Cross-species analysis of temperature-dependent phosphoglycolate phosphatase activity and dynamics

Luke Sharpe[1,2], Duncan Boren[1,2], Emily Stringham-Turner[1], Matt Stata[3], Josh Vermaas[1,2], Berkley J. Walker[1,4]

[1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, United States,

[2]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, United States,

[3]Plant Resilience Institute, Michigan State University, East Lansing, MI, United States

[4]Department of Plant Biology, Michigan State University, East Lansing, MI, United States

sharpelu@msu.edu

Photorespiration occurs when Rubisco catalyzes the fixation of O instead of CO , producing the toxic metabolite 2-phosphoglycolate (2-PG). This compound must be rapidly recycled into 3-phosphoglycerate (3-PGA) to reenter the Calvin Benson Bassham (CBB) cycle. In most plants, Rubisco oxygenation occurs once for every four to five carboxylation reactions, making photorespiration the second-highest flux pathway on Earth, surpassed only by carbon fixation and CBB itself. The pathway is thought to have originated in ancestral cyanobacteria and has been conveyed to plants and algae via endosymbiosis. Individual photorespiratory enzymes have diversified to suit distinct ecological and environmental niches. One of the most conserved and essential enzymes in this pathway, phosphoglycolate phosphatase (PGPase), catalyzes the dephosphorylation of 2-PG to glycolate. Because this reaction is both energetically demanding and thermally sensitive, PGPase presents a key target for improving the robustness of the photorespiratory cycle under heat stress. Here, we surveyed PGPases from a phylogenetically and environmentally diverse panel of organisms, including mesophilic species such as *Arabidopsis thaliana* and *Brassica rapa*, as well as thermotolerant taxa from extreme habitats, such as *Rhazya stricta* (desert plant) and *Cyanidioschyzon merolae* (acidothermophilic red alga). Through heterologous expression and temperature-dependent kinetic assays, we identified isoforms exhibiting distinct thermal performance profiles that correlate with their native environmental regimes. To probe the structural underpinnings of these differences, we performed molecular dynamics simulations and quantified the flexibility of different regions of the enzymes using root mean square fluctuation (RMSF) analyses. Complementary evolutionary analyses identified residues under positive selection at the base of a flexible loop region on the periphery of the PGPase. Site-directed mutagenesis of this residue in *R. stricta* supports its role in enhancing structural stability via a hydrogen-bonding network that potentially contributes to elevated thermotolerance. Together, these data highlight adaptive strategies by which photorespiratory enzymes achieve thermotolerance and provide mechanistic insights to guide engineering of more heat resilient carbon recycling pathways in plants.