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# Regulative phosphorylation of plastocyanin and cytochrome b6f subunit IV: Insights into photosynthetic electron transfer and STT7 kinase feedback control

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In this study, we examined plastocyanin (PC) binding and electron transfer with both photosystem I (PSI) and cytochrome b6f (cyt b6f), and show the synergetic adaptations between these three enzymes. Furthermore, we explored the effects of PC phosphorylation on these interactions. To do so, we generated several recombinant variants of PC, in which we genetically engineered two of the phosphorylated residues (S10 & S49). We studied the kinetics of both Cytf oxidation and P700 re-reduction by measuring fast optical spectroscopy. We also conducted chemical protein crosslinking and structural proteomics to gain further insights on the interaction between PC and cyt b6f. Our results show that the phosphorylation mode of PC alters the conformation in which they establish binding and electron transfer, and generated new models which elaborate the mechanism of this adaptation. To address the role of STT7 dependent phosphorylation of Thr4 in the N-terminal domain of cyt b6f subunit IV, we generated site-directed mutants in the N-terminal domain of cyt b6f subunit IV by chloroplast transformation. The phosphomimic mutation PetD Thr4/Glu effectively inhibits STT7 kinase activity, as shown by phosphoproteomic analyses, resulting in the PetD Thr4/Glu strain being locked in State 1. These findings reveal a novel feedback regulation mechanism that controls the phosphorylation capacity of STT7 kinase. Similarly, deletion of five N-terminal amino acids cyt b6f subunit IV also disrupts STT7 function, retains cells in State 1, and, in contrast to the PetD Thr4/Glu mutation, significantly impairs electron transfer within cyt b6f. These data reveal that the PetD N-terminus has crucial functions in cyt b6f electron transfer and STT7 regulation.

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