

RESURRECTING ANCESTRAL RUBISCO *IN SILICO*. A. L. Donovan¹ and B. Kaçar²¹Department of Chemistry, Colby College, Waterville, ME²Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA

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Earth's early environments were not the hospitable worlds familiar to extant organisms; the atmosphere was almost certainly anoxic and of unknown pressure, temperature and composition compared to modern norms. 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 [1-4].

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 carboxylase activity is plagued by a competing oxygenation reaction [5-8]. Modern atmospheric oxygen levels are orders of magnitude greater than those of carbon dioxide, but this was not always the case. Thus the dual enzymatic activity of RuBisCO introduces an evolutionary conundrum: the enzyme partially responsible for creating the oxygenated atmosphere is greatly hindered by oxygen [7].

The evolutionary history of RuBisCO may provide insight into the conditions necessary for life and habitability. Across RuBisCO species different organisms display extremely diverse biochemical characteristics, but the origin of this diversity remains unknown due to scant geologic evidence [8-10]. Reconstruction of ancient RuBisCO phenotypes provides a greater understanding of how evolutionary changes in the protein were correlated with the oxygenation of the atmosphere.

Ancestral sequence reconstruction of several key RuBisCO ancestors was performed using maximum likelihood phylogeny. This method utilizes protein sequences from extant organisms, and provides a window into the metabolic states of their ancestors. Additional evolutionary tests were performed to determine which sites in the enzyme were under positive selective pressure. These sites were then analyzed at the structural level to determine their catalytic importance. This analysis of key residue substitutions offers greater understanding of the correlation between changes at the molecular and environmental levels.

Combining ancestral sequence reconstruction, evolutionary analysis, and structural biochemical studies provides insight into how life on earth evolved with

rapidly changing atmospheric conditions. By mapping these molecular changes onto the evolutionary history of the atmosphere, the conditions necessary for life and habitability may be uncovered.

References: [1] E. G. Nisbet et al. (2007) *Geobiology*, 5, 311-335. [2] A. H. Becker et al. (2004) *Nature*, 427, 117-120. [3] T. W. Lyons et al. (2014) *Nature*, 506, 307-315. [4] J. Farquhar et al. (2011) *Photosynth Res*, 107, 11-36. [5] I. Andersson et al. (2008) *Plant Physiol Biochem*, 46, 275-291. [6] I. Andersson. (2008) *J Exp Bot*, 59, 1555-1568. [7] N. Eckardt. (2005) *The Plant Cell*, 17, 2139-2141. [8] R. J. Spreitzer et al. (2002) *Annu Rev Plant Biol*, 53, 449-475. [9] F. R. Tabita et al. (2007) *Microbiol Mol Biol Rev*, 71, 576-599. [10] F. R. Tabita et al. (2008) *Philos Trans R Soc Lond B Biol Sci*, 363, 2629-2640.