Phosphoregulation within the Photorespiratory Cycle: Regulate Smarter, Adapt Better?

To cope with various abiotic and biotic stress factors, plants must be facile in adapting cellular metabolism. In particular, they have to adjust photosynthesis and balance associated metabolism. Heat and drought stress strengthen plant photorespiration, a metabolic process resulting from Rubisco oxygenase activity (Busch, 2020). Rubisco oxygenase activity results in the production of 2-phosphoglycolate, a toxic intermediate that needs to be rapidly detoxified via the photorespiratory cycle to avoid inhibition of several Calvin-Benson-Bassham cycle enzymes (Fernie and Bauwe, 2020). The interconnection with associated metabolism, in particular the nitrogen cycle, demands a precise regulation of photorespiration depending on the environmental circumstances (Eisenhut et al., 2019). In order to achieve short-term regulation of photorespiratory fluxes, the activities of several photorespiratory enzymes are regulated by posttranslational modifications, including *S*-nitrosylation and phosphorylation (Hodges et al., 2016).

In this issue of *Plant Physiology*, Liu et al. (2020) demonstrate a novel regulatory mechanism that depends on cofactor switching mediated by phosphorylation of the photorespiratory enzyme hydroxypyruvate reductase 1 (HPR1) in Arabidopsis (*Arabidopsis thaliana*; Fig. 1). Under changing environmental conditions, the phosphorylation-dependent changes in HPR1 cofactor specificity allow the regulation of photorespiratory fluxes.

HPR catalyzes the second to last step of photorespiration, converting hydroxypyruvate into glycerate, while oxidizing a pyridine cofactor, preferentially NADH. In photosynthetic leaves, peroxisomal HPR1 accounts for up to 80% of HPR activity (Liu et al., 2020). The central role of HPR1 in photorespiration is supported by the retarded growth phenotype of the *hpr1-1* loss-of function mutant in air (Fig. 1).

Based on previous knowledge of the likely HPR1 phosphorylation site, Liu et al. (2020) demonstrate that mimicking HPR1 phosphorylation at Thr-335 ($HPR1^{T335D}$) shifted HPR activity toward NADPH dependency. Speaking in numbers, the catalytic efficiency for NADPH-dependent HPR1 activity was increased by 35%, while the NADH-dependent activity was reduced by 50%. Consequently, $HPR1^{T335D}$ was more specific for hydroxypyruvate as substrate for HPR activity in the presence of NADPH (31% increase) compared with NADH (56% reduction). Homology-based modeling revealed that the position of the phosphorylated Thr residue is within an α-helix connecting the substrate-binding and cofactor-binding domains. Structural changes upon Thr phosphorylation can explain the observed effects regarding cofactor and substrate specificity (Fig. 1).

Liu et al. (2020) complemented the *hpr1-1* photorespiratory phenotype in planta to assess the effects of HPR1 phosphorylation at Thr-335. Whereas wild-type HPR1 and nonphosphorylated HPR1 fully complemented the photorespiratory *hpr1-1* mutant phenotype in air, $HPR1^{T335D}$ only partially rescued the phenotype (Fig. 1). In planta, $HPR1^{T335D}$ also preferentially catalyzed NADPH-dependent HPR activity, consistent with the biochemical data using purified $HPR1^{T335D}$ protein. The partial complementation of the

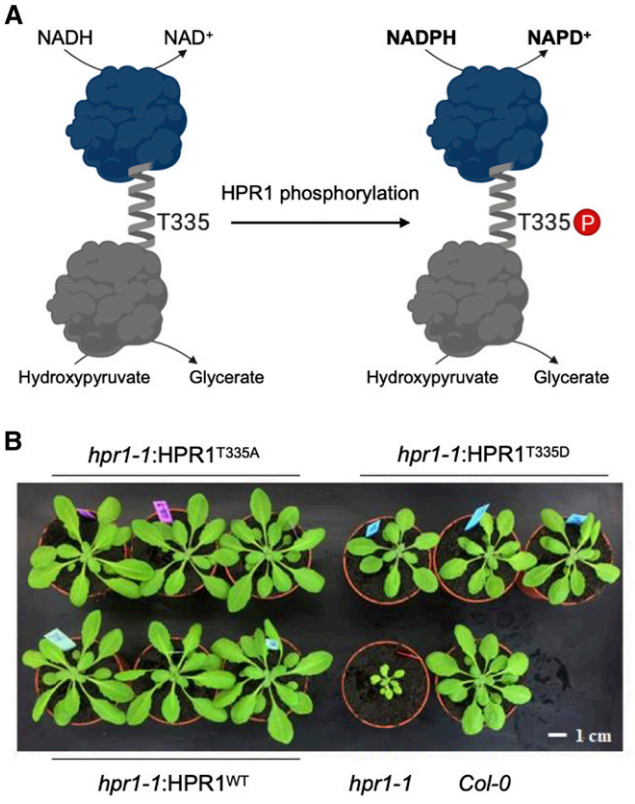

Figure 1. Phosphorylation of HPR1 at Thr-335 alters cofactor specificity and impacts Arabidopsis growth in air. A, HPR1 catalyzes the reduction of hydroxypyruvate to glycerate upon NADH oxidation. Phosphorylation at Thr-335 shifts HPR1 cofactor specificity toward NADPH. B, Complementation of the *hpr1-1* mutant with a nonphosphorylated HPR1 ($HPR1^{T335A}$), a mimicked phosphorylation HPR1 ($HPR1^{T335D}$), and wild-type HPR1 ($HPR1^{WT}$). Columbia-0 (Col-0) served as the wild-type control. A was generated with BioRender and B was adapted from Liu et al. (2020).

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hpr1-1 phenotype implies that constitutive HPR1 phosphorylation is obstructive under optimal growth conditions in air.

Indeed, HPR1 phosphorylation negatively influenced photosynthetic CO_2 assimilation due to disturbed photorespiration, shown by decreased levels of photorespiratory metabolites (glycerate, Gly, and glyoxylate). It remains to be proven if the reduced levels of photorespiratory metabolite are due to a feedback inhibition of the peroxisomal Ser:glyoxylate aminotransferase or reduced chloroplastic export of glycolate via the plastidial glycolate/glycerate transporter1 (Pick et al., 2013).

The study by Liu et al. (2020) provides evidence for a novel regulatory mechanism within the photorespiratory cycle. In contrast to previous studies that focused on altered enzymatic activity of photorespiratory enzymes upon posttranslational modifications, phosphorylation of HPR1 changes cofactor specificity. The shift from NADH toward NADPH for the HPR1-catalyzed step of photorespiration allows the adaptation of photorespiratory fluxes in response to altered environmental conditions that directly influence cellular NADH/NADPH ratios. Moreover, cofactor switching allows the cell to modulate the peroxisomal NADH/NADPH ratio by HPR1 activity. However, the advantages of altering cofactor specificity as an adaptive mechanism under changing environmental conditions have to be proven. Furthermore, open questions remain regarding the in vivo functions of the other HPR isoforms (Timm et al., 2011) as well as the regulation of both isoforms in the context of photorespiration.

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