149a Construction and Evolution of an Artificial Microbial Symbiotic System

Nikos Reppas, Xiaoxia (Nina) Lin, Jay Shendure, Greg Porreca, and George M. Church

Mutualistic symbiosis exists widely in nature, for example, between plants and fungi in nutrient-poor soils, and between aphids and their endosymbiotic bacteria. In this work, we artificially engineered a microbial symbiotic system comprised of two cross-feeding *E. coli* amino acid auxotrophs and investigated its evolutionary adaptation in amino-acid-free minimal medium in serial batch cultures. This can be viewed as a simplified experimental model of much more complicated natural mutualistic ecosystems that are often refractory to straightforward experimental analysis.

We constructed seven types of auxotrophs by deleting genes or operons in different amino acid biosynthesis pathways. Each of them on its own is incapable of growth in minimal medium due to the absence of the required amino acid, but a pair of different auxotrophs are potentially able to co-grow by obtaining the needed nutrient from each other. Twenty one such pairs of auxotrophs were co-cultured and their overall fitnesses were measured. Many of these pairs exhibited considerable co-growth.

We then evolved the fastest pair in the above experiment as three independent lineages by daily passaging into fresh minimal medium over the course of approximately 150 days, which corresponded to roughly 600 generations. We measured the growth rate of all three lineages at different time points of the evolution. It was observed that all three lineages showed an overall trend of improving fitness. The highest improving rate took place during the early period of the evolution. One lineage improved its growth rate by over 50% in a short period of 40 days. Interestingly, the growth rate did decrease occasionally throughout the whole evolutionary process. The evolution experiment ended when one of the auxotrophs regained the ability to grow independently in the minimum medium and became dominant in all three lineages.

To help understand the interactions between the two auxotrophs in the system and the evolution process, we developed an ordinary differential equation (ODE) based model to investigate the population dynamics of the system. The model considered two layers of relationships of the system: competition among each subpopulation and cooperation between the two subpopulations. Quantitative analysis revealed that the fitness of the overall system depends only on how well the two auxotrophs support each other, though in each subpopulation it is the fastest growing individuals that possess selective advantage. Therefore, the observed increase of overall fitness of the system indicated correlation between changes of the properties of growth and cross-feeding during the evolution in one or both auxotrophs. Further work are being carried out to test whether the theoretical predictions agree with experimental data.

To discover the genetic basis for the observed co-culture mutualistic adaptation, we first sought simply to identify any gross changes in genome structure due to chromosomal amplifications or deletions in either of the two auxotrophs. To this end, we analyzed the hybridization signatures of genomic DNA isolated from the different lineages and passage numbers on Affymetrix Antisense Genome arrays, relative to the wild type genomic DNA. We observe a consistent ~163kb amplification centered at 2.1 MB in all cross-feeding genomic DNA samples, strongly suggesting that the amplification took place in the single ancestral co-culture that was used to start all three lineages. Although the 163 kb region contains many genes, a gene encoding a relevant amino acid transporter is of particular interest and indicated that this amplification is associated with one specific auxotroph.

In an effort to perform higher resolution genetic analysis, we are utilizing new polony based whole-genome sequencing technology to analyze an isolated clone of one of the auxotrophs after 40 rounds of

passaging in the evolution. A number of potential mutations have been identified and we are currently carrying out further analysis to confirm them and to investigate the phenotype-genotype relation.