CHARACTERIZATION OF PLASMA MEMBRANE MIP PROTEINS IN MAIZE

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The requirements of maize and other major crops for water have been studied at the organism and at the tissue level in great detail, resulting in a fairly complete picture of how the transpiration stream moves through the plant. Yet, little is known about the water relations of individual cells: how water influx drives specific processes such as cell enlargement or how water movement is regulated for the cytosolic osmoregulation that is necessary for maintaining normal metabolic processes. Aquaporins are proteins that enable water to pass through biological membranes. In plant cells, aquaporins are found in the tonoplast (TIPs) and in the plasma membrane (PIPs). The proteins are members of the major intrinsic protein (MIP) family but not all MIPs are aquaporins. Some transport small solutes in addition to water.

We started to study aquaporins in maize. A preliminary search of the maize EST database shows that there may be as many as 50 differently expressed MIP genes. We recently reported on the characterization of the tonoplast aquaporin ZmTIP1 (Chaumont *et al.*, 1998; Barrieu *et al.*, 1998). We are currently characterizing three plasma membrane MIP proteins (ZmPIP1, ZmPIP2 and ZmPIP3). The water channel activity of these proteins is determined with a *Xenopus* oocyte swelling assay. Oocytes that express an aquaporin swell and burst rapidly when shifted to a hypotonic solution. Using this assay we demonstrated that ZmPIP3 is an aquaporin, but ZmPIP1 and ZmPIP2 apparently have no water channel activity. Their primary function is still unknown. We are investigating the function of ZmPIPs by heterologous expression in yeast *Saccharomyces cerevisiae*.

The plasma membrane localization of ZmPIPs relies entirely on amino acid sequence comparisons with proteins previously localized in the plasma membrane and needs to be demonstrated. We fused *ZmPIP2* cDNAs to the reporter gene coding for the Green Fluorescence Protein (GFP) and introduced these constructs into tobacco. Strong green fluorescent signals were observed in the plasma membrane surrounding the cell and in the perinuclear region. Filaments extending from the nucleus to the plasma membrane were also labeled and could be ZmPIP-GFP proteins travelling through the secretory pathway.

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References

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