Background

The evolution of ancient organisms both shaped and was shaped by drastic global environmental changes such as the Great Oxygenation Event (GOE). This rapid atmospheric transition is believed to be broadly coincident with the diversification of cellular life on earth.1,2 The enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes a key reaction of oxygenic photosynthesis. It is the primary catalyst of biological carbon fixation, but its carboxylation activity is plagued by a competing oxygenation reaction.3-6 Modern atmospheric oxygen levels are orders of magnitude greater than those of carbon dioxide, but this was not always the case. Thus this dual enzymatic activity of RuBisCO introduces an evolutionary conundrum: the enzyme partially responsible for creating the oxygenated atmosphere is greatly hindered by oxygen.7 Reconstruction of ancient RuBisCO phenotypes provides a greater understanding of how evolutionary changes in this protein are correlated with the oxygenation of the atmosphere. Previous work has been done to reconstruct ancestral RuBisCO large subunits. Here we present the reconstruction of the small subunit, which is more sequentially divergent, only present in one form of the enzyme, and not directly involved in catalysis.

Ancestral Sequence Reconstruction

Ancestral sequences were inferred based on maximum likelihood phylogeny for three ancestors: MRCA(IAB), MRCA(IA/B), and MRCA(IB). At calculations performed using Phylobit8 Alignment method and evolutionary model highlighted in yellow (see left). Though all autotrophs express RuBisCO, only one evolutionary group expresses the small subunit. Its origins and function remain highly mysterious. There is strong support for the existence of MRCA(I), MRCA(A/B), and MRCA(IB). These ancestors were reconstructed in this study.

Coevolution of Subunits

Ancestral structures were inferred by homology modeling.9 In this method, structural models for the ancestors were created based on extant small subunit templates. Ancestral sequences can be directly modeled.

Future Directions

1. Resurrect ancestral RuBisCO in vitro

   - small subunit (blue) termini maintain high level of contact with large subunit (gray)
   - These terminal residues most likely to be positively selected
   - Are contacted residues both positively selected?
   - How are these changes related to the evolving atmosphere?

2. Functional analysis of ancestral RuBisCO activity

   We will measure the biochemical activity of ancient RuBisCO enzymes under different atmospheric and environmental conditions to mimic the evolving atmosphere.

References