

Peer Review

Review of: "Predicting the Effects of Coevolution on Rubisco: A Study on Carbon Dioxide Fixation by Bryophytes"

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This is an interesting attempt to explore the structural and functional biology of Rubisco from a purely computational level in bryophytes, which constitute half of plant phylogenetic diversity. The coevolution analyses are well-performed, although I am not sure about the functional applications that the authors suggest. I feel that more effort can be made to connect the structural diversity of Rubisco that the authors observe to known kinetic properties in experimental studies of isolated enzymes and of bryophyte photophysiology:

1. Some hornworts are known to have pyrenoids, which may have been differentially lost or re-evolved independently many times <https://www.pnas.org/doi/10.1073/pnas.1213498109>, which impact significantly on RuBisCo activity versus specificity. How is this reflected in protein sequence evolution?
2. How many of the residues that the authors identify as having functions (e.g., in CABP binding) are known from in vitro kinetic studies to impact the Kcat or Km for CO₂/O₂ of RuBisCo?
3. Are the authors sure that the coevolving residues observed in RbcS and rbcL only interact with other residues in the RuBisCo holoenzyme, or may they interact with other proteins in the plastid stroma? This is particularly important for species in which RuBisCo is structurally arranged in a pyrenoid, where we might expect important interactions with carbonic anhydrases <https://www.pnas.org/doi/10.1073/pnas.1603112113> or Calvin cycle proteins.
4. What are the Ka/Ks frequencies associated with the residues inferred to coevolve? Can the authors find any evidence at a nucleotide level that these residues are under positive selection?

More broadly, I am not sure of the direct application of bryophytes for green carbon capture, although I do agree on their evolutionary and ecosystem significance. What applications do the authors think their data have for synthetic biology and agronomy, e.g., engineering enhanced primary production in future crop species?

Declarations

Potential competing interests: No potential competing interests to declare.