ENGINEERING MODERN MICROBES WITH ANCIENT GENES TO UNDERSTAND FUNCTIONAL INNOVATION

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Introduction: Evolution is unarguably a historical process in which a lineage's past evolution alters its future evolution. To what degree, however, does the evolutionary past truly shape future evolutionary pathways? A comprehensive understanding of how past evolution might have influenced the evolutionary pathways of modern organisms requires access to both modern and ancestral genotypes so as to map sequence-level information to structural-level outcomes. We used a novel combination of paleogenetics (ancestral gene resurrection) and experimental evolution to provide insights into historical evolutionary pathways. Paleogenetics is a phylogenetic method in which Bayesian inferences of extant sequence data permit reconstruction of putative ancestral gene and protein sequences [1,2]. In experimental microbial evolution, researchers evolve microbial populations under controlled, laboratory conditions for thousands or even tens of thousands of generations and generate a "fossil record" for each population by preserving the samples of evolving populations at regular intervals [3].

We sought to better understand the historical evolutionary constraints on an essential ribosomal protein using a reconstructed 0.7 billion-year-old Elongation Factor (EF-Tu gene) in a modern microbial cell [4,5]. We monitored the co-adaptation between the ancient gene and the modern bacterial genome by experimentally evolving the ancient-modern hybrid system for 2000 generations with 8-fold replication. To identify the changes that occurred over the course of evolution, we characterized the viable modern-ancient hybrid bacteria's biological, biochemical and genetic properties. The majority of lineages displayed parallel evolution through *cis*-regulatory adaptation in the EF-Tu promoter. Surprisingly, one lineage exhibited structural adaptation only within nodes of the EF-Tu protein-protein interaction network, not within the pleiotropic EF-Tu hub itself. Our results suggest that *cis*-regulatory adaptations are more accessible than structural adaptation, at least when modern organisms respond to the presence of ancient essential genes. Further, connecting "ancient with modern" allows us to address the issue of historical contingency in a unique way as we, to a degree "replay life's tape" [6] for a genetic fragment representative of the evolutionary past.

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