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综 述

水孔蛋白在细胞延长、盐胁迫和光合作用中的作用

杨淑慎, 崔丽荣

西北农林科技大学生命科学学院, 杨凌 712100

摘 要: 水孔蛋白属于一个高度保守的、能够进行跨生物膜水分运输的通道蛋白 MIP 家族。水孔蛋白作为膜水通道, 在控制细胞和组织的水含量中扮演重要角色。本研究的重点是属于 PIP 亚家族的 GhPIP1;2 和属于 TIP 亚家族的γTIP1 在植物细胞延长中的作用。使用特异基因探针的 Northern 杂交和实时荧光 PCR 技术证明 GhPIP1;2 和 GhγTIP1 主要在棉花纤维延长过程中显著表达, 且最高表达量在开花后 5 d。在细胞延长过程中, GhPIP1;2 和 GhγTIP1 表达显著, 表明它们在促使水流迅速进入液泡这一过程中扮演重要角色。而且也研究了盐胁迫植物中钙离子对水孔蛋白的影响。分别或一起用 NaCl 或 $CaCl_2$ 处理原生质体或细胞质膜。结果发现在盐胁迫条件下, 水渗透率值在原生质体和质膜颗粒中都下降了, 同时 PIP1 水孔蛋白的含量也下降了, 表明 NaCl 对水孔蛋白的功能和含量有抑制作用。同时也观察了 Ca^{2+}的两种不同的作用。感知胁迫的胞质中游离钙离子浓度的增加可能导致水孔蛋白的关闭。而过剩的钙离子将导致水孔蛋白的上游调控。同时实验已经证明大麦的一类水孔蛋白-HvPIP2;1 有更高的水和 CO_2 转移率。本研究的目标是确定负责转运水和 CO_2 的关键水孔蛋白的类型, 并且提高植物对水的利用率、抵抗胁迫的能力, 促进 CO_2 的摄取及吸收、提高植物的产量。

关键词: 水孔蛋白, 细胞延长, 盐胁迫, 光合作用

The action of aquaporins in cell elongation, salt stress and photosynthesis

Shushen Yang, and Lirong Cui

Chinese Northwest A & F University College of Life Science, *Yangling* 712100, *China*

Abstract: Aquaporin belongs to a highly conserved group of membrane proteins called major intrinsic proteins (MIPs) that facilitate water transport across biological membranes. Aquaporins are membrane water channels that play critical roles in controlling the water content of cells and tissues. We focused on GhPIP1;2 which belongs to the PIP subfamily and GhγTIP1 which belongs to the γTIP group of the TIP subfamily. Northern blot analysis with gene-specific probes and real-time PCR demonstrated that GhPIP1;2 and GhγTIP1 are predominantly expressed during cotton fiber elongation, with the highest expression levels at 5 days post anthesis. The high and preferential expression of GhPIP1;2 and GhγTIP1 suggests that they may play important roles in supporting the rapid influx of water into vacuoles during cotton fiber cell expansion. Also, the effects of Ca^{2+} on aquaporins in salinity-stressed plants were studied. Researchers treated the protoplasts and plasma membrane with NaCl or $CaCl_2$, alone or in combination. Under saline conditions, osmotic water permeability (Pf) values decreased in protoplasts and plasma membrane vesicles, and the same reduction was observed in the PIP1 aquaporin abundance, indicating inhibitory effects of NaCl on aquaporin functionality and protein

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Corresponding author: Lirong Cui. E-mail: andycui198303@163.com
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abundance. Two different actions of Ca^{2+} were observed. Increase in free cytosolic calcium concentrations associated with stress perception may lead to aquaporin closure, however, the extra-calcium would lead to an upregulation of aquaporins. Meanwhile, experiments have demonstrated HvPIP2;1, one of barley aquaporins, has a higher water and CO_2 transport activity. The goal of our plant aquaporin research is to determine the key aquaporin species responsible for water and CO_2 transport, and to improve plant water relations, stress tolerance, CO_2 uptake or assimilation, and plant productivity.

Keywords: aquaporin, cell elongation, salt stress, photosynthesis

1 Introduction

Water uptake and flow across cellular membrane is a fundamental requirement for plant growth and development. If water uptake through the roots is reduced or blocked by water-related stress such as drought, salt stress, or low temperatures, plant growth is seriously or lethally inhibited. There are three different components of water flow in plant tissues: symplastic movement of water via plasmodesmata, transcellular movement across cell membranes, and apoplastic flow through the cell walls. The relative contribution of each water movement pathway to flow across the composite structure of tissues and organs in plants varies, which provides a useful mechanism by which the plant can respond to changing environmental conditions[1]. Moreover, we focused on the aquaporins that are responsible for the transcellular movement of water across the cell membrane. Although the biophysical bases of water transport across biological membranes have been enounced as early as in the 1950s, it is until 1992 that Dr. Agre discovered and reported the first aquaporin (AQP1) from erythrocytes[2]. Aquaporin (AQP) belongs to a well-conserved and ancient family of proteins called major intrinsic proteins (MIPs) that facilitate the flow of small molecules like water and/or glycerol across the hydrophobic interior of membranes[3−5]. The AQPs different forms have been found in organisms ranging from bacteria and fungi to animals and plants, and the structure, molecular biology, biophysics, and the role of AQPs in plant water transport have been reviewed[6]. In this review, we will rather focus on the most recent findings concerning the molecular and cellular properties of aquaporins. We will discuss how these findings open new perspectives for understanding the multiple function of aquaporins in plants[7].

2 Aquaporins structure and classification

Aquaporins are small and highly hydrophobic transmembrane proteins, and most of them are 250−300 amino acids, in length with a molecular mass between 26 kD and 34 kD[8]. All aquaporins have six transmembrane domains with the N- and C-termini facing the cytosol. The six transmembrane domains were predicted to be α-helices, which are connected by five loops (A-E). Loop B and loop E each contains a helical domain, HB and HE, both of which posses a conserved asparagine-proline-alanine (NPA) motif[9,10]. The two halves of the protein show observe symmetry, with the hydrophobic loops containing the NPA motif overlapping in the middle of the lipid bilayer to form two hemipores that together creat a narrow channel[11,12]. Water molecules passing the channel are forced, by the protein's electrostatic forces, to flip at the center of the channel.

Aquaporins can be subdivided into four groups with highly conserved amino acid sequences and intron positions in each group: the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the nodulin 26-like intrinsic proteins (NIPs) and the small intrinsic proteins (SIPs)[13]. The PIPs subfamily, thought to be localized in plasma membrane, can be further divided into two classes named PIP1 and PIP2 with the specific arrays of amino acids at the N-and C-termini. According to the nomenclature by Jang JY *et al*[14], in *Arabidopsis*, the PIP1 subgroup representing five members of aquaporins is named PIP1;1 to PIP1;5, and PIP2 subgroup consisting of eight members of aquaporins is named PIP2;1 to PIP2;8. The TIPs subfamily, thought to be presented in vacuolar membranes, can fall into several classes including αTIP, βTIP, γTIP, δTIP, and so on. The subcellular location of membranes from the NIP and SIP families is still uncertain, but recent studies have shown that SIPs are localized in the ER fraction of *Arabidopsis* cells [11].

3 Aquaporins functions

A lot of aquaporins found in plants suggests their importance during plant development and adapting to the different environment conditions[13]. Many processes in plants are dependent on massive water

flow into and out of cells, and therefore it is reasonable that aquaporin plays an important role in several cellular processes, including the cell elongation, response to stress and plant photosynthesis[14].

4 Aquaporins functions in the cell elongation

Cotton fiber is a good model and provides a unique experimental system to study cell differentiation and cell elongation[15]. The study of cotton fiber development not only provides a basic understanding of plant cell elongation, but also identifies the importance of aquaporins in this process. Because high expression of aquaporin genes preferentially observed in dividing and elongating cells[16]. Recently, numerous genes specifically or preferentially accumulated during fiber development have been isolated and characterized[17]. GhPIP1;2, encoding plasma membrane intrinsic protein, and GhγTIP1, encoding tonoplast intrinsic protein, are obtained from the cultivated specie of cotton, G.hirsutum. Phylogenetic analysis showed that GhPIP1;2 belongs to the PIP1 group of the PIP subfamily and GhγTIP1 belongs to the γTIP group of the TIP subfamily. The high and preferential expression of GhPIP1;2 and GhγTIP1 suggests that they may play important roles in supporting the rapid influx of water into vacuoles during cotton fiber cell expansion. So, in order to investigate the role of PIP and TIP in the regulation of water movement during fiber development, we choose these two aquaporin genes for further study.

First constructed a suppression subtractive hybridization cDNA library of 10 day post anthesis (DPA) fiber, and then isolated the full-length cDNA fragments using 5′ RACE (rapid amplification of cDNA ends)[18] Through 5′ RACE, the 5′ terminals of the two partial sequences were cloned and the corresponding full-length genes were isolated by long-distance PCR. The genomic sequences of GhPIP1;2 and GhγTIP1 are 1825 bp and 1456 bp in length, respectively. According to the sequence comparison between genomic sequence and cDNA, GhPIP1;2 contains four exons and three introns, whereas GhγTIP1 has three exons and two introns. Northern blotting was used to analyse the accumulation patterns of GhPIP1;2 and GhγTIP1. Because the 3′ end regions in their full-length cDNAs share the lowest homology to their closed subfamily members in cotton, these regions were used as gene-specific probes. At the same time, researchers make use of real-time PCR using gene-specific primers and QRT-PCR(Quantitative reverse transcription-PCR) to analyze the expression patterns of the two aquaporin genes during fiber development[19].

From the results of Northern blotting analysis, we know that the expression pattern of GhPIP1;2 and GhγTIP1 reaches the highest level at 5 DPA. After 5 DPA, GhPIP1;2 transcripts decrease gradually, but GhγTIP 1 transcripts decrease quickly. Because the rapid expansion of cotton fiber aslo happens from 5 DPA to 15 DPA, we think that aquaporins play important roles in cotton fiber elongation.

Aquaporins are ancient channel proteins that allow plants to rapidly alter their membrane water permeability in response to environmental cues. Plant PIP and TIP subfamilies are abundant in plasma membrane and tonoplast, and they play pivotal roles in maintaining cell turgor and water potential of the cytoplasm[20]. Because TIPs and PIPs can rapidly transport water into the protoplast (Vacuole), the transport of water across membranes by aquaporin has an important function in rapid plant cell elongation[20]. Cotton fiber cell expansion not only needs turgor pressure as a major regulator, but also requires that the rigidity of the primary cell wall which is relaxed by cell wall proteins such as expansins and endo 1,4-β-glucanase[21]. The rapid influx of water into the central vacuole is driven by high turgor pressure generated by the solute accumulation in the vacuole, and this is functionally linked to the cell enlargement.

Changes in turgor can induce changes in the conformation of the plasma membrane, or even in the aquaporins themselves. Water-channel activity, inferred from the membrane permeability parameters, was seen to decrease with increasing osmotic pressure and with increasing osmolyte size[22]. We assume that since rapid cell expansion requires a high hydraulic permeability of the tonoplast to support water entry into the vacuole, proteins of the aquaporin superfamily may mediate a large part of water movements across the tonoplast and plasma membrane during cotton fiber elongation. In the present study, the transcripts from GhPIP1;2 and GhγTIP1 are maintained at high levels from 5 DPA to 10 DPA corresponding to the opening of fiber plasmodesmata and the import of soluble sugars, K^+, and malate[23,24]. All results showed that the fragments are aquaporin genes preferentially expressed during cotton fiber elongation. Some hypothesis mentioned above can provide an explanation of many basic processes in which aquaporins are already implicated.

5 Aquaporins function in environmental stress

Land plants have evolved to cope with rapid changes in the availability of water by regulating all aquaporins which represent a major path during water uptake by roots[6]. Regulation of aquaporin may also represent a way to modulate membrane water permeability, and the factors affecting and regulating aquaporin behaviors possibly involve phosphorylation, pH, Ca^{2+}, environment stresses like salinity and drought. The abundance and activity of aquaporins in the plasma membrane and tonoplast may be regulated, hence enabling the plant to tightly control water fluxes into and out of cells. Under salt stress, a number of plasma membrane-type aquaporins were down-regulated, which can prevent continuous dehydration resulting in cell death[25].

As a step toward understanding the aquaporin function in plants under various environmental stimuli, the expression of the gene families encoding aquaporins under various abiotic stress conditions were investigated. The expression of the aquaporin genes responded significantly and differently to environmental stress conditions. One of the primary responses of plants to salt stress is the inhibition of their root water uptake capacity[26]. Salinity has been shown to decrease the amounts of mRNA encoding PIP aquaporins in *Arabidopsis*[27]. It has been observed that changes of certain aquaporin isoforms are regulated by salt-stress[28]. Many laboratories have started to study the molecular and cellular mechanisms for regulation of aquaporins under normal or stress conditions[29]. Different stimuli can induce changes in aquaporin phosphorylation or protonation, which playing an important role in aquaporin gating under stress conditions. Experiments have shown that the water permeability of *Arabidopsis* and *Beta vulgaris* plasma membrane is down-regulated by Ca^{2+}, in addition to low pH[30]. Ca^{2+} plays an important role in the up-regulation of water channel activity. On root water transport, the inhibitory effects of $HgCl_2$ (a common aquaporin blocker but also a general metabolism inhibitor), were only seen provided that the plant nutrient solution contained Ca^{2+}[31]. Recent results with pepper plants have also shown that calcium seems to be involved in plasma membrane aquaporin regulation via a chain of processes within the cell but not by alteration of the stability of the plasma membrane[32].

To characterise water transport in both intracellular and plasma membranes of plant cells, osmotic water permeability values (Pf) were measured on isolated protoplasts and purified plasma membrane vesicles[33]. To study how NaCl affects water transport and the role of calcium under salt stress, we treated the isolated protoplasts and plasma membrane with NaCl or $CaCl_2$, alone or in combination.

The results can be seen as follows: treatment of plants by calcium alone had no significant effect on protoplast Pf. Pf values were significantly decreased in NaCl-treated plants, but they can be restored in a pretreatment of plants by Ca^{2+}. Moreover, the overall Pf results obtained from membrane vesicles of NaCl-treated plants showed the same tendencies as those obtained from protoplasts and both were consistent with the PIP1 abundace[34]. In all membrane preparations except in those from NaCl-treated plant, an external Ca^{2+} supply to the vesicle medium can decrease Pf. Acidic pH had similar effects on membrane Pf. Tornroth-Horsefield *et al*[35] provided an explanation of the mechanisms of plant plasma membrane aquaporin inhibition by pH and calcium. In summary, it has been proposed that in the closed aquaporin conformation the second cytoplasmic loop (D) caps the channel from the cytoplasm occluding the pore , whereas in the open conformation the loop D is displaced, and this movement opens a hydrophobic gate blocking the channel entrance from the cytoplasm[35]. In one word, externally supplied Ca^{2+} would act directly to block the aquaporin pore.

The interacting effects of salt stress with calcium homeostasis and signalling has been reported. For example, NaCl stress can affect membrane polarity and Ca^{2+} uptake in plant cells[36]. In *Arabidopsis*, the cytosolic Ca^{2+} gradient can be reduced by NaCl[37]. The confocal microscope studies showed that extended NaCl stress can reduce intracellular Ca^{2+}. This provides a supportion of the idea that NaCl affects cell Ca^{2+} homeostasis which in turn would result in aquaporin inhibition. Many environmental factors, such as salinity, also critically interfere with aquaporin abundance. Many examples that stress increased the level of aquaporins have been observed. A recent report showed that, after NaCl treatment, the protein amounts of PIP2;1, PIP2;2 and PIP2;3 in *Arabidopsis* suspension cells increased several fold, whereas the abundance of PIP1 homologues did not change[38]. By contrast, a significant decrease in the abundance of PIP1 proteins can be observed in *Arabidopsis* plants after salt exposure. The amounts of PIP1 in pepper root plasma membrane also decreased with salt stress. This pointed to a rapid response to salt through dynamic control of PIP1 aquaporin translation and

/or degradation. Aquaporin transcriptional regulation provides an explanation of long-term regulation of root water transport. The PIP1 abundance was in agreement with the Pf results, indicating that Ca^{2+} plays an important role in the restoration of PIP1 abundance under saline conditions. Ca^{2+} dependent blockade of PIPs and Ca^{2+}-dependent phosphorylation (activation) can be effective for adjustment of the cellular water balance. Accordingly, cytosolic Ca^{2+} was reduced after long-term exposure to salinity stress and seems to be associated to an overall inhibition of aquaporins. The different regulation of aquaporin gene expression during salt stress may play roles in limiting initial water loss during the early stage of salt stress and assisting the subsequent uptake of water to maintain water homeostasis in high cellular salt conditions. The aquaporin gene increases in the roots to uptake water from environment and to maintain reasonable water status during salt stress. The complex expression pattern of PIP genes suggests that maintenance of a proper water status under salt, drought, or cold stress requires both increased water transport via aquaporins in some cells and reduced water transport via aquaporins in other cells and tissues, which awaits further verification by biochemical and genetic analyses. Phosphorylation of certain serine residues activates some PIPs and dephosphorylation rapidly closes them. This aquaporin inactivation can prevent water loss from cells under drought or strong salt stress[39]. This aquaporin inactivation can prevent water loss from cells under drought or strong salt stress.

6 Aquaporins function in photosynthesis

Photosynthesis in chloroplasts within mesohyll cells is the most basic and important function of plants. Except for chemoautotrophic bacteria, all plants and animals, including humans, depend on photosynthetic products. H_2O and CO_2 are the most important materials for photosynthesis which is essential for plant life. Because the atmospheric CO_2 concentration is low, adding that CO_2 is rapidly consumed internally if CO_2-fixing enzymes (Rubis CO_2) function properly, it will be inadequate to maintain photosynthesis that the simple diffusion of CO_2 without an efficient transport system.

To date, we have known that aquaporins take part in the transport of many essential molecules in plants, like H_2O and CO_2[3,40,41]. Maki Katsuhara *et al* pointed that PIPs are the most important factors regarding the characteristics of cellular water uptake or water loss[42].

HvPIP2;1, HvPIP1;3 and HvPIP1;5, three PIPs from barley, were isolated and analyzed by Katsuhara M *et al*[43]. The results showed that transcripts of HvPIP2;1 (22 million copies/ug total RNA) were tenfold more abundant than those of HvPIP1;3 (2 million copies/ug total RNA) and HvPIP1;5 (1 million copies/ug total RNA). Compared with HvPIP1;3, HvPIP2;1 markedly increased the water permeability (Pf) of oocytes[44]. This is in agreement with the general feature of plant PIPs, whereby aquaporins of the PIP2 subfamily have higher water transport activity than PIP1 aquaporins in the oocyte system[45]. Hense, members of PIP1 group showed lower water channel activity in this expression system. The difference in water permeability can be attributable to the changes in specific amino acid sequences of PIP1 and PIP2 aquaporins in the conserved amino acid residues of six membrane-spanning α-helixes and NPA motifs. Besides these, PIP2 proteins are characterized by a shorter N-terminal extension than PIP1 proteins and a longer C-terminal end that contains putative phosphorylation site. The adequate regulation of water homeostasis via aquaporins is common in plant cells adjusting to various environment. Moreover, continuous over-expression of HvPIP2;1 increased the water permesbility of roots and raised the salt sensitivity of transgenic rice plants[46]. Recently, ten novel barley PIP genes (HvPIPs) have been found. Based on the sequence homology, five genes were classified into the sub-class PIP1 and the others into the sub-class PIP2. Except HvPIP1;2, HvPIP2s were markedly higher than HvPIP1s. The down-regulation of PIP gene expression by drought stress may result in reduced membrane water permeability, and may promote cellular water conservation during periods of dehydration stress[47].

Internal conductance gi, conductance regarding CO_2 diffusion from stomata to chloroplasts, is a limitation of photosynthesis. Among many factors, aquaporins are probably the most important factor determing gi. In the pioneering work of Terashima and Ono, a significant decline of gi in the presence of $HgCl_2$, an inhibitor of most of the aquaporins, was demonstrated[48]. The leaves of transgenic rice plants that expressed the largest amount of HvPIP2;1 showed a 40% increase in gi compared with the leaves of wild-type rice plants. A recent relation between aquaporins and gi can be seen from the above result. Flexas *et al* confirmed an increase in gi of leaves of transgenic plants over-expressing NtAQP1[39,49]. A high gi and increased CO_2 assimilation can be shown in transgenic rice leaves. A high CO_2 assimilation rate

is the result of a rise in gi which is supposed to effectively promote the CO_2 supply to the photosynthetic center in chloroplasts. These results suggest that CO_2 assimilation is commonly limited by gi, which depends on aquaporins, and the enhancement of aquaporin activity is a potentially promising way to promote plant CO_2 assimilation via improving gi[40].

7 Summary and perspective

From the discovery of the first aquaporin until now, tremendous progresses have been made in understanding the structure and function of aquaporins in animals, plants, and microbes. The aquaporin family in plants is large, indicating complex and regulated water transport within the plant in order to adapt to different environmental conditions. The role of aquaporins in plant water status under water stress is a complex issue, because the expressions of different aquaporin genes may be stimulated, reduced, or changed under abiotic stress. Recent, researchers found some aquaporins can mediated other low molecular weight compounds such as silicon, boron ammonia and H_2O_2 [50,51]. We specifically exame how our current understanding of aquaporin structure and genetic analyses have allowed to delineate a variety of functions for aquaporins in roots, leaves and during plant reproduction. However, at present, we know only a little about the genes encoding aquaporins, and we need more practice. Hence, this is the point of our research in the future.

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