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## Symposium 17. C<sub>4</sub>

Paper S17-013:

# C<sub>3</sub> origins of the C<sub>4</sub> pathway regulatory enzyme, PPDK-RP

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A current view of C<sub>4</sub> and CAM evolution holds that all of the constituent enzymes of the C<sub>4</sub> pathway preexisted in C<sub>3</sub> plants, albeit functioning in nonphotosynthetic capacities. Problematic in this view is the C<sub>3</sub> origin of presumably dedicated C<sub>4</sub> pathway regulatory enzymes, such as PPDK regulatory protein (RP). RP is an unusual, bifunctional Ser/Thrkinase/ phosphatase that mediates light/dark regulation of C<sub>4</sub>/CAM PPDK activity via reversible phosphorylation of an active-site Thr. Because of its unique substrate requirements and localization in the stroma of C<sub>4</sub> mesophyll cells, RP appears to be specific to C<sub>4</sub> PPDK regulation. However, in this presentation we show that an RP-like activity exists in chloroplasts of C<sub>3</sub> leaves. Specifically, immunoblot analysis of phospho- and dephospho- PPDK from illuminated and dark adapted C<sub>3</sub> 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<sub>4</sub> plants, with light activation occurring rapidly ( $\leq 15$  min) and dark deactivation more slowly ( $\geq 1$  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<sub>4</sub> RP, adenylates and stromal pools of Pi likely regulate the opposing bifunctional activities of the C<sub>3</sub>-like RP activity. Thus, evolution of RP into its C<sub>4</sub>/CAM role may have been no more problematic than for other C<sub>4</sub>/CAM pathway enzymes, as it apparently pre-exists in chloroplasts of C<sub>3</sub> plants.