Engineering the Photosynthetic Enzyme RuBisCO for Enhanced Carboxylation Efficiency

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Carbon assimilation by photosynthesis is facilitated by the enzyme Ribulose-1,5-bisphosphate Carboxylase/Oxygenase (RuBisCO), which fixes atmospheric CO_2 into sugar for biomass accumulation. Despite this key role, RuBisCO has several severe biochemical shortcomings (slow catalysis, promiscuous reaction with oxygen, and self-inhibition) that limit photosynthetic efficiency and make it an attractive target for protein engineering. Our work towards the application of synthetic biology technologies to improve RuBisCO isoforms from both bacterial and plant species will be presented. We demonstrate improvement of a chemolithoautotrophic bacterial RuBisCO from *Gallionellaceae* using laboratory directed evolution. We utilized *in vivo* mutagenesis combined with RuBisCO-dependent *E. coli* (RDE) screening to make targeted mutations and explore deep sequence space within the enzyme, identifying mutations in a key structural region that improve carboxylation efficiency. Our ongoing efforts to improve plant RuBisCO utilize machine learning tools to predict catalysis-enhancing mutations in the enzyme from *Nicotiana tabacum* (tobacco) that are screened for activity using RDE. We plan to test these predicted mutations both *in vitro* and *in planta* to determine their impact on catalysis and photosynthetic efficiency. If successful, improvements to RuBisCO carboxylation, together with other advances in photosynthetic engineering, have the potential to increase crop yields and provide agricultural resiliency in a changing climate.