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## **Aquaporins**

Proteins in the aquaporin family (AQPs) are important for the transport of water, ammonia, and possibly other small neutral molecules in symbiotic interactions. AQPs are membrane proteins in the major intrinsic protein (MIP) family. In plants, MIPs are found in five subfamilies: the plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and the poorly defined X intrinsic proteins (XIPs) (Maurel et al., 2015). Small neutral solutes transported by some AQPs include ammonia, glycerol, carbon dioxide, hydrogen peroxide, urea, boric acid, silicic acid, selenite, and various species of arsenic (Maurel et al., 2015; Deshmukh et al., 2020). In addition, some AQPs function as ion channels (Kourghi et al., 2018).

MIPs are found in all organisms with the exception of a few prokaryotic lineages (Abascal et al., 2014). All MIPs share a common structure, which includes six transmembrane domains with five connecting loops (Fig. 4A; Mitsuoka et al., 1999). Based on sequence similarity of the N- and C-terminal halves of MIPs, it is likely that they arose from a gene duplication event leading to a tandem repeat (Maurel et al., 2015). In biological membranes, MIPs form tetramers in which each monomer contains a pore (Daniels et al., 1999; Fotiadis et al., 2001). A possible fifth pore in the center of the tetramer may also function in transport (Ozu et al., 2018). Most MIPs can form functional homotetramers as indicated by their activity when expressed heterologously. The predominance of heterotetramerization is not known, but it can have an important impact on activity. PIP1 and PIP2 from plants and AQP4M1 and AQP4M23

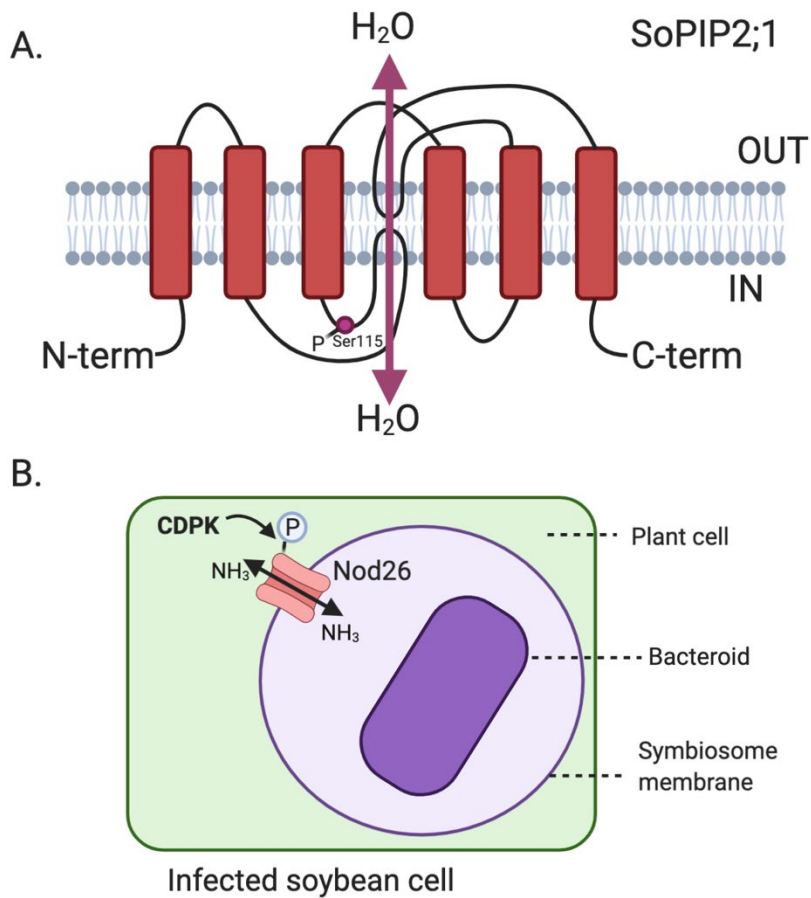
from animals are known to form heterotetramers and, for those examples, heterotetramerization is important for membrane localization, regulation by pH, substrate specificity, and modulating water transport activity (Jozefkiewicz et al., 2017).

In addition to heteromerization, protein phosphorylation is an important form of posttranslational regulation of AQPs and is important for AQP function in symbioses. In soybean (*Glycine max*), nodulin 26 is specific to the symbiosome membrane, where it plays an integral role in the legume-rhizobia symbiosis as a water and ammonia channel (Hwang et al., 2010). Nodulin 26 is phosphorylated on Ser262 by a calcium-dependent protein kinase (CDPK) associated with the symbiosome membrane (Roberts and Routray, 2017). The CDPK is activated by calcium signaling pathways that respond to osmotic stress (Guenther et al., 2003). Therefore, the phosphorylation of nodulin 26 on Ser262, which increases water permeability, likely occurs in response to salinity and drought stress (Guenther et al., 2003). Phosphorylation could also play a role in regulating ammonia flux via nodulin 26 from the bacteroid to the host (Fig. 4B).

The establishment of mycorrhizal symbiosis modulates AQP phosphorylation in the host plant under stress conditions. Inoculation of *Phaseolus vulgaris* with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* decreases in hydraulic conductivity due to various stresses (cold, drought, salinity). In response to stress, the phosphorylation of PIP2s on Ser113 increases in *Phaseolus vulgaris* roots inoculated with the mycorrhizal fungus whereas the phosphorylation level of PIP2s in uninoculated roots decreases (Aroca et al., 2007).

In addition to phosphorylation, protein interactions play an important role in AQP regulation. Phosphorylation of S280 and S283 of AtPIP2;1 is important for regulation by 14-3-3 proteins. 14-3-3- proteins GRF3, 4, 8, and 10 regulate the water permeability of AtPIP2;1 in a phosphorylation-dependent manner (Prado et al., 2019). Other protein interactions also affect AQP activity. The ER-resident proteins *ZmVAP27-1* and *ZmVAP27-2* interact with the maize aquaporin *ZmPIP2;5* at ER-plasma membrane contact sites. *ZmVAP27-1* interaction with

*ZmPIP2;5* increases membrane water permeability through the *ZmPIP2;5* and the interaction may additionally have a function in endocytosis of *ZmPIP2;5* under salt stress conditions (Fox et al., 2020).



**Figure 4. Aquaporins (AQP) are uniporters for water and other small neutral molecules that are regulated by protein phosphorylation.** A. Example of AQP structure using SoPIP2;1 showing phosphorylation site at Ser115. AQPs have 6 transmembrane spans, the N- and C-terminal halves are homologous and both contain loops that enter the membrane and form part of the pore. B. In the legume-rhizobium interaction NH<sub>3</sub> is thought to be delivered from the bacteroid to the plant via Nod26, an AQP in the symbiosome membrane. (Figures made with BioRender.)

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