duct, glandular epithelia, airways, skeletal muscle, stomach and retina [103]. In the brain, AQP4 null mice showed altered cerebral water balance with protection from brain edema (see below) [108]. AQP5 is permeable to water and expressed in glandular epithelia, corneal epithelium, alveolar epithelium and gastrointestinal tract [103]. Saliva production in the parotid gland is a sympathetically controlled phenomenon which involves the re-localization of AQP5 into the plasma membrane of the secretory cells [109]. The important role of AQP5 in homeostasis is evidenced by AQP5-null mice which have reduced fluid secretions [103]. Defective cellular trafficking of AQP5 in salivary [110] and lacrimal [111] glands has been associated to the Sjögren's syndrome, an autoimmune disease characterized by deficient secretion of tears and saliva. For a review on the expression, localization and function of AQP's in salivary and lacrimal glands and pancreas, and the role of multiple water and ion transporters and channels in exocrine fluid secretion see [112]. AQP6 is thought to be involved in chloride permeability and is expressed in kidney collecting duct intercalated cells [103]. AQP7 is involved in water and glycerol permeability and is expressed mainly in adipose tissue, and in testis, heart, skeletal muscle and kidney proximal tubule [103,113]. In adipocytes, AQP7 is known to facilitate the secretion of glycerol, which constitutes a key metabolite in the control of fat accumulation and glucose metabolism [114]. AQP8 is permeable to water and is expressed in liver, pancreas intestine, salivary gland, testis and heart [103]. It was suggested to be involved in cAMP stimulated bile secretion by Garcia et al. [115]. AQP9 is expressed in liver, white blood cells, testis and brain, and is involved in water and small solutes permeability [103]. The functional role of AQP9 in the brain is still unknown, but its permeability to glycerol and lactate indicates that it may play a role in energy metabolism [102,116]. Studies performed with mice lacking AQP9 and leptin receptor function, developed obese mice, with type II diabetes and increased lipolysis and glycerol production [117]. AQP10 is thought to be involved in water and glycerol permeability and is expressed in small intestine [103]. AQP11 is expressed in kidney, testis, liver, brain, intestine, heart, and adipose tissue, but its function is still unknown [103,106,118]. AQP12 seems to be expressed specifically in pancreatic acinar cells, and as well as AQP0 and AQP11, it has not been characterized functionally. AQP12-knock-out mice have not been generated, and no human diseases have been credited to mutations in the AQP12 gene [103,106]. The involvement of AQP's in many human diseases such as glaucoma, obesity or cancer, is now well reported. Indeed, AQP's are strongly expressed in tumor cells and promote cell migration [119]. Migration is a fundamental property of cells that

occurs during many physiological and pathological processes including organogenesis in the embryo, repair of damaged tissue after injury, the inflammatory response, formation of new blood vessels, and the spread of cancer. AQP dependent cell migration has been found in a variety of cell types in vitro and in mice in vivo [120]. AQP1 deletion reduces endothelial cell migration, limiting tumor angiogenesis and growth. AQP4 deletion slows the migration of reactive astrocytes, impairing glial scarring after brain stab injury. AQP's are conducive to migration by facilitating the rapid cell volume changes and augmenting cell propulsion. The emerging roles of water movement in cell migration are not only important in the mechanistic understanding of the migration process, but may also have a wide range of therapeutic implications (for a review, see [121]). Thus, the modification of AQP function or expression constitutes a promising target to develop new drugs and provide a treatment of several diseases (reviewed by [122]). The following paragraphs focus on AQP's in kidney and brain that illustrate well the diversity, regulation and physiological roles of these proteins in mammals.

5.1. Aquaporins in the kidney

In the kidney, at least 8 AQP's homologues are expressed: AQP1 to 4, and AQP6 to 8 and AQP11 (for a review see [123,124]). AQP1 is most particularly expressed in the nephrons where it allows the retrieval of water from the primary urine. To remove toxic wastes and the high concentration of ions that alters the pH, blood is filtrated in the glomerulus of the nephron. The passage of ions is accompanied by osmosis, thus the resulting primary filtrate is rich in water that must be reabsorbed. Passing through the Henle's loop, water is retrieved from the filtrate in the proximal convoluted tubules and the thin descending limbs that exhibit high water permeability. Indeed, AQP1 proteins are constitutively expressed in these epithelia, at both the apical brush border facing the tubule lumen and the basolateral membranes of these cells. Water is passively reabsorbed, driven by osmotic gradients [125]. However, a major difference in AQP1 expression exists between the types of nephrons that compose the kidney: in short loop variety, AQP1 is expressed only in proximal tubules but not in the descending thin loop, whereas it is expressed in both sites in long loop nephrons [123,126,127]. Recently, Zhai et al. [128] suggested that the role of AQP1 as the major water re-absorption mechanism should be re-investigated. The gene AQP1 is disrupted in some human patients impairing the production of the corresponding aquaporin. Thus, these individuals are true AQP1-null mutants. Their red blood cells present basal but sufficient permeability not compromising a normal life. However their urine concentration cannot reach more than half the maximal concentration reached by the urine of a normal person, expressing AQP1, but such a defect is balanced by the relative free access to water in our modern societies [8,129].

AQP2 is expressed in the collecting duct of the nephron whose permeability to water is induced by the physiologic concentration of vasopressin, an anti-diuretic brain secreted hormone [125,130–132]. In normal conditions, AQP2 proteins were shown to be very poorly expressed at the apical membrane of the epithelia cells but were located in membranes of intracellular vesicles. In dehydration conditions, as fluid volume decreases and plasma osmolality increases in the body, vasopressin is released from the brain and AQP2 is re-located by exocytose to the apical border, increasing the water permeability and the re-absorption of water from the urine. Water is then transferred to the vascular space via AQP3 and AQP4 proteins expressed at the basolateral membranes of the conduction duct cells [126,132–134]. AQP3 is also responsive to vasopressin but not AQP1 nor AQP4 [135]. AQP2 and AQP3 are also inducible by ethanol [136].

Several pathogenesis result from an incorrect level of AQP2 in the conducting duct principal cells. An overexpression of AQP2 is triggered by heart failure and leads to water retention [137]. A defect in AQP2 function has been shown to cause the congenital nephrogenic

diabetes insipidus disease (NDI), a human hereditary disorder that impairs the concentration of urine in response to vasopressin. Studied mutants in AQP2 presented amino acid substitutions that affect the folding of the protein and maturation thus impairing its trafficking to the plasma membrane [132,138].

5.2. Aquaporins in the brain

A detailed overview regarding brain aquaporins is presented in two excellent reviews [139,140]. Here we will briefly recall some important aspects regarding the involvement of brain AQPs in fluids production and homeostasis.

At least 6 AQPs are present in the rodent brain: AQP1, AQP3 to 5, AQP8 and AQP9, AQP1, 4 and 9 being the best studied [136]. Brain AQPs play an important role in the production, circulation and homeostasis of the cerebrospinal fluid (CSF). Fluid balance (secretion, removal, fluxes and homeostasis of salts) is important for the brain survival but also neuronal excitability. Its role to the body is compared to that of the lymphatic system [141]. CSF is formed by the secretion of water and salts by the choroid plexus epithelium. AQP1 is expressed at the apical pole of these cells, whereas the mechanism of water flow through the basolateral membrane from the blood is still unknown. Interestingly, AQP1 from the choroid plexus appears to be a non-selective cation channel in *Xenopus* oocytes. Pharmacological and protein modelling tools suggest that the passage of Na^+ through AQP1 occurs through the central pore formed in the AQP1 tetramer, and that this functionality is regulated by a gating mechanism that involves intracellular cGMP. The relevance of this functionality is still not clear and seems tissue-specific [52,140,142,143]. This mechanism can participate in the rate of CSF secretion. However, some data in the literature contradicts both the putative role of cGMP as an ion channel activity enhancer, as well as the function of AQP1 as a cation channel [144]. Because choroid plexus is essential for CSF production and balance, but also a source and a target for many regulatory molecules, it is not astonishing that its functional decline is being associated with Alzheimer's disease. The putative involvement of AQP1 in this process, as a cause or a consequence is not known but it is under investigation (for review, see [140]).

AQP4 is predominantly expressed in the brain, at the blood–brain and brain–cerebrospinal fluid barriers [107,145], suggesting that it controls the water flow into and out of the brain, contributing to the maintenance of brain water homeostasis [102]. AQP4 is widely expressed in the brain glial cells, mainly in astrocytes, and most particularly at their end-feet structures surrounding blood vessels. Studies using AQP4 knock-out mice demonstrated increased protection from cytotoxic brain oedema induced by water intoxication and cerebral ischemic injury [108]. In contrast to the findings in cytotoxic brain edema, AQP4 is up-regulated during vasogenic oedema, caused by increased permeability of the blood–brain barrier, playing a key role in fluid clearance [146].

AQP4 is co-ordinately expressed at astrocytes end-feet with Kir4.1, an inward rectifier K^+ channel, suggesting a physiological role in K^+ homeostasis and neuronal activity. Potassium is released after neural excitation for the cell to recover its membrane potential. Excess of extracellular K^+ is retrieved by perineuronal astrocytes and exported to end-feet structures and then to blood capillaries. Astrocytes end-feet present a high K^+ conductance correlated with an enrichment of Kir4.1 channel and AQP4 at their membrane. K^+ is proposed to be released in blood as KCl, thus allowing the efflux of water through AQP4. A coordinate regulation of Kir4.1 and AQP4 is strongly strengthened by the spatial co-localization of these two channels. Furthermore, an interaction between Kir4.1 and AQP4 involving components of the dystrophin-glycoprotein complex has been demonstrated [147]. Indeed, a decrease of AQP4 expression at astrocytes end-feet and the consequent swelling of these structures are associated with the Duchenne's muscular dystrophy disease. It is

proposed that dystrophin plays a role in AQP4 assembly at the plasma membrane. AQP4 expression is related to brain development (post-natal expression). However, even if AQP4 is not expressed in mature neurons, a role in early neural development is suggested by its expression, along with AQP9, in forebrain subventricular zone neural stem cells. Furthermore, AQP4 is expressed in interneurons and then in Müller cells during the development of the retina [147].

A role of AQP9 in Parkinson's disease has been suggested. AQP9 is expressed in astrocytes but also in noradrenergic and dopaminergic neurones, the degenerating of the latest causing the disease. However, a putative involvement of AQP9 in this process, as a cause or as a consequence, is purely speculative and is still under investigation [139,140].

6. Plant aquaporins

The tonoplast protein from *Arabidopsis* γ -TIP (AtTIP1;1) was the first plant aquaporin to be characterized. Its water channel activity was demonstrated by heterologous expression in *Xenopus* oocytes [148]. Since then, many other plant AQPs have been identified and cloned, and functional studies have shown their important role for the selective movement of water through the cell membranes. In fact, AQP-mediated water transport in plants is an important step in various physiological processes such as cell elongation, seed germination, and osmoregulation [149]. Comparatively with other organisms, plants appear to have a remarkable large number of MIP homologues, ubiquitously expressed [150,151]. Genome sequencing revealed the presence of 35 MIP-encoding genes in *Arabidopsis* [152], 36 in maize (*Zea mays*) [153] and 33 in rice (*Oryza sativa*) [154], thus far more than in mammals (13), in *E. coli* (2), in *C. elegans* (11) and in *S. cerevisiae* (9). The high number of plant MIP isoforms and their relative ubiquity in plant tissues may emerge from the need of continuous water absorption, flux and subsequent evaporation during plant growth and development but may also reflect a possible involvement in the transport of other solutes [149,155,156]. Four MIP subfamilies are distinguished from their sequence similarity in plants (Fig. 3), and usually reflect their subcellular location: i) the plasma membrane intrinsic proteins (PIPs), ii) the tonoplast intrinsic proteins (TIPs), iii) the nodulin26-like intrinsic proteins (NIPs) and iv) the small basic intrinsic proteins (SIPs). PIPs and TIPs are located in plasma membrane and tonoplast, respectively, whereas the subcellular location of NIPs and SIPs is still uncertain [51]. Indeed, some studies reported that some NIPs are inserted in the plasma membrane whereas other isoforms are located in intracellular membranes [157]. SIPs are located in endoplasmic reticulum (ER) [97,158]. Regardless their subcellular location, most of the studied plant MIPs confirmed their function as water channels in *Xenopus* oocytes. However, some MIPs are able to carry some small neutral molecules, such as glycerol, urea and even gases like CO_2 but also display permeability to ammonia, hydrogen peroxide and the metalloids silicon, boron, arsenite, and antimony [97,157,159–162].

6.1. PIP subfamily

PIPs are plasma membrane MIPs with a molecular weight around 30 kDa and an isoelectric point of 9.0, in part due to the presence of several basic amino acids at the C-terminal extremity. PIPs have been studied in many species and the involvement of many of them in water transport successfully demonstrated. Among the 35 full-length aquaporin genes present in the *Arabidopsis* genome, 13 encode for PIPs. On the basis of their sequence similarities, two PIP subgroups can be considered: PIP1 and PIP2. The PIP1 subgroup has five members (PIP1;1 to PIP1;5), whereas the PIP2 subgroup possesses eight isoforms (PIP2;1 to PIP2;8) [149,152]. PIP1 proteins have longer N-terminal but shorter C-terminal tails compared with PIP2. Furthermore, all PIP proteins possess conserved S (Ser) amino acid residues

proposed to be essential for the protein gating by phosphorylation [163,164]. The SoPIP1 from spinach (*Spinacia oleracea*) displayed low water transport activity in *Xenopus* oocyte whereas its PIP2 homologue strongly increased the membrane water permeability [36,165,166]. Such results were verified for almost all plant PIPs aquaporins: with the exception of *Arabidopsis* PIPs isoforms, members of the PIP1 subgroup show no or poor permeability to water, whereas PIP2 related proteins function as efficient water channels [167–169]. However, recent studies performed in oocytes revealed that maize ZmPIP1;2 can be functional if it interacts with ZmPIP2s: indeed, ZmPIP1;2 is able to increase membrane water permeability when co-expressed with the functional channel ZmPIP2 in *Xenopus* oocytes. The observed increase in water permeability was proportional to the amount of ZmPIP1;2 cRNA injected in the oocytes *i.e.* directly linked with a water channel activity mediated by ZmPIP1;2 [51,170]. Recently, the localization and interaction of ZmPIP1s and ZmPIP2s fused to fluorescent proteins have been characterized in maize cells. When expressed alone, ZmPIP1 fusion proteins were retained in the endoplasmic reticulum, whereas ZmPIP2s were found in the plasma membrane [171]. Interestingly, when co-expressed with ZmPIP2s, ZmPIP1s were re-localized to the plasma membrane. This re-localization results from protein interactions and these data suggest that hetero-oligomerisation of ZmPIP1s and ZmPIP2s is required for trafficking of the former to the plasma membrane to modulate the cell permeability. Accordingly, the interaction between NtPIP1;1 and NtPIP2;1 results in an increase in water transport activity in *Xenopus* oocytes [172]. On the other hand, it has been suggested that PIP1 members can also transport other molecules such as glycerol, boric acid, urea and CO₂ [173]. For instance, NtAQP1, a PIP1 member from tobacco, is able to mediate glycerol transport but also permeates CO₂. The capacity of NtAQP1 to mediate gas transport has been compared to AQP1: indeed, both MIPs are expressed in membranes involving high CO₂ traffic (red blood cells, plant cells of leaves) [174–176]. Recent evidence have pointed out the contribution of PIP1s to CO₂ conductance in parenchyma cells and allowed the re-evaluation of the regulatory mechanisms of photosynthesis. In fact, changes in NtAQP1 expression by antisense inhibition or overexpression affect the CO₂ conductance, photosynthesis and plant growth [174,177,178]. Interestingly, NtAQP1 is detected in the plasma membrane and chloroplast inner membranes where a decrease in protein amount lowers CO₂ permeability [177]. This role might not be restricted to the tobacco AQP1 since many PIPs are expressed at the leaf level, such as SoPIP1;1, which has a high constitutive level of expression in guard cell plasma membrane [179,180].

Finally, PIPs are generally localized in organs and tissues characterized by large fluxes of water *i.e.* vascular tissues, guard cells and flowers. For instance, AtPIP2;2 is abundantly expressed in roots, cortex, endodermis, and stele. SoPIP1;2 is highly expressed in phloem sieve elements of leaves, roots and petioles and SoPIP1;1 is present in stomatal guard cells [150,173,179]. Interestingly, AtPIP2;1 is able to transport hydrogen peroxide (H₂O₂), a reactive oxygen species (ROS) involved in oxidative stress but also as a messenger in plant responses to wounding or pathogen attack. PIPs would then play an important role in cell to cell communication [181].

Genetic techniques modifying the expression of PIP genes in the plants brought precious information regarding the role of these aquaporins in water transport (reviewed in [155]). Down-expression of plasma membrane AQP genes usually results in a decrease in cell water permeability but, this does not necessarily result in lower tissue water conductivity, probably as a consequence of the different mechanisms used by plants to deal with lower cell water permeability, for instance by increasing their root mass [150,155,182].

6.2. TIP subfamily

TIPs are expressed mainly in tonoplast membrane although other subcellular locations cannot be ruled out. The vacuole occupies as much as 90% of most mature plant cells, and tonoplast is then a barrier

to intracellular transport. TIPs are thus essential for intracellular movement of water and possibly of other small molecules [95]. Aquaporins are the most abundant proteins of the tonoplast [183]. This explains why the water permeability of the tonoplast is higher than that of the plasma membrane, allowing a rapid osmotic adjustment of the cytoplasm, thus maintaining osmolality and cell turgor [184]. TIPs possess a molecular weight between 25 to 28 kDa and an isoelectric point around 6.0 [185]. They are functional water channels but some of them also permeate urea, glycerol and ammonia. Interestingly, it has been recently demonstrated that AtTIP1;1, AtTIP1;2 and AtTIP2;3 can transport H₂O₂ [186,187]. The tobacco NtTIPa (TIP4), for instance, transports water, urea and glycerol [188]. A common feature to all TIPs studied so far is their classical sensitivity to heavy metals when expressed in oocytes [185,189]. In maize, *Arabidopsis* and rice, TIP subfamily consists of five subgroups regarding their sequence homologies: TIP1 (the former γ TIP), TIP2, TIP3, TIP4 and TIP5. TIPs are predominantly located at the tonoplast, but some isoforms are markers of specialised organelles such as protein storage vacuoles, lytic vacuoles and small vacuoles [190,191]. Furthermore, some TIP isoforms are more related to developmental stages and/or organ specificity: for example, the expression of AtTIP2;1 (δ TIP) is especially high in the vascular system of the shoot but is barely detectable in the root [192]. AtTIP3;1 (α TIP) is highly expressed in cotyledons and associated to the membrane of protein storage vacuoles. This suggests that a specific TIP isoform is related to a specific vacuole function [190]. Furthermore, during germination, the stored proteins are degraded and there is a shift from TIP3;1 to TIP1;1 isoforms [152,164,193]. In addition, TIPs are differentially expressed during fruit maturation, for instance, in pear TIP1;1 homolog is highly expressed in the young fruit whereas in grape, TIP proteins levels gradually increase along with ripening [194,195].

Vacuole participates in cell expansion and thus, its enlargement by water compartmentation is essential to provoke the rapid fruit growth characteristic of the ripening process. The lack of specific regulation observed along fruit ripening for PIPs isoforms points out the essential role of TIPs in this process [196].

The various aspect of their expression, together with their wide specificity suggested that TIPs might play additional roles besides water transport or even an entirely different function. TIP1;1 is the most expressed AQP in both maize and *Arabidopsis*, and is expressed in all plant organs, but mainly in roots and leaves, and seems associated with cell elongation [153,197,198]. Reduction of AtTIP1;1 expression in *Arabidopsis* by RNA interference has been shown to result in early senescence, plant death, modification in primary metabolism and carbohydrate distribution whereas the relative water content of the whole plantlet is not significantly affected [199]. It has been proposed that TIP1;1 might be involved in vesicle-based metabolite routing. However, these data remain controversial as the different phenotypes have not been observed in AtTIP1;1 knock-out *Arabidopsis* lines [200].

6.3. NIP subfamily

The name of this subfamily comes from the sequence similarity of these proteins with the nodulin-26, a protein expressed at the symbiosome membrane during root infections of leguminous plants like soybean (*Glycine max*) by rhizobacteria. In such structures, nodulin-26 ensures the communication between the bacteria and the host plant, giving passage to water at the symbiotic interface. Nodulin26-like proteins (NIPs) are found in both leguminous and non-leguminous plants, located in plasma and intracellular membranes and are structurally and functionally different from other plant MIPs. They usually display low water permeability, in contrast with their nodulin-26 archetype, but are permeable to small solutes (*i.e.* glycerol, urea). In fact, these MIPs are considered as plant aquaglyceroporins [149]. The NIP subfamily can be subdivided into two

subgroups, based on the structure of their ar/R selective filters: i) NIP1 comprises six members in *Arabidopsis* permeable to water and glycerol, ii) NIP2 proteins have a wider pore than those of NIP1 subgroup and are permeable to large solutes like urea. Two NIPs of the second class also present a variation in the loop B NPA motif [42,201]. The overall level of NIP expression is lower than the expression of PIPs and TIPs, and is usually associated with specialised organs and cells. For instance, AtNIP2;1 is specifically expressed in the endoplasmic reticulum (ER) of roots, whereas AtNIP5;1 is a plasma membrane MIP mainly expressed in root elongation zones [157,202]. Because of their particular structure and locations, NIPs appear to be involved in a striking range of processes which are still poorly known. However, studies pointed out the amazing possibilities of these particular MIPs: for instance, AtNIP5;1 (a NIP2 isoform) is transcriptionally induced by boron deficiency. Boron, and most particularly its predominant form at physiological pH, boric acid, is important for plant growth since it participates in cell wall expansion. It is taken from the soil and boron deficiency is associated with reduced shoots surface, root expansion and plant fertility. AtNIP5;1 protein efficiently transports boric acid and allows its accumulation in *Xenopus* oocytes whereas it displays a weak channelling capacity for water; *nip5;1* mutants plants (T-DNA insertion lines) present a less developed root systems and a decreased uptake capacity of boric acid. Furthermore, these plants are more sensitive to a limitation of this specific compound. AtNIP6;1 and AtNIP7;1 may also be involved in boric acid transport in planta [157].

AtNIP2;1, a MIP expressed in differentiated roots, mediates lactic acid transport, and its expression is transcriptionally induced by anoxia, being very important to the adaptation to lactic fermentation under anaerobic stress [203].

The Rice gene Lsi1 (OsNIP2;1), related to the *Arabidopsis* NIP7;1, permeates silicon (Si), a compound involved in defence mechanisms against biotic and abiotic stresses [159,204]. In rice, Si accumulates in the shoots where silica constitutes a mechanical barrier against biotic and abiotic stresses. The expression of Lsi1 in *Xenopus* oocytes allowed the specific influx of Si, and the suppression of this gene in plant resulted in a reduction of Si uptake [159]. In contrast, Lsi2 is an efflux transporter [204]. Recently, Lsi6 (OsNIP2;2), which is responsible for the export of Si from the xylem to the shoots, has been cloned and characterized [205]. Interestingly, OsNIP2;1 is also able to transport arsenite, a form of arsenic present in culture soils [205,206]. This ability of transporting arsenite is reported for AtNIP7;1 [207] and AtNIP1;1 [208] and some mammalian and bacterial MIPs (see above).

6.4. SIP subfamily

The SIP aquaporins constitute a new subfamily of MIPs identified by database mining and phylogenetic analyses [209]. Because of their low homology with other MIPs (about 20%) they were recently classified within a superfamily of intracellular AQP [95]. They have a basic pI and highly diverge from other MIPs regarding their unusual loop B NPA sequence and shorter N-terminus that classify them into two different subgroups: i) the SIP1, subdivided into the SIP1;1 type bearing the NPT sequence instead of the classical NPA, and the SIP1;2 type bearing either the NPC or the NPT sequences, and ii) the SIP2,

which differs from classical MIPs in its NPL sequence. These amino acids substitutions confer a wider aperture of the pore and hence a potentially different substrate specificity [42,158]. Each type of SIP is only represented by one clone in both *Arabidopsis* and maize. The grape genome has only 2 SIP genes, *VvSIP1*, encoding a SIP1 subtype, and *VvSIP2* [210]. Fig. 4 shows the deduced amino acid sequence of OeSIP1;1 recently cloned in the Portuguese lab which reveals the presence of the NPT sequence in loop B and putative motives involved in endoplasmic reticulum retention. Indeed, first experiments regarding subcellular location demonstrated that SIPs are associated with intracellular membranes and more particularly ER [97]. In this regard, they tend to strengthen the idea of a subclass of intracellular MIPs, recently suggested by the cloning and study of the mammalian AQP11 isoform. These AQPs have in common, at their C-terminal extremity, a sequence rich in positively charged amino acids (K, Lys), which is a potential ER retention signal [95,149,153] (Table 1). Yet, no sequence responsible for the retention in ER membranes has been clearly identified whereas proteins bearing the KDEL motive are retained in the lumen of the ER [158]. Myristoylation of N-terminal amino acid residue of the growing polypeptide is a co-translational modification that plays a pivotal role in membrane targeting and signal transduction in plant responses to environmental stress [211]. Myristoylation signal peptide was identified in SIP1 proteins from olive (OeSIP1;1 and OeSIP1;2), grapevine (*VvSIP1*) and in maize (*ZmSIP1;1*) and its physiological role deserves further investigation (Fig. 4, Table 1).

Water channel activity has been demonstrated for the two AtSIP1s in yeast vesicles but no activity was detected in *Xenopus* oocytes. However, AtSIP2;1 does not exhibit any water permeability in the investigated conditions and is supposed to have other function [97]. In *Arabidopsis*, each SIP seems to be expressed in a preferred range of tissues including young roots, flowers and pollen. It is remarkable that SIPs are also strongly expressed in suspension cultured cells compared to other MIPs [158]. In grape, *VvSIP1* is strongly expressed in the berry at the green stage, i.e. when cells division is active (Agasse, unpublished data).

The potential role of such intracellular MIPs is to facilitate the intracellular water movement. As suggested for TIPs at the tonoplast, SIPs could participate in the passage of water through the ER membranes and regulate both the volume of its lumen and the ionic concentration, as well as the morphology of the organelle. As mentioned before, the crucial role of mammal AQP11 in ER integrity has been demonstrated [188].

7. Regulation of aquaporin function and expression

The regulation of water transport through MIPs is complex: in a general view, mechanisms of rapid control affect the protein itself (activity, gating, and amount) whereas slower adaptative-responses through the regulation of gene expression are also involved. Structure features and experimental studies elucidated some mechanisms for substrate exclusion by MIPs but also gating, and/or turn-over. Most of the functional studies are based on heterologous expression in *Xenopus* oocytes because these cells are naturally poorly permeable to water and allow the rapid translation of the injected cRNA and targeting of the protein. They are also relatively resistant enabling application of

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>OeSIP1;1
MRAIKVAIADAIVTCLWVFCASSLGLTFVVASAFVGSQGLPTLLITTFLLFVLLFVFGFIGDALG
GATFNPTGPAAFYAAGVGGTESLVAAVRFPQAAGAVGGALGILEVMPVQYKHMLDCPSLK
VDLNTGAI AEGVLTFIITFAVLYIVLRGPTNPFVKNWLLAMSTVALVAGSSYTGPSMNPANAF
GWAYVNNHTKWEQFYVYVICPFIGAILAAWVFRFLFLPPPVKKQKKA
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Fig. 4. Deduced amino acid sequence of OeSIP1;1 (accession number EF211089) aquaporin showing putative N-myristoylation (yellow), ER retention signals (red) and the two “NPA” repeats (blue). The putative transmembrane domains are underlined. The SIP1;1 type aquaporins contain in the loop B a NPT sequence instead of the classical NPA sequence. Sequence analysis was performed with Wolf PSORT (<http://wolfpsort.org/>) and SosuiSignal (http://bp.nuap.nagoyau.ac.jp/sosui/sosuisignal/sosuisignal_submit.html) programmes.

pharmacological compounds in the study of channel functionality [212]. However, several other studies have been processed in yeasts and yeast vesicles, taking advantage of the poor expression of endogenous MIPs in most of laboratory yeast strains and their relatively easy transformation and manipulation [67]. Yet, the protein expressed in the foreign environment may behave differently, in the absence of natural regulatory compounds and/or partners and may develop non-specific interaction [213]. In plants, these problems can be circumvented using protoplasts, a useful tool to check the functionality of MIPs as well as for the study of their subcellular location by GFP-fusion [171,214]. Nevertheless, it has become clear that many signalling pathways regulate the expression of MIP genes through complex transcriptional and posttranscriptional controls and that a general pattern of expression cannot be distinguished, especially in plants [155].

A rapid way to modulate membrane water permeability is by regulating the activity of the MIP constitutively expressed. Several works report the involvement of both H^+ and Ca^{2+} in the MIP closure. For instance, in *Arabidopsis*, a decrease in pH causes the protonation of a conserved H (His)-197 residue of the loop D, closing the pore [36,215,216]. Such a pH influence on MIPs activity has been demonstrated in members of other kingdoms, for instance in mammals, but the location of the targeted H is generally not conserved since it can be placed at loops A or C [217]. Additionally, protein phosphorylation and dephosphorylation events participate in the gating of MIPs from several plant species (i.e. bean seed PvTIP3;1, spinach SoPIP2;1, soybean NOD26, and *Arabidopsis* and maize PIP1 and PIP2 isoforms). In spinach, SoPIP2;1 closes to reduce water loss in response to drought stress. Indeed, molecular dynamics simulations indicate that the dephosphorylation of two S residues (the loop B S-115 and the C-terminal S-274) modifies the interaction between several amino acid residues and induces a modification of the loop D conformation creating a hydrophobic barrier blocking the pore. In normal conditions, the two S residues are phosphorylated and the channel is open [36]. Phosphorylation events also occur to regulate AQP1-water permeability [218].

The inhibition of MIP transport activity by mercury ($HgCl_2$) is a well-known tool used to study the function of the protein. It is based on its binding to SH-groups of C residues, and most particularly a quite conserved C at the vicinity of the first NPA repeat. This triggers a conformational change and therefore the decrease of water flow through the membrane, reversed by a β -mercaptoethanol treatment [219,220]. Other heavy metals, such as silver nitrate or gold, are also able to block MIPs function. These compounds also interact with protein sulfhydryl groups [221]. However, this property is not a general feature of all aquaglyceroporin channels since many of them do not have the C residue and thus are insensitive to $HgCl_2$ (ex: AqpZ, [222]). Furthermore, a direct role in MIP gating of abscisic acid (ABA), an important phytohormone involved in the response to environmental stresses such as drought and salinity, has been speculated although no consistent evidence is brought so far. By binding to the transport protein or by modifying the heterogeneity of the surrounding lipid bilayers, ABA might stabilise an open conformational state of the channel [219,223].

Another rapid way to modulate the membrane water permeability is by adjusting the number of MIP in the membrane. In normal physiological conditions the targeting of the MIP is dependent on its structure and more precisely on the presence/absence of specific sequences that allow their retention in intracellular organelles or their trafficking to the plasma membrane (e.g. SIPs, AQP11, Aqp1). However, phenomena of re-location in response to hormonal stimuli or environmental stresses have been described in mammals, allowing the fast mobilization or removal of these proteins in order to control the flux of water at the membrane. AQP2 regulation by vasopressin at the kidney level is a well known example, extensively described. Vasopressin is an antidiuretic hormone released from the brain in response to the dehydration of the body. In the kidneys, the

fixation of the hormone on V2-receptors at the basolateral membranes of the conducting duct principal cells, triggers a signal cascade (cAMP synthesis, protein kinase A activation) that results in the exocytosis of AQP2-membrane containing vesicles and their incorporation at the apical membrane. The mechanism involves the phosphorylation of S-256 residues of the protein and seems relatively widespread for other AQPs (for instance the translocation to the plasma membrane, however not associated by hormonal stimuli, requires the phosphorylation of a S (Ser) residue [224]. The phosphorylation step of AQP2 is important for the translocation but does not significantly affect the activity of the protein. Ca^{2+} has also been involved in the translocation of AQP2 to the plasma membrane. Vasopressin is also able to regulate the number of AQPs at the membrane and then finely and quickly tunes the re-absorption of water by the conducting duct epithelium. This involves endocytosis and degradation of the protein through both lysosomal and proteosomal pathways. However, after stimulation, AQP2 is refractory to endocytosis whereas the vasopressin receptor is recycled by re-internalisation [123,225–227]. The phosphorylation status of other C-terminus S residues (S-261 and S-264) may affect both protein subcellular location and abundance in response to vasopressin [228,229]. Recently, the role of a heat shock protein in the trafficking of AQP2 has been demonstrated [230]. AQP5 is also re-localized to the plasma membrane of secretory cells of salivary glands in response to neurotransmitter stimulation. This mechanism, involving lipid rafts as well, results in the control of fluid secretion [109]. In hepatocytes, AQP8 is mainly localized in intracellular vesicles and its translocation to plasma membrane is induced by cAMP and facilitates osmotic water movement during canalicular bile secretion. Thus, defective expression of AQP8 may be associated with secretory dysfunction of hepatocytes caused by extrahepatic cholestasis [231].

In plants, MIP activity is also regulated by salt and osmotic stresses, acting on the phosphorylation status and thus the aperture of the channel, altering translation and/or degradation of the protein; in addition, some mechanisms of re-location in response to salt treatment have been reported for some TIP and PIPs isoforms. Indeed, these proteins, initially present in the tonoplast, were re-located into intravacuolar invaginations or other vesicular membranes after salt or mannitol-induced stress in *Arabidopsis* and *Mesembryanthemum crystallinum*, respectively [155, 232,233]. In *Arabidopsis* the phosphorylation of the S-283 residue is necessary for the subcellular trafficking of the AtPIP2;1 protein in response to stress [234]. Also, it has been shown that AtPIP1 proteins may be present in plasma membrane invaginations called plasmalemmasomes, even if under no stress conditions [235]. In *M. crystallinum*, the redistribution of McTIP1;2 correlates with its glycosylation and is abolished by tunicamycin, an inhibitor of N-glycosidic linkages formation. McTIP1;2 re-location is also disturbed by brefeldin A, which triggers disassembly of the Golgi and disruption of vesicular trafficking, and by wortmannin and cytochalasin D, which inhibit endocytosis and the disassembly of the actin cytoskeleton, respectively [232,233]. This could be a mechanism for fast intracellular water fluxes control [232,233,236]. Interestingly, in *Arabidopsis* roots, the signalling molecule H_2O_2 induced a decrease in hydraulic conductivity through the internalisation of PIPs proteins [237].

Although controversial, AQP1 has been suggested to function as a non-selective cation channel [144]. The passage of cations would occur through the central axis of the AQP1 tetramer. This specific function may be controlled by gating mechanisms involving the action of PKA or cGMP [143,238]. Furthermore, it was demonstrated that AQP6 functions not as a water channel but as an anion-selective channel, suggesting that subtle differences in the sequence of the protein may lead to major differences in biophysical function. The amino acid residue N-60 was identified for anion permeability of AQP6 and its substitution for G(Gly)-60 switches the function of

AQP6 from that of an anion channel to that of a water-selective channel [239]. However, it has been suggested that, rather than being ion channels themselves, some AQPs could participate in protein complexes together with functional ion channels. Indeed, ability of some MIPs to interact with each other (hetero-oligomerisation, e.g. ZmPIP1s and ZmPIP2s, NtPIP1;1 and NtPIP2;1 in plants), together with the closely related expression of AQP4 and Kir4.1 in specific cells of the brain, strengthen this idea [140,170–172]. Moreover, a PDZ domain is found in the C-terminal extremity of AQP1. This sequence has been described as an interaction site with membrane proteins involved in ionic regulation [240,241], opening new insights regarding the function of MIPs and the complexity of their regulation at the protein level.

The control of gene expression is a longer-term way for the cell to answer to a stimulus by adjusting the amount of available protein in the membrane. Transcriptional regulation is rather an adaptation mechanism because the gene has to be transcribed, the subsequent RNA translated and the protein matured and correctly targeted. In mammals, AQP2 transcription is controlled by both vasopressin-dependent and independent mechanisms. After vasopressin V2-receptor stimulation, AQP2 gene transcription is enhanced. Thus, vasopressin acts both at transcriptional and posttranscriptional levels and causes somehow contradictory answers that control the level of active proteins at the plasma membrane [226]. Other hormones are also able to control the transcription of mammalian AQPs: AQP1 and AQP9 are regulated by oestrogens [242], and an increase in AQP2 expression has been observed in early pregnancy (for a review see [243]). Pregnancy has been shown to increase AQP2 mRNA and protein expression in the renal collecting ducts of rats, resulting in expansion of extracellular fluid volume and water retention that occurs during normal pregnancy [244].

In plants, MIP gene expression is regulated developmentally in a cell-specific manner, via hormones, and by environmental conditions, such as water stress, nematodes infection, low temperature, and salinity. However, a general expression pattern of MIP genes cannot be distinguished, the different isoforms being either up- or down-regulated depending on the stimulus and/or the organ studied [155,245]. The absence of a unique expression pattern represents an important tuning mechanism by which the plant is able to adapt to unfavourable conditions. For instance, in response to water deprivation, most of AtPIPs in *Arabidopsis* leaves are transcriptionally down-regulated, however the expression of AtPIP1;4 and AtPIP2;5 is induced, and mRNA levels of AtPIP2;6 remain constant [173]. As in the case of drought stress, salt exposure also results in significant and differential regulation of MIP gene expression: in salt-tolerant rice lines some AQP genes have been shown to be up-regulated following a 150 mM NaCl treatment [246]. Conversely, in *Arabidopsis* roots, all the PIPs and TIPs – highly expressed in non-treated plants – are transcriptionally down-regulated within few hours after the addition of 100 mM NaCl [233]. This difference in transcriptional regulation suggests that each MIP isoform has a distinct role on stress response. Indeed, it has been suggested that, as water content decreases in tissues submitted to salt treatment, MIP genes expression is down-regulated to reduce the water loss within 2–5 h after stress exposure. Then, stressed-cells would recover by up-regulating their MIP stress-responsive isoforms, allowing water influx and promoting ions uptake, and compatible osmolytes synthesis to lower the cellular water potential [247]. As ABA is involved in water stress-related answers, it is not surprising that ABA regulates the transcription of many AQPs isoforms [173]. For instance, *PIP1* genes is transcriptionally controlled by ABA and blue light in guard cell [248,249] and *Lis1* (OsNIP2;1) transcription is down-regulated by dehydration stress and ABA [250]. Some other phytohormones such as gibberellins and possibly brassinosteroids have been shown to control aquaporin expression [184], as well as the circadian rhythm (ex: OsPIP2;4, [154]).

8. Conclusions and perspectives

AQP1 discovery by Peter Agre brought first the answer to a brain-teasing problem – how can water effectively cross biological membranes? – and generated a large interest from the scientific community. Over the past twenty years, after the discovery of the first AQP, many members of the MIP family from all living kingdoms have been cloned and functionally studied. Data collected have shown that these proteins exhibit a striking structural diversity, specificity and regulation mechanisms. In mammals, the involvement of AQP1 in Alzheimer's diseases is being investigated while the role of AQP2 in diabetes insipidus disorder is well-known and opens promising clinical perspectives. On the other hand, AQP0 was showed to be involved in congenital cataracts in humans and mouse [251,252], AQP4 in cerebral water transport and oedema [253,254], AQP5 in Sjögren's syndrome [110,111,112] and AQP7 in obesity [255–257]. Indeed several studies are now staring at AQPs as potential targets for drugs elaboration or genetic engineering strategies [8,123,258,259] to cure and/or prevent human diseases. Ongoing work on the study of the expression of AQP genes and regulation will elucidate in more detail the involvement of AQPs in pathophysiological processes. In plants, the identification of water channels able to transport silicon, a compound involved in abiotic and biotic stress responses, highlights the potential involvement of these proteins in plant growth and survival. Because it has been observed that AQPs are not specific to water transport, since new substrates and new roles, albeit sometime speculative, are assigned to these proteins, the ongoing intense investigation will indeed provide, in a short time, astonishing surprises. For instance, the possible involvement of an aquaporin-like channel on glucose transport in plant cells has been suggested [260], implying a direct role of AQPs in organic nutrition. In a near future, the numerous ongoing studies will certainly reveal a multi-functional role of these proteins in humans and plants.

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