EXPERIMENTAL EVOLUTION OF AN ANCIENT EF-Tu HOMOLOG. M. Kinnersley[1], M. Garner[1], F. Rosenzweig[3] and B. Kacar[4], [1]University of Montana, Missoula, MT, [3]Georgia Institute of Technology, Altanta, GA and [4] Harvard University, Cambridge, MA.

Introduction: The study of the evolution of gene sequence, protein function and regulatory networks has traditionally been approached via phylogenetics, which is comparative and retrospective. A prospective approach using experimental evolution can provide fruitful insights into these processes. To address questions regarding the flexibility of regulatory networks, as well as contingency and determinism in the evolution of protein function we have integrated the ancestral γ-proteobacterial gene sequence for elongation factor Tu (EF-Tu) into modern *E. coli* and followed its evolution under sub-optimal temperature at slow growth rates.

Experimental Approach: Replacement of the extant gene for EF-Tu (*tufA*) with the "ancient" sequence (*tufAanEF1*) diminishes growth rate in modern *E. coli* K12 MG1655 [1]. While this deficit may be partly due to impaired translation efficiency, temperature, may also play a causal role, as ancient EF-Tus have a higher Tms than modern versions [2]. Here we employed a glucose-limited accelerostat run at 30°C to select for *tufAanEF1* derivatives with enhanced resource utilization efficiency.

Results: Over 200-300 generations, mutants with improved growth characteristics appeared in both *tufAanEF1* and tufA wild-type accelerostats. Maximum specific growth rates under glucose limitation (2% weight/volume) were increased from 0.5 to 0.8 hr^{-1}, which corresponds to a reduction in generation time from 120 minutes to 75 minutes. Comparison of *tufAanEF1* evolved strains to their ancestor via targeted gene sequencing suggested SNPs in the *tufA* promoter region likely played a role in adaptation. Efforts to characterize changes affecting other binding partners such as the actin-like protein MreB are currently underway.

References:

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