Genetically Engineering Cyanobacteria Into Chloroplasts. G. S. Gavelis[1] and G. H. Gile[2], [1]School of Life Sciences, Arizona State University, ggavelis@asu.edu, [2]School of Life Sciences, Arizona State University, ggile@asu.edu.

Introduction: Chloroplasts produce the vast majority of oxygen and organic carbon in the biosphere. The ancestry of these photosynthetic organelles has been traced back to a free-living cyanobacterium, which was engulfed and subsequently "domesticated" by a single eukaryotic cell (1, 2). This ancient endosymbiotic event—called the "primary endosymbiosis"—was a turning point in the history of life, for it allowed for eukaryotes to harness photosynthesis, and eventually gave rise to plants and algae. How this symbiosis began and ultimately became permanent is a matter of great debate (3, 4, 5). While many hypotheses have been proposed, they are confounded by the fact that the primary endosymbiosis occurred very remotely in the history of life (~1 BYA) (6). Given its rarity, scientists are unlikely to witness the early stages of a primary endosymbiosis unfold in nature ever again.

Owing to advances in synthetic biology, another avenue of research is possible. By re-tailoring a cyanobacterium with genes that predispose it to endosymbiosis, the onset of a primary plastid acquisition can be observed within a human timescale.

Building an artificial endosymbiosis is no longer hypothetical. Recently, a strain of the cyanobacterium *Synechococcus elongatus* was engineered to express proteins that allowed it to enter into the eukaryotic cytoplasm, where it continued to grow, divide, and photosynthesize (7). While the cyanobiont showed no evident harm to either host type (human macrophages and embryonic zebrafish), it was not heritable due to the segregation of the germ line in these multicellular animals. Therefore, transgenic cyanobionts should be inoculated into unicellular eukaryotes (protists), which are liable to transfer endosymbionts over several generations with each cell division.

We present progress in engineering a new primary plastid. a model system for the field of chloroplast evolution, which has amassed a number of hypotheses but currently lacks experimental tools to test them. Questions about chloroplast origins are fundamental to understanding the present state of the biosphere, for they concern the origin of eukaryotic photosynthesis, the rise of multicellular plants and the domination of the sea by eukaryotic phytoplankton, which together drive modern climatic and biogeochemical patterns. This work will also provide bioinspiration for a major undertaking in applied biology, which is the bioengineering of synthetic organelles. In principle, synthetic organelles would allow for the installation of bioengineered compartments into natural hosts, as a vehicle for self-contained genetic circuits and biochemistry.

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