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C_3 origins of the C_4 pathway regulatory enzyme, PPDK-RP

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A current view of C_4 and CAM evolution holds that all of the constituent enzymes of the C_4 pathway preexisted in C_3 plants, albeit functioning in nonphotosynthetic capacities. Problematic in this view is the C_3 origin of presumably dedicated C_4 pathway regulatory enzymes, such as PPDK regulatory protein (RP). RP is an unusual, bifunctional Ser/Thrkinase/ phosphatase that mediates light/dark regulation of C_4/CAM PPDK activity via reversible phosphorylation of an active-site Thr. Because of its unique substrate requirements and localization in the stroma of C_4 mesophyll cells, RP appears to be specific to C_4 PPDK regulation. However, in this presentation we show that an RP-like activity exists in chloroplasts of C_3 leaves. Specifically, immunoblot analysis of phospho- and dephospho- PPDK from illuminated and dark adapted C_3 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 C_4 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 C_4 RP, adenylates and stromal pools of Pi likely regulate the opposing bifunctional activities of the C_3-like RP activity. Thus, evolution of RP into its C_4/CAM role may have been no more problematic than for other C_4/CAM pathway enzymes, as it apparently pre-exists in chloroplasts of C_3 plants.