Reductive evolution towards primitive life: What will we see?

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Abstract
Observing the gradual transition from “life” to “non-life” tells us a lot about the features of living systems. Based on this idea, we started simplifying natural cells by inactivating their genes randomly through experimental evolution. *Escherichia coli* (*E. coli*) cells were cultured in a high-mutagenicity environment to accumulate replication errors on their genomes. As a result, we observed dozens of mutations, which were supposed to deactivate gene expression. In addition, the gene inactivation accumulates time proportionally without growth defects so far. These results suggest that naturally isolated cells are highly redundant in an experimental environment—implying the possibility of further simplification deleting hundreds or thousands of genes.

Introduction
What is life? In order to answer this fundamental question, there have been many attempts to create primitive living systems from simpler components—with many challenges. For example, building artificial cells from chemical materials in a test tube provided experimental platforms to observe the unsophisticated cellular behaviors (Kurihara, et al. 2015). This bottom-up approach (blue arrow in Fig.1) would allow us to observe the gradual transitions from non-life to life. However, there are many technical difficulties in this approach to upgrading such highly primitive artificial cells toward complex and modern forms. On the other hand, the top-down approach (green arrow in Fig.1) can avoid such difficulties. This approach simplifies the modern cells by reducing their sophisticated genes to only retain primitive cellular functions. For example in nature, *Pelagibacter ubique*, which has smallest number of genes among free-living bacterial strains (1354 genes, Luo, 2015), is supposed to have lost thousands of genes through reductive evolution. This bacteria shows slower growth compared to other related species regardless of the amount of environmental resources—implying the loss of the sophisticated ability to respond to environmental changes. In the same way, gene loss in bacterial genomes would result in more primitive behavior in various aspects. Thus, both approaches are significant in exploring the transition between living and non-living systems.

Evolutionary Experiments
Ultra violet (UV) irradiation was used in increasing doses as to increase the mutation rate. Previous research showed experimental evolution with periodic UV irradiation can increase gene inactivation in the *E. coli* genome (Shibai, et al. 2014). We used the *E. coli* MDS42 strain where 15% of its genes were manually deleted from the progenitor (Pósfai, et al. 2006). Cells were cultured in minimal medium and showed exponential growth. On the other hand, increasing dosages of UV irradiation kills cells at an exponential rate. Thereby, cell concentration was constantly measured to control the timing of UV irradiation and prevent both extinction and saturation. The cells were subcultured every four days and glycerol-stocked at the same time. The experiment was replicated six times and continued for 168 days.

Analysis of Evolutionary Changes
We conducted whole genome resequencing for the evolved lineages on the 56th and 168th days. Mutations were detected by comparing the DNA alignments with the ancestor’s. Mutations that caused stop codons at abnormal positions were counted as nonsense mutations. Additionally, the mutations that caused hazardous gaps in codon reading frames were counted as missense mutations. We regarded nonsense and...
missense mutations as inactive mutations. Genes with at least one inactive mutation were counted as inactivated genes, though it is still not certain whether their functions were completely lost. As a result, 19 to 94 genes were inactivated over 168 days in each lineage (Fig.2). The cell lineage with the least number of inactivated genes (Series-1) had highly aggregative growth by the 56th day already. The maximum growth rate of each lineage after the evolutionary experiment was 0.68 to 0.86 [h⁻¹]—not significantly declined from ancestor’s (0.73 [h⁻¹]).

![Number of inactivated genes during evolution](image)

Figure 2: Number of inactivated genes during evolution

**Discussion**

Cells were estimated to grow more than 3000 generations over 168 days, assuming the growth rates higher than 0.6 [h⁻¹] were maintained. Dozens of genes accounting for 0.5 to 2.5% of the all genes on the ancestral genome displayed traits for accumulated inactivation. Surprisingly, even though the loss of function occurred at such a scale, a significant drop in the maximum growth rate was not observed. This implies that many of the genes impacting the growth of *E. coli* in this evolutionary experiment were redundant. One of the possible causes of this redundancy is that the natural environments were more complex than the experimental environments, so that the *E. coli* can still function with the loss of those genes obtained through natural history. To examine this, additional analysis on the function of the inactivated genes is needed.

The lineage with the least number of gene inactivation (Series-1) obtained the trait of increased aggregation of cells. We inferred that the outer cells of the aggregates protected inner cells from the harmful UV irradiation, so that the outer cells died and inner cells survived with a decreased number of inactive mutations. Such an adoptive evolution obtaining multicellular-like behavior is interesting though it does not meet the purpose of this study.

What will we see if we continue this evolutionary experiment over a longer period of time? Through first-order approximation, the number of functional genes will be about 1500 in 20 years. That is comparable to *Pelagibacter ubique*. With 10 years of further evolution, the number of functional genes would reach the level of *Mycoplasma genitalium*, which has the smallest genome even among parasites (487 genes, Choe, et al. 2016)—though the time proportional accumulation of gene inactivation would not last so long. How would the behavior of the reductively evolved *E. coli* differ from the behavior of natural organisms with a similar number of genes? Also, as a complex adaptive system, how would its features, like energy efficiency or evolvability change? In order to answer these open questions, discussing how living systems should be understood is needed side by side with the decades-long experimental evolution.

**Materials and Methods**

In all the experiments of this study, cells were cultured in mM63 minimal medium at 37 °C with shaking.

**Evolutionary Experiments**

*E. coli* MDS42 was used as the ancestral strain. The bacteria were incubated in a quartz test tube. Optical density (OD) of the cells were measured every 3 min. Intensive UV irradiation was conducted when the OD value increased by 0.002 from the former irradiation. We irradiated UV from the bottom of the tube. Irradiation was applied for the dosages that killed the ancestral cells so that the survival rates were $10^2 \sim 10^4$ at a time. Aliquot of the culture was transferred into a new tube with fresh media at 4-day intervals, so that dilution rate was $10^2$. The cells were glycerol-stocked at the same time.

**Analysis of Evolutionary Changes**

Purified genomic-DNA samples of the cells were sequenced by Illumina Miseq. Base-pair substitutions and short insertions/deletions were identified using SAMtools.

Maximum growth rates were measured as the increased rate of the OD value during the exponential growth phase in the absence of UV irradiation.

**References**


