The phosphorylated redox proteome of *Chlamydomonas reinhardtii*: revealing novel means for regulation of protein structure and function

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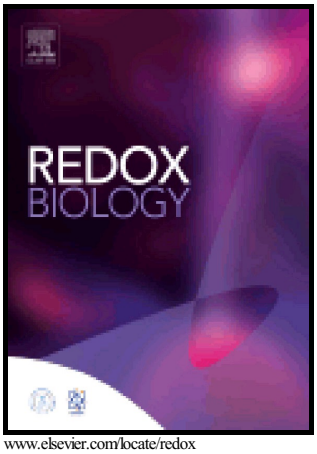

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Abstract

Post-translational modifications (PTMs) are covalent modifications to protein residues which may alter both conformation and activity, thereby modulating signaling and metabolic processes. While PTMs have been largely investigated independently, examination into how different modification interact, or crosstalk, will reveal a more complete understanding of the reciprocity of signaling cascades across numerous pathways. Combinatorial reversible thiol oxidation and phosphorylation in eukaryotes is largely recognized, but rigorous approaches for experimental discovery are underdeveloped. To begin meaningful interrogation of PTM crosstalk in systems biology research, knowledge of targeted proteins must be advanced. Herein, we demonstrate protein-level enrichment of reversibly oxidized proteoforms in *Chlamydomonas reinhardtii* with subsequent phosphopeptide analysis to determine the extent of phosphorylation in the redox thiol proteome. Label-free quantification was used to quantify 3,353 oxidized Cys-sites on 1,457 enriched proteins, where sequential phosphopeptide enrichment measured 1,094 sites of phosphorylation on 720 proteins with 23% (172 proteins) also identified as reversibly oxidized. Proteins identified with both reversible oxidation and phosphorylation were involved in signaling transduction, ribosome and translation-related machinery, and metabolic pathways. Several redox-modified Calvin-Benson cycle proteins were found phosphorylated and many kinases/phosphatases involved in phosphorylation-dependent photosynthetic state transition and stress-response pathways had sites of reversible oxidation. Identification of redox proteins serves as a crucial element in understanding stress response in photosynthetic organisms and beyond,