Cloning and Functional Analyses of Water Channels from a Conifer Stem

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Trees play an important role to control CO_2 in the atmosphere because the wood is the most abundant biomass on the earth. Water is essential for the assimilation of carbon dioxide, and trees over ten meters in height need to pull water and ions up to the leaves in their crowns. Most widely accepted theory how to pull water up to their crown is so-called cohesion theory (Zimmermann 1983). According to the theory, cohesive strength of water can maintain in a meta-stable water column beyond 10 meters in height under substantial hydrostatic tension, which is driven by transpiration of water from leaves. However, metastable state is vulnerable to cavitation in xylem (Tyree and Sperry, 1989). Refilling of embolized conduits requires that water enters the embolized tracheids while pressurizing the gas phase until it is forced back into solution.

The sap ascent has long been considered as a process simply physical and no living cell involved. However, a conifer wood consists of tracheids, or water conduits, which are always connected with a living cell, or ray parenchyma cells, on their radial walls. Considering the topological location of the living cells in the xylem, they are probably involved in the water refilling in the conifer stems, into an embolized area (Canny, M. J., 1997), which may reduce hydrostatic tension in the xylem conduits. Only a little molecular basis has been reported in the water conduit through the living cells in the xylem (Kuroda 2000). Aquaporins are known as a water channel or the other solute, and universally distributed in organisms. Here, the study was focused the molecule located in a conifer xylem trunk.

So far no functional aquaporin was reported in the wood trunks, especially in mature secondary xylem in spite of abundant aquaporin fragments in trunk EST libraries. Thus, the trunk of a 22-year-old Japanese red pine (*Pinus densiflora*) was used for the mRNA source. In the RT-PCR cloning, primers were designed by using a few hypothetical contig sequences, which were connected EST sequences in the genus *Pinus* by using a soft ware. The KOD plus™ amplified products were ligated into a vector, pT7Blue-2. Finally, three different clones have obtained with full coding sequences in the mature xylem. They are assigned as a member of Plasma membrane Intrinsic Proteins, two PIP1s and a PIP2. Xylem PIP1 had two isoforms that is distinct from only a base or one amino acid residue. They are probably on a homologous chromosome. On the contrary, xylem PIP1 was different in 3'-UTR from the other PIP1, which has also cloned separately from the seedlings. In 5'RACE, one of the isolated clones was mainly expressed in the xylem. All of the cloned PIPs contain six putative membrane-spanning regions. A conserved motif NPA is located in loop B and E. A deduced phosphorylation site is also found in the clones. The sequence analyses clearly show that the cloned PIPs are an aquaporin. The cloned PIP1 and PIP2 were respectively transcribed *in vitro* after subcloning into an expression vector pTNT™, which carries a 5'ß-globin leader sequence and a poly(A) tail. The obtained mRNAs with pol(A) tail were respectively micro-injected into *Xenopus* oocytes and measured water transport by analyzing the volume changes in response to the hyper- and hypotonic solutions. The cloned PIPs were demonstrated water transport in response to the osmolarity.

Together with these findings, functional aquaporins are first demonstrated in the mature xylem. The living cells in the xylem, which was the RNA source in this study, only consist of ray and epitherial cells. The latter surrounds resin cannel and produces hydrophobic isoprenoids. Thus, the aquaporin probably functional in the ray cells participating water ascent of the xylem conduit.

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