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Pyruvate,Orthophosphate Dikinase in Leaves and Chloroplasts of C_3 Plants Undergoes Light-/Dark-Induced Reversible Phosphorylation[1]

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Pyruvate,orthophosphate (Pi) dikinase (PPDK) is best recognized as a chloroplastic C_4 cycle enzyme. As one of the key regulatory foci for controlling flux through this photosynthetic pathway, it is strictly and reversibly regulated by light. This light/dark modulation is mediated by reversible phosphorylation of a conserved threonine residue in the active-site domain by the PPDK regulatory protein (RP), a bifunctional protein kinase/phosphatase. PPDK is also present in C_3 plants, although it has no known photosynthetic function. Nevertheless, in this report we show that C_3 PPDK in leaves of several angiosperms and in isolated intact spinach (*Spinacia oleracea*) chloroplasts undergoes light-/dark-induced changes in phosphorylation state in a manner similar to C_4 dikinase. In addition, the kinetics of this process closely resemble the reversible C_4 process, with light-induced dephosphorylation occurring rapidly ($\leq$15 min) and dark-induced phosphorylation occurring much more slowly ($\geq$30-60 min). In intact spinach chloroplasts, light-induced dephosphorylation of C_3 PPDK was shown to be dependent on exogenous Pi and photosystem II activity but independent of electron transfer from photosystem I. These in organello results implicate a role for stromal pools of Pi and adenylates in regulating the reversible phosphorylation of C_3-PPDK. Last, we used an in vitro RP assay to directly demonstrate ADP-dependent PPDK phosphorylation in desalted leaf extracts of the C_3 plants *Vicia faba* and rice (*Oryza sativa*). We conclude that an RP-like activity mediates the light/dark modulation of PPDK phosphorylation state in C_3 leaves and chloroplasts and likely represents the ancestral isoform of this unusual and key C_4 pathway regulatory "converter" enzyme.

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