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C₃ origins of the C₄ pathway regulatory enzyme, PPDK-RP

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A current view of C₄ and CAM evolution holds that all of the constituent enzymes of the C₄ pathway preexisted in C₃ plants, albeit functioning in nonphotosynthetic capacities. Problematic in this view is the C₃ origin of presumably dedicated C₄ pathway regulatory enzymes, such as PPDK regulatory protein (RP). RP is an unusual, bifunctional Ser/Thrkinase/ phosphatase that mediates light/dark regulation of C₄/CAM PPDK activity via reversible phosphorylation of an active-site Thr. Because of its unique substrate requirements and localization in the stroma of C₄ mesophyll cells, RP appears to be specific to C₄ PPDK regulation. However, in this presentation we show that an RP-like activity exists in chloroplasts of C₃ leaves. Specifically, immunoblot analysis of phospho- and dephospho- PPDK from illuminated and dark adapted C₃ leaves (rice, *F. pringlei*, *V. faba*, spinach) revealed that PPDK phosphorylation/dephosphorylation is regulated in a light/dark-dependent manner. Further, the kinetics of the reversible activation process are similar to C₄ plants, with light activation occurring rapidly (≤ 15 min) and dark deactivation more slowly (≥ 1 h). In vitro experiments with isolated intact spinach chloroplasts show the same light/dark modulation of PPDK phosphorylation state occurs, with light-induced dephosphorylation of phospho-PPDK being Pi dependent, inhibited by DCMU, but insensitive to MV. Hence, as with C₄ RP, adenylates and stromal pools of Pi likely regulate the opposing bifunctional activities of the C₃-like RP activity. Thus, evolution of RP into its C₄/CAM role may have been no more problematic than for other C₄/CAM pathway enzymes, as it apparently pre-exists in chloroplasts of C₃ plants.