Phosphate Accumulation in Plants: Signaling

The availability of mineral nutrients is vital for plant growth and survival, and, as such, plants have evolved elaborate mechanisms to maximize the sequestering and uptake of scarce minerals. This is especially true in the case of phosphorus (P), which, when limiting, induces changes in root architecture to aid in the uptake of this mineral nutrient. How this limiting condition is sensed and propagated in the plant is of much interest. The elucidation of the signaling pathway is the focus of two articles, from independent labs, published in the July 2006 issue: Aung et al. (2006), “pho2, a Phosphate Overaccumulator, Is Caused by a Nonsense Mutation in a MicroRNA399 Target Gene”; and Bari et al. (2006), “PHO2, MicroRNA399, and PHR1 Define a Phosphate-Signaling Pathway in Plants.”

WHAT WAS SHOWN

Arabidopsis (Arabidopsis thaliana) pho2 mutants overaccumulate P in leaves when P is not limiting and display P toxicity due to increased P uptake and translocation of P from roots to shoots (Delhaize and Randall, 1995; Dong et al., 1998). P remobilization in the leaves, however, is impaired. When P is limiting, P starvation-induced genes are up-regulated in pho2 mutants. However, after P addition, P starvation-induced genes are not repressed as they are in the wild type. This phenotype is similar to what is seen in UBC24 knockout plants as well as miR399-overexpressing plants, suggesting that PHO2 is UBC24. Aung et al. (2006) sequenced UBC24 from pho2 plants and found a single base pair substitution resulting in early translational termination and no UBC24 protein accumulation. Functional complementation of pho2 by the wild-type genomic copy of UBC24 strongly supports the identity of the pho2 locus as UBC24 (Aung et al., 2006). Using map-based cloning, Bari et al. (2006) also found the same single base pair substitution, identifying pho2 as At2g33770. Bari et al. (2006) found potential orthologs of PHO2 in rice (Oryza sativa), Medicago truncatula, and poplar (Populus trichocarpa; Bari et al., 2006), suggesting that PHO2 is conserved among angiosperms.

Micrografting experiments with pho2 mutants and wild-type root and shoot stocks demonstrated that the pho2 root genotype is necessary and sufficient for increased P accumulation in leaves under P-replete conditions (Bari et al., 2006). Due to this observation, Bari et al. (2006) chose to use roots from pho2 mutants for further molecular characterization of the mutants to determine the role of PHO2 in P status signaling.
pathway. Wild-type and pho2 plants were grown under +Pi and −Pi conditions for ATH1 genechip analysis. PHO2 was found to be expressed in all tissues examined by ATH1 genechip and, as expected, expression was low in pho2 mutants regardless of Pi status. Mutant pho2 roots grown under +Pi conditions had 12 genes with greater than 2-fold change in expression compared with the wild type. These genes are most likely involved in Pi transport, acquisition, and signaling during Pi starvation. This set of genes was also induced in wild type under Pi deprivation, suggesting that in pho2 mutants, part of the Pi-deprived signaling network is still active in Pi-replete conditions. In the pho2 mutants, miR399 is still up-regulated as a result of Pi deficiency.

PHO2 expression is regulated by miR399, which has been shown to be phosphate dependent (Chiou et al., 2006). Interestingly, the miRNA-binding sites are located in the 5’ untranslated region of the message. There are five miR399 primary transcripts, a through f, all of which are expressed when Pi is limiting. The 21-nt miR399 level decreases six to 12 hours after readdition of Pi to Pi-deprived plants (Bari et al., 2006). The primary transcripts of miR399 are “strongly induced” by low Pi and repressed by elevated Pi, while PHO2 transcripts act reciprocally to miR399 in Pi-deprived plants as well as miR399 overexpressors. However, there is evidence of PHO2 regulation other than by miR399.

To investigate why an increase in Pi uptake in pho2- and miR399-overexpressing plants was observed, Pi transporter expression (Aung et al., 2006; Bari et al., 2006) and uptake rates were examined (Aung et al., 2006). Using quantitative reverse transcription-PCR, Bari et al. (2006) looked at the members of the Arabidopsis Pht1 Pi transporter gene family for changes in gene expression in the pho2 mutants and wild type under the two Pi regimes, sufficient and limiting. In pho2 roots, Pht1;8 and Pht1;9 transporters were not down-regulated under +Pi conditions, whereas in the wild type most of the transporters were down-regulated. The authors interpret this as two closely related phosphate transporters contribute to the Pi accumulations observed in pho2 leaves. An increase in Pht1;8 expression in the roots of Pi-sufficient plants was also observed by Aung et al. (2006). The increase Aung et al. (2006) found in PHT1;8 was only 10% of what was observed under Pi deficiency. There was also an increase in the expressions of various organelle or Pi transporters, possibly to aid in the redistribution of intracellular Pi. While looking at changes transporter activity, Aung et al. (2006) found evidence for a dual transporter system—high and low affinity—and concluded that the observed increase in Pi uptake activity in plants overexpressing miR399 or pho2 mutants could be due to an elevated $V_{\text{max}}$ in both high- and low-affinity transporter systems without alteration of $K_m$. For this study, Aung et al. (2006) used whole seedlings to measure transporter activity; thus, $V_{\text{max}}$ and $K_m$ are a sum of all transporters. This increase in $V_{\text{max}}$ could be due to the 10% increase in transporter expression; nevertheless, it is not know if this increase is enough to account for the observed increase in $V_{\text{max}}$.

Promoter::reporter analysis demonstrated that UBC24 and miR399 were colocalized to the vascular cylinder (Aung et al., 2006). This colocalization correlates well with the putative role of UBC24 and miR399 in regulation of Pi translocation and remobilization. A recent study micrografting miR399-overexpressing and wild-type plants suggests that miR399 is able to move from shoots to roots (Lin et al., 2008; Pant et al., 2008), most likely through the phloem. In addition to expression in the vascular system, miR399 expression was found in root tips and mesophyll cells, suggesting the possibility of additional, unknown targets of miR399 in these tissues. GUS staining driven by these promoters highlighted the antagonistic relationship between these two genes: miR399 driven staining was basipetal while UBC24 was acropetal (Aung et al., 2006).

Two of the genes that Bari et al. (2006) found to be up-regulated under Pi-sufficient conditions in pho2 mutants, AtIPS1 and AT4, have also been shown to be regulated by the MYB-like transcription factor PHOSPHATE STARVATION RESPONSE1 (PHR1; Rubio et al., 2001), suggesting that these two proteins, PHO2 and PHR1 (Franco-Zorrilla et al., 2007), have targets in common. The expression levels of a set of highly Pi-responsive genes, including the miR399 gene family, were compared between Pi-deprived phr1 mutants and Pi-sufficient pho2 mutants. Interestingly, the increase in expression of all five miR399 primary transcripts typically seen under Pi deprivation was partially inhibited in the phr1 mutants (Bari et al., 2006), suggesting that PHR1 is required for miR399 expression and thus upstream of PHO2 in Pi signaling.

THE IMPACT

The MYB-related transcription factor PHR1 has been demonstrated to mediate Pi starvation response in plants. It functions as a dimer to bind an imperfect palindromic sequence found in many Pi starvation-inducible genes (Rubio et al., 2001; Franco-Zorrilla et al., 2004). Under Pi starvation, phr1 mutants accumulate less Pi in the leaves and there is less of a change in the shoot to root ratio compared with the wild type. A subset of Pi-responsive genes, including miR399, is also not up-regulated under Pi starvation. In their study, Bari et al. (2006) showed that PHR1 was involved in the regulation of PHO2 and miR399d, and this was further investigated by Nilsson et al. (2007), who examined Pi starvation response of a phr1 T-DNA knockout and, in parallel, the overexpression of PHR1 in wild-type and phr1 mutant backgrounds. The overexpression of PHR1 partially complemented the phr1 mutation and resulted in an increase in Pi accumulation in the shoots, similar to what is seen in pho2 mutants.
Although much has been learned about PHR1 in Arabidopsis and its importance in the regulation of the Pi starvation response, studies in monocots, which are some of the most important agricultural crops, have been scarce. Using the AtPHR1 protein sequence, Zhou et al. (2008) isolated two homologous genes in rice: OsPHR1 and OsPHR2. They made transgenic lines that either overexpressed or had reduced expression of both of the genes to determine if these two genes had similar functions to AtPHR1. Of the two genes, OsPHR2 was believed to have functional homology with AtPHR1 as overexpression lead to an accumulation of Pi in the shoots. Several Pi transporters were up-regulated in the shoots of OsPHR2-overexpressing plants under high Pi levels, which could account for the increased accumulation of Pi in the shoots. An increase in OsmiR399 expression in the PHR2 overexpressors suggests that in rice, PHR2 positively regulates miR399 expression as in Arabidopsis.

CONCLUSION

PHO2 is surfacing as an important participant in the regulation of plant Pi homeostasis. Knockout mutants in pho2 overaccumulate Pi in the shoots, resulting in Pi toxicity. Overexpression of miR399, a regulator of PHO2, leads to the same condition. Work on AtPHR1 (Nilsson et al., 2007) and OsPHR2 (Zhou et al., 2008) demonstrate that they are upstream of PHO2 in the Pi signaling pathway and act on miR399, confirming the work from Bari et al. (2006). A recent study by Lin et al. (2008) furthers their study on the vascular localization of miR399 and PHO2 (Aung et al., 2006) and supports the suppression of PHO2 by miR399 by the movement of miR399 from the shoots to roots. This study also found that in addition to miR399, PHO2 is also suppressed by small interfering RNAs, lending support to the observation by Bari et al. (2006) that PHO2 may be regulated by different pathways.

LITERATURE CITED


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