Heteromerization of Plant Aquaporins

Cintia Jozefkowicz, Marie C. Berny, François Chaumont, and Karina Alleva

Abstract The discovery of plasma membrane intrinsic protein (PIP) heterotetramerization has opened a new field of research. This phenomenon was first observed between PIPs belonging to two phylogenetic groups (PIP1 and PIP2) with ubiquitous expression in different plant tissues. These isoforms present few differences in their primary sequence but show major differences in their functionality when expressed in heterologous systems.

Many reports in recent years shed light on the PIP1 and PIP2 interaction as a regulatory mechanism to modulate their trafficking and biological activity. In this regard, PIP heterotetramerization has been proposed as a way of achieving a diversification in the water transport capacity and in the control of net solute transport. Also, acidification conditions were shown to act as a mechanism to control the opening and blockage of these channels in native tissues, and their proton-dependent gating can be affected depending on the presence of PIP2 homotetramers or PIP1-PIP2 heterotetramers in the target membrane.

In the present chapter, we report the state-of-the-art knowledge about PIP heterotetramerization in the context of protein oligomerization. We emphasize the main experiments that help to understand the existence of some relevant structural elements involved in PIP oligomerization and the conditions necessary for these hetero-oligomers to occur in the cell.

C. Jozefkowicz • K. Alleva (✉)
Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Química y Fisicoquímica Biológicas (IQUIFIB). Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina., Buenos Aires, Argentina
e-mail: karina.alleva@gmail.com

M.C. Berny • F. Chaumont
Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud 4-L7.07.14, B-1348, Louvain-la-Neuve, Belgium

F. Chaumont, S.D. Tyerman (eds.), *Plant Aquaporins*, Signaling and Communication in Plants, DOI 10.1007/978-3-319-49395-4_2

1 Aquaporin Hetero-oligomerization

Protein-protein interactions play diverse roles in biology, and, in particular, protein multimerization confers many different functional advantages such us diversifying cellular signalling transduction, modulation of biological activity by protein conformational changes, functional diversity and increases in protein stability. Multimerization is common among proteins that generally exist in a crowded environment where many potential binding partners with different surface properties are available. Nooren and Thornton (2003) point out that the specificity for protein-protein interaction derives mainly from the complementarity of shape and chemistry that determine the free energy of binding, but the protein localization also has a role to play. Interestingly, despite the fact that there are many protein-protein interactions between proteins from different families, interactions are also frequent between proteins belonging to the same family. Many proteins are very specific in their choice of partner, assembling as homo- or hetero-oligomers. In the case of homo-oligomers, protein interaction occurs between identical protein chains, while, in the case of hetero-oligomers, the interaction occurs between non-identical protein chains.

Aquaporins are integral membrane proteins that allow the transport of water and non-charged solutes. All aquaporins have six transmembrane helices and N- and C-termini located intracellularly. Despite the fact that each aquaporin monomer forms a channel with its own pore, they have been shown to adopt a quaternary structure organizing mainly as tetrameric homo-oligomers in membranes (see chapter "Structural Basis of the Permeation Function of Plant Aquaporins"). Many mammal aquaporin structures have been resolved, as is the case of AQP1 (Walz et al. 1994; Murata et al. 2000; Sui et al. 2001; Ruiz Carrillo et al. 2014), AQP0 (Gonen et al. 2004; Harries et al. 2004; Palanivelu et al. 2006; Hite et al. 2010), AQP2 (Frick et al. 2014), AQP4 (Ho et al. 2009) and AQP5 (Horsefield et al. 2008). The tetrameric arrangement was first observed for plant AQPs by cryoelectron microscopy of two-dimensional crystals for α-TIP (TIP, tonoplast intrinsic protein) from bean (Daniels et al. 1999) and SoPIP2;1 (PIP, plasma membrane intrinsic protein) from spinach (Kukulski et al. 2005). Later, SoPIP2;1 structure was resolved by X-ray crystallography of 3D crystals in an open conformation to 3.9 Å resolution and in a close conformation to 2.1 Å (Törnroth-Horsefield et al. 2006). More recently, the structure of an ammonia-permeable TIP aquaporin (AtTIP1;2) from *Arabidopsis thaliana* was obtained at 1.8 Å resolution (Kirscht et al. 2016).

All of the resolved structures correspond to homotetrameric assemblies despite hetero-oligomerization having been described for some family members. Among non-plant aquaporins, some cases of hetero-oligomerization have been reported. For example, a mutated AQP2 is able to form a hetero-oligomer with the wild-type form of AQP2 (Sohara et al. 2006). Also, hetero-oligomerization was reported for AQP1, where a non-functional AQP1 mutated in the loop B or E forms mixed oligomers with a truncated AQP1 mutant (D237Z) (Jung et al. 1994). A striking example of hetero-oligomerization among mammal aquaporins is the case of AQP4. This protein exists in two splicing variants, AQP4M1, starting with Met1, and AQP4M23, starting with Met23, which can assemble in the plasma

membrane as heterotetramers (Lu et al. 1996; Neely et al. 1999). Interestingly, AQP4M23 homotetramers alone or together with AQP4M23-AQP4M1 heterotetramers can aggregate into supramolecular structures known as orthogonal arrays (OAPs) (Rash et al. 1998; Sorbo et al. 2008; Rossi et al. 2012). It is not clear what is the precise function of this kind of AQP clustering, but the predominant localization of OAPs in cells facing a basal lamina and the type of molecules that interacts with these aggregates of AQP4 suggest that this channel arrangement may be involved in establishing and maintaining cell polarity (Wolburg et al. 2011). Interestingly, the difference in the ability to form OAPs between AQP4M23 and AQP4M1 seems to be due to a specific amino acid sequence located in the native N-terminus (Hiroaki et al. 2006).

In contrast to the reported cases of hetero-oligomerization of mammal aquaporins, where the assembly was obtained by the interaction of different aquaporin splicing variants or between a wild-type AQP and its mutated versions, plant PIP aquaporins have the particularity of forming hetero-oligomers that include different PIP isoforms.

It has been described that proteins that are part of complexes tend to evolve at a relatively slow rate in order to improve the co-evolution with their interacting partners (Mintseris and Weng 2005). Interestingly, the molecular phylogenetic profiling of AQPs from nine genomes of flowering plants has shown that the PIP subfamily has a low evolutionary rate (Soto et al. 2012); this high evolutionary constraint may be due to a functional constraint related to the physical interaction that occurs between different members of the PIP subfamily. In the following sections, the state-of-the-art knowledge of PIP heterotetramerization will be presented and discussed.

2 Plant Plasma Membrane Intrinsic Proteins (PIP): The Paradigmatic Case for Hetero-oligomerization of Aquaporins

The first step in the study of the biological activity of PIP aquaporins consists in water transport assays by expressing them in a heterologous system such as *Xenopus laevis* oocytes (Preston et al. 1992). Intriguingly, while PIP isoforms belonging to the PIP2 group are able to reach the oocyte plasma membrane as functional oligomers to increase the osmotic water permeability coefficient (P_f) of the membrane, most PIP1 isoforms do not (Fetter et al. 2004; Sakurai et al. 2005; Bellati et al. 2010). Only a few cases of functional PIP1 facilitating water diffusion when expressed alone in oocytes have been reported (Tournaire-Roux et al. 2003; Suga and Maeshima 2004; Zhang et al. 2007). The lack of water transport activity when *PIP1* cRNA is injected into the oocytes was first interpreted as PIP1 being inactive or having very low water permeability (Daniels et al. 1994; Yamada et al. 1995; Weig 1997; Johansson et al. 1998; Biela et al. 1999; Chaumont et al. 2000; Marin-Olivier et al. 2000; Moshelion et al. 2002). However later on, it was demonstrated that most PIP1 proteins fail in reaching the oocyte plasma membrane but are retained in intracellular compartments (Fetter et al. 2004; Bienert et al. 2012; Jozefkowicz et al. 2013; Yaneff et al. 2014).

Interestingly, Fetter and co-workers (2004) showed that if maize ZmPIP1;2 is co-expressed with ZmPIP2;1, ZmPIP2;4 or ZmPIP2;5, an increase in P_f, which is dependent on the amount of *ZmPIP1;2* cRNA injected, is observed compared to the P_f of oocytes injected with ZmPIP2;5 cRNA alone (Fetter et al. 2004). Moreover, confocal microscopy analysis of oocytes expressing ZmPIP1;2-GFP alone or ZmPIP1;2-GFP plus ZmPIP2;5 showed that the amount of ZmPIP1;2-GFP present in the plasma membrane is significantly higher in co-expressing cells. A physical interaction was proposed to explain these results. Nickel affinity chromatography purification of ZmPIP2;1 fused to a histidine tag leads to the co-elution of ZmPIP1;2-GFP demonstrating the physical interaction of both channels. Also, immunoprecipitation experiments provided additional evidence for the association of ZmPIP1;2 and ZmPIP2;1 in vivo in maize roots and in suspension cells in the absence of any PIP overexpression. Finally and importantly, when co-expressed in maize protoplasts with ZmPIP2, ZmPIP1 proteins, which are retained in the endoplasmic reticulum (ER) when expressed alone, are re-localized to the plasma membrane (Zelazny et al. 2007).

All of these data demonstrated for the first time that some PIP1 requires a PIP2 partner to reach the plasma membrane. Thus, PIP1-PIP2 interaction is relevant for trafficking and re-localization of PIP1 proteins to the plasma membrane as a consequence of their physical interaction with some PIP2s. A similar conclusion was later drawn in epidermal cells of transgenic *Arabidopsis* roots (Sorieul et al. 2011). So, as a consequence of the physical interaction with PIP2 proteins, PIP1s are correctly targeted to the plasma membrane; otherwise, these PIP1s remain retained in the ER. The observation that PIP1 plasma membrane localization relies on the concomitant presence of PIP2s was extended to many PIP2/PIP1 pairs from several plant species and has been observed in plants, oocytes and yeast expression systems (Zelazny et al. 2007; Mahdieh et al. 2008; Vandeleur et al. 2009; Alleva et al. 2010; Bellati et al. 2010; Otto et al. 2010; Ayadi et al. 2011; Bienert et al. 2012).

Though most PIP1-PIP2 pairs studied can functionally interact (the oocyte P_f increases when they are co-expressed) by physical interaction (contacts between both channels that promote PIP1 re-localization), it is worth mentioning that there are some exceptions. For instance, the co-expression of ZmPIP1;1 with ZmPIP2;5 does not result in a P_f increase greater than the P_f measured after the expression of ZmPIP2;5 alone, indicating that ZmPIP1;1 does not functionally interact with ZmPIP2;5 in *Xenopus* oocytes (Fetter et al. 2004). Also, BvPIP2;1 is not able to functionally interact with BvPIP1;1 (Jozefkowicz et al. 2013). Additionally, in this subset of noninteracting plant aquaporins should be included the pairs OsPIP2;3/OsPIP1;3 (Matsumoto et al. 2009) and PvPIP2;3/PvPIP1;1 (Zhou et al. 2007). While in most cases it is still not clear which are the structural differences between these noninteracting PIP1 and PIP2 pairs and the interacting pairs, in the case of BvPIP2;1, it was shown that the first extracellular loop (named loop A) could be responsible for the lack of interaction with BvPIP1;1 (Jozefkowicz et al. 2013). Furthermore, while the finding of PIP2 and PIP1 forming hetero-oligomeric assemblies has been prevalent, hetero-oligomerization between different PIP2 and different PIP1 isoforms has also been demonstrated, such as ZmPIP2;6 with ZmPIP2;1 (Cavez et al. 2009) and ZmPIP1;1 with ZmPIP1;2 (Fetter et al. 2004).

Interestingly, the amino acid identity between the PIP1 and PIP2 isoforms is quite high (~80 %), with the main differences located at the C- and N-terminal domains and the loop A. However, this high percentage identity between paralogues is not due to the recent emergence of them, since PIP1 and PIP2 subfamilies separated long before the divergence of monocots and dicots occurred; indeed, PIPs representative of the PIP1 and PIP2 groups are found in the moss *Physcomitrella patens* (Danielson and Johanson 2010). The high identity between PIPs can be due to a high evolutionary and functional constraint of this subfamily (Soto et al. 2012).

3 Structure-Function Relationships in PIP Hetero-oligomerization

The precise way in which PIP1 and PIP2 interact is not yet fully elucidated, but strong experimental evidence is emerging and shedding light on this issue. The results obtained by the co-expression of the PIP1 and PIP2 channels, obtained in the first research stages on this topic, were compatible with protein complexes formed either by interactions between different PIP homotetramers or by interactions between different PIP monomers organized within an heterotetramer. Over the years the terms 'hetero-oligomer' and 'heterotetramer' were both used in the research literature, and many times they were even taken as synonymous. Recently, some experimental data indicated that PIP1 and PIP2 co-assemble in heterotetramers (different monomers interacting within the same tetramer). Still, even though the high sequence identity among all the PIP clusters explains the possibility that PIP1 and PIP2 assemble together into the same tetramer, as if they were almost 'identical' molecules, not all of the PIP1-PIP2 pairs follow this behaviour. Indeed, site-directed mutagenesis experiments together with crystallography and molecular modelling revealed the existence of some crucial structural elements involved in PIP oligomerization that can also control hetero-oligomerization.

3.1 Loop A

The prevalent role of the loops in protein function is due to their flexibility and location on the surface of the protein. Besides their role in protein function, intra- and extracellular loops are also important as structural elements mediating protein interactions, especially in membrane proteins. In particular, for PIP aquaporin oligomers, the first extracellular loop A has been reported as mediating the interactions between different monomers within a tetramer. Indeed, a highly conserved cysteine residue and the hinge connecting the first transmembrane domain (TM1) to the loop A have a relevant role in tetrameric organization (Bienert et al. 2012; Jozefkowicz et al. 2013). Early observations by Barone and co-workers (1998) provided experimental evidence that *Beta vulgaris* PIP dimers are connected by a

disulphide bridge. In the SoPIP2;1 tetrameric structure, the position of the C-terminus of helix 1 and the N-terminus of helix 2 orients the loop A towards the fourfold centre of the tetramer (Kukulski et al. 2005). In this configuration, the nearness of the four-loop A cysteines suggested the formation of a disulphide bond between two monomers (Kukulski et al. 2005). In accordance with these data, molecular dynamic simulation (MDS) of BvPIP2;1 also showed that the position of loop A is oriented towards the centre of the tetramer, with each of the four loops A being flexible parts of the monomers but having a different solvation pattern and different movement along the MDS (Jozefkowicz et al. 2013).

The demonstration of the existence of a disulphide bond linking the loops A of two monomers was conducted by Bienert et al. (2012) and showed that this disulphide bond is not an artefact of the sample preparation but that they occurred in plant cells. Interestingly, mutation of the loop A cysteine residue does not alter the water channel activity of PIP1 or PIP2 in homo- or heterotetramers, hetero-oligomerization or trafficking to the plasma membrane but does modify its mercury sensitivity (Bienert et al. 2012). Incubation of oocytes expressing ZmPIP2;5 alone with $HgCl_2$, a well-known aquaporin inhibitor, leads to a decrease in the P_f. However, the P_f of oocytes co-expressing ZmPIP1;2Cys85Ser and WT ZmPIP2;5 or ZmPIP2;5Cys75Ser is not inhibited in response to $HgCl_2$ treatment, indicating that when ZmPIP2;5 is co-expressed with ZmPIP1;2Cys85Ser, ZmPIP2;5 becomes Hg^{2+} insensitive. This data suggests that the loop A cysteine of ZmPIP1;2 is involved in the mercury sensitivity of hetero-oligomers and indicates that the conformational arrangement of the PIP2 monomers in the PIP1-PIP2 hetero-oligomers is different from that in the PIP2 homo-oligomers (Bienert et al. 2012). These data point to the potential functional and conformational interaction between PIP1 and PIP2 in hetero-oligomer complexes mediated by the loop A structure (Bienert et al. 2012). The presence of a disulphide bridge could affect the stability of the tetramers in some conditions, but this process needs to be investigated in more detail. The results obtained for BvPIP2;1, a PIP2 unable to interact with BvPIP1;1 and presenting non-conserved amino acid residues in the N-terminus of the loop A, are in accordance with this hypothesis (Jozefkowicz et al. 2013). The mutation of non-conserved loop A residues (NETD) of BvPIP2;1 promoted the recovery of its interaction with BvPIP1;1, resembling the PIP2-PIP1 classical interaction (Jozefkowicz et al. 2013). In agreement, Hayward and Kitaos (2010) stress the importance of the first and last residues of the protein loops, remarking that this constraint might influence the dynamical behaviour of the loop.

Altogether, these data are strong evidence for an important role of the loop A in controlling the interactions between contiguous monomers in PIP homo- or heterotetramers.

3.2 Loop E

In all aquaporins, loops B and E containing the NPA motif (asparagine, proline, alanine) form half transmembrane helices that fold into the channel from opposite sides of the membrane. This creates a seventh transmembrane helix, with the NPA

motifs located at the centre of the pore (Jung et al. 1994; Murata et al. 2000; Törnroth-Horsefield et al. 2006).

Early studies on AQP1 suggested that loop E together with loop B is not only involved in water permeation through the pore but is also critical for the tetrameric assembly of the channel (Jung et al. 1994; Mathai and Agre 1999). Later, a mutational analysis conducted in plant aquaporins demonstrated the important role of the C-terminal part of loop E in the interaction of PIP monomers (Fetter et al. 2004). To explore the reason for the different behaviour of ZmPIP1;1 and ZmPIP1;2 regarding their functional interaction with ZmPIP2;5 in *Xenopus* oocytes, a ZmPIP1;1 mutant containing the loop E of ZmPIP1;2 (named ZmPIP1;1LE) was constructed (Fetter et al. 2004). The replacement of the loop E of ZmPIP1;1 by that from ZmPIP1;2 modifies the behaviour of the protein when co-expressed with ZmPIP2;5: a positive synergistic effect on the P_f is observed, while it is absent with the WT ZmPIP1;1 (Fetter et al. 2004). The molecular explanation of this functional observation arises from ZmPIP1;1LE MDS, which shows a different position of the loop in ZmPIP1;1LE affecting the structure of the pore. This conformational change in loop E is proposed to affect the structure of the semi-helix E and the TM6, which can in turn impact the oligomerization of this protein (reviewed in Chaumont et al. 2005). However, it was not shown whether loop E from ZmPIP1;2 is required for ZmPIP1;1 interaction with ZmPIP2;5 or only for the activation of ZmPIP1;1 water channel activity. Similarly, exchanging the loop E of GlpF, a bacterial glycerol facilitator of the MIP/aquaporin family, with loop E of the insect AQPcic, alters either oligomer assembly or tetramer stability (Duchesne et al. 2002). Other evidence supporting the involvement of the aquaporin loop E in oligomerization is the crystallographic and molecular modelling data, showing that TM5 participates in the interactions between monomers within the tetramer and can affect the spatial positions of loops B and E in AQP1 and GlpF (Ren et al. 2000; Murata et al. 2000; Fu et al. 2000; Jensen et al. 2001). Moreover, in mammal AQPs, functional analyses of AQP0-AQP2 chimeras have demonstrated that stability of loop E is crucial for the channel activity (Kuwahara et al. 1999; Suga and Maeshima 2004).

3.3 Transmembrane Domains

In addition to the loops, the TM domains from neighbour monomers interact within a tetramer. In AQP1, TM1 and TM2 interact with TM4 and TM5 of an adjacent monomer by coiled-coil interactions in a left-handed fashion (Murata et al. 2000). This type of interaction is common to tightly pack structures, thanks to van der Waals interactions between side chains of close residues. In addition, hydrogen bonds between TM1 and TM5 or between TM2 and TM4 stabilize the structure. The role of specific TM amino acid residue in oligomerization is still unknown, but some TM residues have been shown to turn an inactive PIP1 into an active one without any co-expression in oocytes. It is the case of substitutions in the rice OsPIP1;1 and OsPIP1;3 (A103V and A102V, respectively) that allowed an increase in water channel activity for non-functional PIP1 isoforms (Zhang et al. 2010). In this work, the authors identified by homology modelling and mutagenesis one residue in TM2

of OsPIP1;3 located at the interface between monomers that induced upon mutation a change of conformation within the pore. Indeed, a change of orientation of Ile101 was caused by the substitution of Ala102 into Val and allowed the widening of the constriction region within the pore.

Comparative modelling on the basis of SoPIP2;1 X-ray structure was used to build heterotetramers containing ZmPIP1;2 and ZmPIP2;5 and identify amino acid residues in the TM domains that putatively interact at the interfaces between monomers (Berny et al. 2016). Mutational analysis of these residues showed single residue substitution that either inactivates ZmPIP2;5 (W85A, F92A and F210A) or activates ZmPIP1;2 (Q91L and F220A) without affecting their interaction when express in *Xenopus* oocytes. Interestingly, the activating F220A mutation in TM5 of ZmPIP1;2 inactivates, at the same time, the water channel activity of the interacting ZmPIP2;5 within a heterotetramer (Berny et al. 2016). Altogether, these data highlight the importance of single specific TM amino acid residues in the activity of the channels within a heterotetramer without affecting the interaction between monomers. Multiple mutations might be required to affect the oligomerization state.

4 Co-expression of PIP: A Condition for Oligomerization

The protein localization, concentration and local environment are parameters affecting the interaction and controlling the composition and oligomeric state of protein complexes (Nooren and Thornton 2003). Monomers participating in obligate interactions to form oligomers are supposed to be co-expressed and to co-localize upon synthesis. This time and space synchronization should be valid for homo-oligomers as well as for hetero-oligomers.

The interactions between proteins which allow complex formation are usually driven by the concentration of the components and the free energy of the complex relative to the alternative states (Nooren and Thornton 2003). So, there are different conditions that can control the oligomerization phenomenon, including encountering the interacting surfaces. In this regard, the association of two proteins relies on co-localization in time and space and the adequate concentration of the interacting proteins, where control mechanisms that alter the effective local concentration such as gene expression, protein degradation rates or diffusion rates, among others, are relevant. In the competition between binding partners for protein-protein interactions, different factors can influence oligomerization. As PIP2 and PIP1 can ensemble either as homotetramers or heterotetramers, different elements should be involved in the ruling of the processes that govern each option.

PIPs are expressed in organs and tissues that present large fluxes of water, i.e. vascular tissues, guard cells and suberized endodermis and bundle sheath cells, among others (Gomes et al. 2009; Chaumont and Tyerman 2014). Interestingly, there is not a homogeneous expression pattern for all PIPs, and this has been interpreted as a tuning mechanism by which the plant is able to adapt to different conditions (Gomes et al. 2009). Nonetheless, there are several stimuli that promote a

coordinate response in the expression of different PIPs. For example, upon water deprivation, most *Arabidopsis* PIPs expressed in leaves are reported as transcriptionally downregulated, with the exception of AtPIP2;6 which remains constant, and AtPIP1;4 and AtPIP2;5 which are both induced (Alexandersson et al. 2010). A reverse genetic study performed for AtPIPs showed that knock-out of three PIP isoforms belonging to the PIP1 and PIP2 groups contributed individually to the same hydraulic conductivity as the corresponding triple *PIP* mutant (Prado et al. 2013). Microarray studies during grape berry development showed that PIP1 and PIP2 are expressed in the same tissues at the same time, but each one has a particular pattern of expression (Fouquet et al. 2008). All these results indicate synchronization between some *PIP1* and *PIP2* gene expression, but the physical or functional interactions of all the mentioned PIPs have not been studied yet.

Conversely, there are other cases for which information about both tissue expression and protein interaction in heterologous systems were recorded. For instance, *Fragaria x ananassa* PIPs interact to form heterotetramers (Alleva et al. 2010; Yaneff et al. 2014), and both *PIP1* and *PIP2* mRNA are expressed during the whole ripening process in fruits (Alleva et al. 2010). Interestingly, the expression of *FaPIP1* was low in the first ripening stages and later increased; while in the case of *FaPIP2*, the expression was markedly high in the first stages and decreases progressively until the end of ripening or remained approximately constant and low, depending on the cultivar. This dissimilar expression pattern of FaPIP2 and FaPIP1 can be compatible with the reported random stoichiometry suggested for *F. x ananassa* PIP heterotetramers after FaPIP oocyte expression and mathematical analysis of the results (Yaneff et al. 2014). Furthermore, information about the expression pattern of paradigmatic interacting ZmPIPs is also available. Gene and protein expression was studied in maize roots, leaves and stomatal complexes (Hachez et al. 2006, 2008; Heinen et al. 2014). All *ZmPIP* genes, except *ZmPIP2;7*, are expressed in primary roots and leaves, and their expression is dependent on the developmental stage of the organ (Hachez et al. 2006, 2008). In this regard, it was proposed that some specific pairs of PIP1 and PIP2 have a correlation in their expression pattern in accordance with their functional responses (Yaneff et al. 2015).

In addition to transcriptional and translational regulation, PIP localization and stability are also highly regulated (for a review, see Hachez et al. 2013; Chevalier and Chaumont 2015). For instance, after salt exposure to *Arabidopsis* roots, a decrease in PIP1 protein abundance was observed after 30 min, while PIP2 abundance remained constant even after 6 h of exposure to stress conditions. However, PIP1and PIP2 were both reduced after 24 h of salt exposure (Boursiac et al. 2005).

All of these results indicate that, depending on the conditions, PIP1 and PIP2 can be co- or differentially regulated, but PIP physical interaction could represent an additional cooperative way to respond to different physiological processes or even stresses. Indeed, all reports regarding PIP expression in different plants show that both PIP1 and PIP2 proteins are always present together. It is the ratio between protein (or mRNA) amounts of each group that can vary considerably between tissues or cell types, certainly affecting their physiology. For instance, among all the *PIP* transcripts found in maize stomatal complexes, 85 % were PIP1s (Heinen et al.

2014). In this regard, it was empirically proven that *B. vulgaris* PIP1 and PIP2 can assemble in a flexible fashion into tetramers having different stoichiometries depending on the amount of each *PIP1* or *PIP2* cRNA available (Jozefkowicz et al. 2016). Thus, differences in the expression levels of each paralogue condition the existence of different PIP1 and PIP2 homotetramers or heterotetramers.

5 Biological Relevance of PIP Hetero-oligomerization

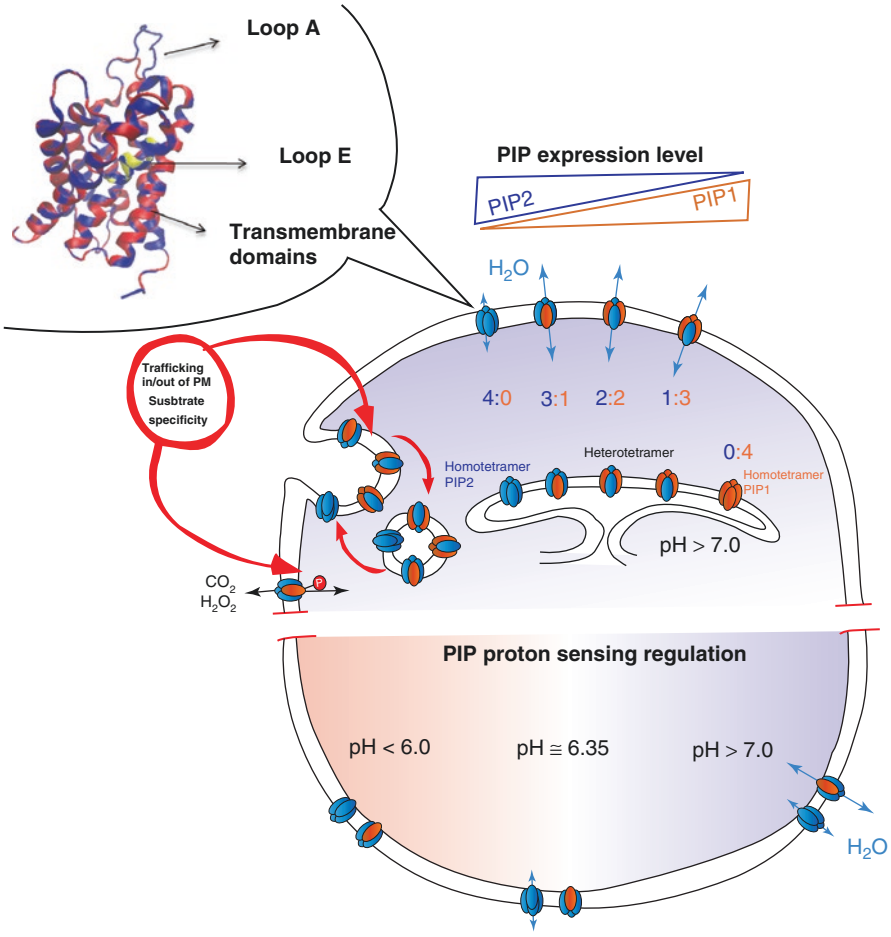
The hetero-oligomerization of membrane proteins is believed to play a fundamental role in the regulation of cellular function. Oligomerization can increase protein stability, and in particular hetero-oligomerization may allow the diversification of biological activity. In this regard, it was postulated that protein-protein interactions may have evolved to optimize functional efficacy (Nooren and Thornton 2003). However, it is also possible that an interaction with no functional reason evolved, and this interaction survives due to the absence of selective pressure to be rejected from the evolutionary path. Often, the functional rationale of oligomerization is not clear, and a happenstance of oligomerization can be supposed (Nooren and Thornton 2003). However, in the case of PIP1-PIP2 hetero-oligomerization, this kind of supramolecular assembly plays an important role in the subcellular PIP1 localization and modulates the cell membrane hydraulic conductivity (Fig. 1).

Fig. 1 PIP heterotetramerization in the plant cell. Scheme of a plant cell where the PIP1 monomers are shown in *orange*, and the PIP2 monomers are shown in *blue*. In the *lower part* of the scheme, the cell is under three different pH units: <6.0, ~ 6.5 and >7.0; in the *upper part* of the scheme, the cell is under pH >7.0. The water capacity of each tetramer is represented as proportional to the length of the *light-blue arrow*, while the solute capacity of each tetramer (i.e. to dioxide carbon) is represented with a *black arrow*. In the *box* on *top* at the *left*, a structural alignment of a representative PIP1 (in *red*) and PIP2 (in *blue*) monomer is shown, pointing out the major differences between these channels (regarding loop A and the N- and C-termini) and some structural elements relevant for heterotetramerization. All reports regarding PIP expression in different tissues or cell types found the simultaneous expression of both PIP1 and PIP2. However, the ratio between mRNA (or protein) amounts of each group can vary considerably between different cell types, conditioning the formation of different PIP1 and PIP2 homo- or heterotetramers. All PIP heterotetramers with different stoichiometries and PIP2 homotetramers are able to reach the plasma membrane, but they show different water transport capacities when heterologous expressed, being the water transport capacity of PIP1-PIP2 heterotetramers higher than the water transport capacity found for PIP2 homotetramers. On the contrary, PIP1 homotetramers are unable to reach the plasma membrane when heterologous expressed. Thus, upon regulation of PIP1-PIP2 expression levels, the plant cell can modulate the localization of different tetrameric species and the osmotic water permeability and substrate permeability of the plasma membrane by the assembly of PIP heterotetramers with different stoichiometries. Since each plant species has a variety of PIP1 and PIP2 isoforms, another point of regulation would be given by the expression of different types of each of these paralogues. Since not all PIP1 PIP2 are capable of interacting, locating PIP1 into the plasma membrane would depend on their capacity to interact with certain PIP2. In this regard, some structural elements involved in PIP1 and PIP2 contacts in PIP heterotetramers – loop A and loop E containing the second NPA motif (show in *yellow*) – and some TM have been shown to be relevant. At the level of the gating of the channel, the intracellular pH changes can differentially regulate the activity of PIP tetramers found in the plasma membrane, either as homo- or heterotetramers.

5.1 *Trafficking*

As mentioned above, immunocytochemistry, immunodetection and the expression of PIP fused to fluorescent proteins showed that most PIP2 aquaporins are localized in the plasma membrane, while most PIP1 aquaporins are found in the ER unless co-expressed with PIP2 (Zelazny et al. 2007; Li et al. 2009; Luu et al. 2012; Besserer et al. 2012; Jozefkowicz et al. 2013). The prevalence of PIP2 homotetramers vs PIP1-PIP2 heterotetramers can be controlled by the regulation of the expression of both paralogues, as described in Sect. 4.

The regulation of PIP1 and PIP2 trafficking to the plasma membrane, as homo- or heterotetramers, is another important mechanism leading to modification of the membrane permeability (Fig. 1) (see also chapter "Plant Aquaporin Trafficking"). Specific trafficking motifs for PIP2 export from the ER have been described. These include a diacidic motif (DxE) located at the N-terminus of some maize and *Arabidopsis* PIP2s (Zelazny et al. 2009; Sorieul et al. 2011) that probably interact with the Sec24 protein of the COPII complex for their recruitment to the moving



vesicle (reviewed in Hachez et al. 2013; Chevalier and Chaumont 2015). However, ZmPIP2;1 or ZmPIP2;2 can reach the plasma membrane even in the absence of a diacidic motif (Zelazny et al. 2009). Recently, a new LxxxA motif located in the TM3 of ZmPIP2;5 has been shown to be required for the ER export (Chevalier et al. 2014). Interestingly, the addition of ZmPIP2;5 diacidic and/or the LxxxA motifs in ZmPIP1;2 is not enough to induce the ER export of the latter, indicating the presence of specific retention signals in ZmPIP1;2 (Zelazny et al. 2009; Chevalier et al. 2014). The observation that all the hetero-oligomers comprising PIP1-PIP2 tested so far are found in the plasma membrane of plant cells and heterologous systems suggests that (i) the ER retention signal of PIP1 is hidden upon an interaction with PIP2 and (ii) the interaction with PIP2 is enough to ensure the routing of PIP1 to the plasma membrane even in the absence of any export signal in PIP1, meaning that the PIP1-PIP2 interaction does not prevent the PIP2 trafficking motifs from being properly recognized by the trafficking machinery.

From an evolutionary point of view, we can wonder whether most PIP1s have no plasma membrane trafficking motifs due to the fact that these motifs are not required as PIP1s are always co-expressed with PIP2s, which contain the information to direct them together as heterotetramers to the plasma membrane.

5.2 Biological Activity and Substrate Specificity

As it was already mentioned above, when PIP1 is co-expressed with PIP2, their interaction is accompanied in oocytes by an increase in the water permeability of the plasma membrane (Fetter et al. 2004; Vandeleur et al. 2009; Alleva et al. 2010; Bellati et al. 2010; Ayadi et al. 2011; Jozefkowicz et al. 2013; Yaneff et al. 2014), indicating that the most prominent physiological modification upon PIP1-PIP2 hetero-oligomerization is the alteration of the hydraulic conductivity of the cell plasma membrane. The source of this increase in the osmotic water permeability can be simply due to the fact that more aquaporins are present in the plasma membrane. However, other scenarios can result in similar final response. A publication studying *F. x ananassa* PIP1-PIP2 heterotramers shed light on this issue showing that PIP1 has a high water transport capacity and that PIP2 water permeability is enhanced when it is part of a heterotetramer (Yaneff et al. 2014). Furthermore, it was recently demonstrated that heterotetramers of BvPIP2;2 and BvPIP1;1 or ZmPIP1;2 and ZmPIP2;5 having 3:1, 1:3 and 2:2 stoichiometries can coexist at the plasma membrane (Jozefkowicz et al. 2016; Berny et al. 2016). In addition BvPIP2;2 and BvPIP1;1 contribute equally to the total plasma membrane permeability, as it was probed that each of these individual heterotetrameric species present equal water transport capacity (Jozefkowicz et al. 2016).

PIP heterotetramerization has also been proposed as a way of achieving a diversification and control of net solute transport. PIP1 members have been shown to also be permeable to other small uncharged solutes such as glycerol (Biela et al. 1999; Moshelion et al. 2002), boric acid (Dordas et al. 2000) and CO_2 (Uehlein et al.

2003), while PIP2s mainly act as water channels, although some of them can also transport H_2O_2, glycerol (Chaumont et al. 2001; Uehlein et al. 2008; Dynowski et al. 2008; Bienert et al. 2014) and CO_2 (Wang et al. 2016) (Fig. 1). CO_2 transport has been studied in depth in *Nicotiana tabacum* PIP1 and in *Zea mays* PIP1 (Uehlein et al. 2003; Otto et al. 2010; Bienert et al. 2014; Heinen et al. 2014). Different transport profiles have been found for NtPIP2 and NtAQP1 (belonging to the PIP1 group) by analysis of their transport characteristics after co-expression in the yeast *Saccharomyces cerevisiae* (Otto et al. 2010). While the expression of NtPIP2;1 increases the water diffusion through the cell membrane but not the CO_2 diffusion (deduced from CO_2-triggered intracellular acidification of the cells), the expression of NtAQP1 only increases the CO_2 diffusion (Otto et al. 2010). Functional analysis of artificial tetramers with a defined proportion of NtAQP1 and NtPIP2;1 indicates that the presence of a single NtPIP2;1 protein within a heterotetramer allows for an increase in the cell water permeability. On the other hand, an increase in the CO_2 diffusion requires the presence of at least three or four PIP1 in the heterotetramer. These data suggest that the stoichiometry of the heterotetramers can influence the substrate specificity of the complexes.

5.3 Proton-Sensing Regulation

Lastly, we consider pH sensing as an additional important modulator of the biological response upon PIP heterotetramerization. PIPs are acid-sensitive water channels both when heterologously expressed and in native tissues (Gerbeau et al. 2002; Tournaire-Roux et al. 2003; Alleva et al. 2006; Verdoucq et al. 2008). On the basis of SoPIP2;1 molecular structure, a gating model was proposed (Törnroth-Horsefield et al. 2006) that shows how conformational changes in the cytosolic loop D promote the opening and closure of the pore and control its water permeability. These conformational modifications are pH dependent since they are mediated by the protonation of a conserved His residue (His193 in SoPIP2;1) (Tournaire-Roux et al. 2003). At acidic pH, the His residue is charged and by interaction with other residues stabilizes the loop D closing the pore (Törnroth-Horsefield et al. 2006; Frick et al. 2013).

A shift in the pH sensing has been reported for PIP1-PIP2 heterotetramers in comparison with the PIP2 homotetramers (Bellati et al. 2010; Jozefkowicz et al. 2013; Yaneff et al. 2014). Water permeability pH dependence presents a low $pH_{0.5}$ (the pH at which the half maximal P_f is found) for PIP2 homotetramers, implying that a high probability of PIP channels in open conformation is found at physiological conditions. On the contrary, for the PIP1-PIP2 heterotetramers, a pH shift of 0.5 towards alkaline values was observed (Fig. 1). Moreover, all PIP1-PIP2 heterotetrameric assemblies of variable stoichiometries present equivalent biological activity in terms of pH gating and cooperative response (Jozefkowicz et al. 2016).

When *B. vulgaris* PIPs were studied, both homotetrameric BvPIP2;2 and BvPIP2;1 presented sigmoidal response curves with a $pH_{0.5}$ of approximately 6.4–6.5, but BvPIP1;1-BvPIP2;2 heterotetramers showed a $pH_{0.5}$ of approximately 6.7–6.8

(Bellati et al. 2010; Jozefkowicz et al. 2013). Interestingly, the plasma membrane of *B. vulgaris* storage roots vesicles shows a pH gating profile with $pH_{0.5}$ values around 6.7 (Alleva et al. 2006). In this way, acidification conditions to control the opening and blockage of water transport in native tissues vary depending on the presence of PIP2 homotetramers or PIP1-PIP2 heterotetramers in the target membrane.

6 Concluding Remarks

The comprehension of the functional relevance of protein oligomerization is not straightforward due to the fact that the proportion of well-studied oligomeric proteins is quite low compared with monomeric proteins. Even harder is the understanding of the biological relevance of hetero-oligomerization. The discovery of the existence of PIP1-PIP2 hetero-oligomers made these proteins of high interest not only for studying the physiological relevance of these complexes at the cell and plant levels in relation to their trafficking, substrate specificity and activity of the channels but also to better understand the biochemical and biophysical mechanisms that drive their physical association to form heterotetramers. They constitute a promising model to elucidate the rules governing protein oligomerization. One of the next challenges will be to obtain high-resolution structural data of heterotetramers.

Acknowledgements This work was supported by grants UBACYT 02042 0159-2013 (to KA) and grants from the Belgian National Fund for Scientific Research (NFRS), the Interuniversity Attraction Poles Programme-Belgian Science Policy (IAP7/29) and the Belgian French community ARC11/16-036 project (to FC). We thank Gerardo Zerbetto De Palma for his contributions with figure design.

References

Alexandersson E, Danielson JAH, Råde J et al (2010) Transcriptional regulation of aquaporins in accessions of Arabidopsis in response to drought stress. Plant J 61:650–660

Alleva K, Marquez M, Villarreal N et al (2010) Cloning, functional characterization, and co-expression studies of a novel aquaporin (FaPIP2;1) of strawberry fruit. J Exp Bot 61:3935–3945

Alleva K, Niemietz CM, Sutka M et al (2006) Plasma membrane of *Beta vulgaris* storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. J Exp Bot 57:609–621

Ayadi M, Cavez D, Miled N et al (2011) Identification and characterization of two plasma membrane aquaporins in durum wheat (*Triticum turgidum* L. subsp. durum) and their role in abiotic stress tolerance. Plant Physiol Biochem 49:1029–1039

Barone LM, Mu HH, Shih CJ et al (1998) Distinct biochemical and topological properties of the 31- and 27-kilodalton plasma membrane intrinsic protein subgroups from red beet. Plant Physiol 118:315–322

Bellati J, Alleva K, Soto G et al (2010) Intracellular pH sensing is altered by plasma membrane PIP aquaporin co-expression. Plant Mol Biol 74:105–118

Berny MC, Gilis D, Rooman M, Chaumont F (2016) Single mutations in the transmembrane domains of maize plasma membrane aquaporins affect the activity of monomers within a heterotetramer. Mol Plant. doi:10.1016/j.molp.2016.04.006

Besserer A, Burnotte E, Bienert GP et al (2012) Selective regulation of maize plasma membrane aquaporin trafficking and activity by the SNARE SYP121. Plant Cell 24:3463–3481

Biela A, Grote K, Otto B et al (1999) The *Nicotiana tabacum* plasma membrane aquaporin NtAQP1 is mercury-insensitive and permeable for glycerol. Plant J 18:565–570

Bienert GP, Cavez D, Besserer A et al (2012) A conserved cysteine residue is involved in disulfide bond formation between plant plasma membrane aquaporin monomers. Biochem J 445:101–111

Bienert GP, Heinen RB, Berny MC, Chaumont F (2014) Maize plasma membrane aquaporin ZmPIP2;5, but not ZmPIP1;2, facilitates transmembrane diffusion of hydrogen peroxide. Biochim Biophys Acta 1838:216–222

Boursiac Y, Chen S, Luu D-T et al (2005) Early effects of salinity on water transport in Arabidopsis roots. Molecular and cellular features of aquaporin expression 1. Plant Physiol 139:790–805

Cavez D, Hachez C, Chaumont F (2009) Maize black Mexican sweet suspension cultured cells are a convenient tool for studying aquaporin activity and regulation. Plant Signal Behav 4:890–892

Chaumont F, Tyerman SD (2014) Aquaporins: highly regulated channels controlling plant water relations. Plant Physiol 164:1600–1618

Chaumont F, Barrieu F, Jung R, Chrispeels MJ (2000) Plasma membrane intrinsic proteins from maize cluster in two sequence subgroups with differential aquaporin activity. Plant Physiol 122:1025–1034

Chaumont F, Barrieu F, Wojcik E et al (2001) Aquaporins constitute a large and highly divergent protein family in maize. Plant Physiol 125:1206–1215

Chaumont F, Moshelion M, Daniels MJ (2005) Regulation of plant aquaporin activity. Biol cell under auspices. Eur J Cell Biol 97:749–764

Chevalier AS, Chaumont F (2015) Trafficking of plant plasma membrane aquaporins: multiple regulation levels and complex sorting signals. Plant Cell Physiol 56:819–829

Chevalier AS, Bienert GP, Chaumont F (2014) A new LxxxA motif in the transmembrane Helix3 of maize aquaporins belonging to the plasma membrane intrinsic protein PIP2 group is required for their trafficking to the plasma membrane. Plant Physiol 166:125–138

Daniels MJ, Chrispeels MJ, Yeager M (1999) Projection structure of a plant vacuole membrane aquaporin by electron cryo-crystallography. J Mol Biol 294:1337–1349

Daniels MJ, Mirkov TE, Chrispeels MJ (1994) The plasma membrane of *Arabidopsis thaliana* contains a mercury-insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP. Plant Physiol 106:1325–1333

Danielson JAH, Johanson U (2010) Phylogeny of major intrinsic proteins. Adv Exp Med Biol 679:19–31

Dordas C, Chrispeels MJ, Brown PH (2000) Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. Plant Physiol 124:1349–1362

Duchesne L, Pellerin I, Delamarche C et al (2002) Role of C-terminal domain and transmembrane helices 5 and 6 in function and quaternary structure of major intrinsic proteins: analysis of aquaporin/glycerol facilitator chimeric proteins. J Biol Chem 277:20598–20604

Dynowski M, Schaaf G, Loque D et al (2008) Plant plasma membrane water channels conduct the signalling molecule H2O2. Biochem J 414:53–61

Fetter K, Van Wilder V, Moshelion M, Chaumont F (2004) Interactions between plasma membrane aquaporins modulate their water channel activity. Plant Cell 16:215–228

Fouquet R, Léon C, Ollat N, Barrieu F (2008) Identification of grapevine aquaporins and expression analysis in developing berries. Plant Cell Rep 27:1541–1550

Frick A, Eriksson UK, de Mattia F et al (2014) X-ray structure of human aquaporin 2 and its implications for nephrogenic diabetes insipidus and trafficking. Proc Natl Acad Sci U S A 111:6305–6310

Frick A, Järvå M, Törnroth-Horsefield S (2013) Structural basis for pH gating of plant aquaporins. FEBS Lett 587:989–993

Fu D, Libson A, Miercke LJ et al (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. Science 290:481–486
Gerbeau P, Amodeo G, Henzler T et al (2002) The water permeability of Arabidopsis plasma membrane is regulated by divalent cations and pH. Plant J 30:71–81
Gomes D, Agasse A, Thiébaud P et al (2009) Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochim Biophys Acta Biomembr 1788: 1213–1228
Gonen T, Cheng Y, Kistler J, Walz T (2004) Aquaporin-0 membrane junctions form upon proteolytic cleavage. J Mol Biol 342:1337–1345
Hachez C, Besserer A, Chevalier AS, Chaumont F (2013) Insights into plant plasma membrane aquaporin trafficking. Trends Plant Sci 18:1–9
Hachez C, Heinen RB, Draye X, Chaumont F (2008) The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. Plant Mol Biol 68:337–353
Hachez C, Zelazny E, Chaumont F (2006) Modulating the expression of aquaporin genes in planta: a key to understand their physiological functions? Biochim Biophys Acta 1758:1142–1156
Harries WEC, Akhavan D, Miercke LJW et al (2004) The channel architecture of aquaporin 0 at a 2.2-A resolution. Proc Natl Acad Sci U S A 101:14045–14050
Hayward S, Kitao A (2010) The effect of end constraints on protein loop kinematics. Biophys J 98:1976–1985
Heinen RB, Bienert GP, Cohen D et al (2014) Expression and characterization of plasma membrane aquaporins in stomatal complexes of *Zea mays*. Plant Mol Biol 86:335–350
Hiroaki Y, Tani K, Kamegawa A et al (2006) Implications of the aquaporin-4 structure on array formation and cell adhesion. J Mol Biol 355:628–639
Hite RK, Li Z, Walz T (2010) Principles of membrane protein interactions with annular lipids deduced from aquaporin-0 2D crystals. EMBO J 29:1652–1658
Ho JD, Yeh R, Sandstrom A et al (2009) Crystal structure of human aquaporin 4 at 1.8 A and its mechanism of conductance. Proc Natl Acad Sci U S A 106:7437–7442
Horsefield R, Norden K, Fellert M et al (2008) High-resolution x-ray structure of human aquaporin 5. Proc Natl Acad Sci 105:13327–13332
Jensen MO, Tajkhorshid E, Schulten K (2001) The mechanism of glycerol conduction in aquaglyceroporins. Structure 9:1083–1093
Johansson I, Karlsson M, Shukla VK et al (1998) Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. Society 10:451–459
Jozefkowicz C, Rosi P, Sigaut L et al (2013) Loop A is critical for the functional interaction of two *Beta vulgaris* PIP aquaporins. PLoS One 8:e57993
Jozefkowicz C, Sigaut L, Scochera F et al (2016) PIP water transport and its pH dependence are regulated by tetramer stoichiometry. Biophys J 110:1312–1321
Jung JS, Preston GM, Smith BL et al (1994) Molecular structure of the water channel through aquaporin CHIP. The hourglass model. J Biol Chem 269:14648–14654
Kirscht A, Kaptan S, Bienert G et al (2016) Crystal structure of an ammonia-permeable aquaporin. PLoS Biol 14:e1002411
Kukulski W, AD S, Johanson U et al (2005) The 5A structure of heterologously expressed plant aquaporin SoPIP2;1. J Mol Biol 350:611–616
Kuwahara M, Shinbo I, Sato K et al (1999) Transmembrane helix 5 is critical for the high water permeability of aquaporin. Biochemistry 38:16340–16346
Li Y, Wu Z, Ma N, Gao J (2009) Regulation of the rose Rh-PIP2;1 promoter by hormones and abiotic stresses in Arabidopsis. Plant Cell Rep 28:185–196
Lu M, Lee MD, Smith BL et al (1996) The human AQP4 gene: definition of the locus encoding two water channel polypeptides in brain. Proc Natl Acad Sci U S A 93:10908–10912
Luu DT, Martiniãre A, Sorieul M et al (2012) Fluorescence recovery after photobleaching reveals high cycling dynamics of plasma membrane aquaporins in Arabidopsis roots under salt stress. Plant J 69:894–905

Mahdieh M, Mostajeran A, Horie T, Katsuhara M (2008) Drought stress alters water relations and expression of PIP-type aquaporin genes in *Nicotiana tabacum* plants. Plant Cell Physiol 49:801–813
Marin-Olivier M, Chevalier T, Fobis-Loisy I et al (2000) Aquaporin PIP genes are not expressed in the stigma papillae in *Brassica oleracea*. Plant J 24:231–240
Mathai JC, Agre P (1999) Hourglass pore-forming domains restrict aquaporin-1 tetramer assembly. Biochemistry 38:923–928. doi:10.1021/bi9823683
Matsumoto T, Lian H-LL, Su W-AA et al (2009) Role of the aquaporin PIP1 subfamily in the chilling tolerance of rice. Plant Cell Physiol 50:216–229
Mintseris J, Weng Z (2005) Structure, function, and evolution of transient and obligate protein-protein interactions. Proc Natl Acad Sci U S A 102:10930–10935
Moshelion M, Becker D, Biela A et al (2002) Plasma membrane aquaporins in the motor cells of Samanea saman: diurnal and circadian regulation. Plant Cell 14:727–739
Murata K, Mitsuoka K, Hirai T et al (2000) Structural determinants of water permeation through aquaporin-1. Nature 407:599–605
Neely JD, Christensen BM, Nielsen S, Agre P (1999) Heterotetrameric composition of aquaporin-4 water channels. Biochemistry 38:11156–11163
Nooren IMA, Thornton JM (2003) Diversity of protein-protein interactions. EMBO J 22: 3486–3492
Otto B, Uehlein N, Sdorra S et al (2010) Aquaporin tetramer composition modifies the function of tobacco aquaporins. J Biol Chem 285:31253–31260
Palanivelu DV, Kozono DE, Engel A et al (2006) Co-axial association of recombinant eye lens aquaporin-0 observed in loosely packed 3D crystals. J Mol Biol 355:605–611
Prado K, Boursiac Y, Tournaire-Roux C et al (2013) Regulation of Arabidopsis leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. Plant Cell 25:1029–1039
Preston GM, Carroll TP, Guggino WB, Agre P (1992) Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science 256:385–387
Rash JE, Yasumura T, Hudson CS et al (1998) Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. Proc Natl Acad Sci U S A 95:11981–11986
Ren G, Cheng A, Reddy V et al (2000) Three-dimensional fold of the human AQP1 water channel determined at 4 A resolution by electron crystallography of two-dimensional crystals embedded in ice. J Mol Biol 301:369–387
Rossi A, Ratelade J, Papadopoulos MC et al (2012) Neuromyelitis optica IgG does not alter aquaporin-4 water permeability, plasma membrane M1/M23 isoform content, or supramolecular assembly. GLIA 60:2027–2039
Ruiz Carrillo D, To Yiu Ying J, Darwis D et al (2014) Crystallization and preliminary crystallographic analysis of human aquaporin 1 at a resolution of 3.28 Å. Acta Crystallogr Sect F Struct Biol Commun 70:1657–1663
Sakurai J, Ishikawa F, Yamaguchi T et al (2005) Identification of 33 rice aquaporin genes and analysis of their expression and function. Plant Cell Physiol 46:1568–1577
Sohara E, Rai T, Yang S-S et al (2006) Pathogenesis and treatment of autosomal-dominant nephrogenic diabetes insipidus caused by an aquaporin 2 mutation. Proc Natl Acad Sci 103:14217–14222
Sorbo JG, Moe SE, Ottersen OP, Holen T (2008) The molecular composition of square arrays. Biochemistry 47:2631–2637
Sorieul M, Santoni V, Maurel C, Luu D-TT (2011) Mechanisms and effects of retention of over-expressed aquaporin AtPIP2;1 in the endoplasmic reticulum. Traffic 12:473–482
Soto G, Alleva K, Amodeo G et al (2012) New insight into the evolution of aquaporins from flowering plants and vertebrates: orthologous identification and functional transfer is possible. Gene 503:165–176
Suga S, Maeshima M (2004) Water channel activity of radish plasma membrane aquaporins heterologously expressed in yeast and their modification by site-directed mutagenesis. Plant Cell Physiol 45:823–830

Sui H, Han BG, Lee JK et al (2001) Structural basis of water-specific transport through the AQP1 water channel. Nature 414:872–878

Törnroth-Horsefield S, Wang Y, Hedfalk K et al (2006) Structural mechanism of plant aquaporin gating. Nature 439:688–694

Tournaire-Roux C, Sutka M, Javot H et al (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature 425:393–397

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R (2003) The tobacco aquaporin NtAQP1 is a membrane CO2 pore with physiological functions. Nature 425:734–737

Uehlein N, Otto B, Hanson DT et al (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO2 permeability. Plant Cell 20: 648–657

Vandeleur RK, Mayo G, Shelden MC et al (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiol 149:445–460

Verdoucq L, Grondin A, Maurel C (2008) Structure-function analysis of plant aquaporin AtPIP2;1 gating by divalent cations and protons. Biochem J 415:409–416

Walz T, Smith BL, Agre P, Engel A (1994) The three-dimensional structure of human erythrocyte aquaporin CHIP. EMBO J 13:2985–2993

Wang C, Hu H, Qin X et al (2016) Reconstitution of CO2 regulation of SLAC1 anion channel and function of CO2-permeable PIP2;1 aquaporin as carbonic anhydrase 4 interactor. Plant Cell 28:568–582

Weig A (1997) The major intrinsic protein family of Arabidopsis has 23 members that form three distinct groups with functional aquaporins in each group. Plant Physiol 114:1347–1357

Wolburg H, Wolburg-Buchholz K, Fallier-Becker P et al (2011) Structure and functions of aquaporin-4-based orthogonal arrays of particles. Int Rev Cell Mol Biol 287:1–41

Yamada S, Katsuhara M, Kelly WB et al (1995) A family of transcripts encoding water channel proteins: tissue-specific expression in the common ice plant. Plant Cell 7:1129–1142

Yaneff A, Sigaut L, Marquez M et al (2014) Heteromerization of PIP aquaporins affects their intrinsic permeability. Proc Natl Acad Sci 111:231–236

Yaneff A, Vitali V, Amodeo G (2015) PIP1 aquaporins: Intrinsic water channels or PIP2 aquaporin modulators? FEBS Lett 589(23):3508–3515

Zelazny E, Borst JW, Muylaert M et al (2007) FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. Proc Natl Acad Sci U S A 104:12359–12364

Zelazny E, Miecielica U, Borst JW et al (2009) An N-terminal diacidic motif is required for the trafficking of maize aquaporins ZmPIP2;4 and ZmPIP2;5 to the plasma membrane. Plant J 57:346–355

Zhang W, Fan L, Wu W-H (2007) Osmo-sensitive and stretch-activated calcium-permeable channels in *Vicia faba* guard cells are regulated by actin dynamics. Plant Physiol 143:1140–1151

Zhang M, Lu S, Li G et al (2010) Identification of a residue in helix 2 of rice plasma membrane intrinsic proteins (PIPs) that influences water permeability. J Biol Chem 285:41982–41992

Zhou Y, Setz N, Niemietz C et al (2007) Aquaporins and unloading of phloem-imported water in coats of developing bean seeds. Plant Cell Environ 30:1566–1577

http://www.springer.com/978-3-319-49393-0

Plant Aquaporins
From Transport to Signaling
Chaumont, F.; Tyerman, S.D. (Eds.)
2017, VIII, 353 p. 30 illus., 29 illus. in color., Hardcover
ISBN: 978-3-319-49393-0