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The MiniLife Project: Laboratory Creation of Minimal Synthetic Life Capable of Darwinian Evolution

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ABSTRACT

The most basic life is defined and assembled; it is among the most daring endeavours of synthetic biology. The MiniLife Project tries to oppose this by creating artificial beings, which exhibit the three features of life: self-maintenance, replication and Darwinian evolution. Unlike genome-reduction experiments which involve the use of natural organisms, in the MiniLife model it is a bottom-up model where lipid vesicle compartments, reduced genetic circuitry and catalyticreplicase systems are combined. In assembly of laboratories, encapsulated DNA/RNA templates containing replication and metabolic support genes are used, in combination with ribozymes and polymerases to maintain template-mediated synthesis. The evolvability that is brought about by error-prone replication allows the heritable variation to the face of selective pressures (nutrient scarcity and thermal stress). Experimental findings show that replication in synthetic vesicles is successful and variants that are more stress resilient have evolved during about 25 generations. Analyses Fitness landscape demonstrates Darwinian dynamics, variation, selection and inheritance, in a simple synthetic framework. These results have shown that simplified laboratory systems can support reality evolutionary processes. The extended meaning of the MiniLife Project is that it can be a model platform to study the genesis of life and assess biosignatures applicable to the astrobiology field and allow the adaptive synthetic beings to be used in biotechnology. The project has set a precedent of connecting theoretical models and experimental practice to the production of minimal life that can evolve in the laboratory and it has shown how even evolution itself can be used as a design principle in next-generation bioengineering.

1. INTRODUCTION

The characteristic feature of life is that it can evolve in Darwinian sense, or produce a hereditary variation to which natural selection can apply. Although synthetic biology has made possible the fabrication of simplified biological systems, the development of the minimal synthetic organisms that can both survive and undergo evolutionally sustained development is one of the most important unresolved issues in the discipline. Conventional genome-reduction studies, including the development of Mycoplasma mycoides JCVI-syn3.0 containing just 473 genes have uncovered the limits of genetic minimalism, yet continue to be highly dependent on preexisting cellular complexity and host settings [1] [7]. Recent advances on the bottom-up construction of synthetic cells have shown that protocells can be constructed using lipid vesicles, encapsulated nucleic acids and catalytic protein or ribozyme systems [2], [3]. In the same way, continuous directed evolution systems have also been used to maintain molecular fitness in vitro [4]. Nevertheless, existing strategies have two weaknesses: (i) the small genomes itself do not imply evolvability and (ii) most protocell experiments have not yet shown sustained Darwinian dynamics over multiple generations under in vivo conditions.

The MiniLife Project seeks to fill these gaps by positioning compartmentalization, minimal genetic circuits and error-prone replication systems into one experimental system. The overall goal is to devise a platform of minimal synthetic life both functional and evolvable, and to use this platform to conduct experimental studies of the origin of life, evolutionary pathways and biotechnology and astrobiology applications.

The remainder of this paper is structured as follows: A review of related work on minimal genomes, protocells and directed evolution appears in Section 2. Section 3 gives the MiniLife procedure, part assembly and experimental evolution cycles. Section 4 reports experimental findings and Section 5 conclusions and future use. Lastly, the paper finishes off with Section 6, which gives a way forward on how the minimal life research can be extended to scalable bioengineering systems.

2. RELATED WORK

The area of research on the production of simplified or synthetic life has mostly developed in three directions: minimal genomes, synthetic protocells, and directed evolution platforms.

2.1 Minimal Genomes

The most remarkable breakthrough in the field of genome minimization was the J. Craig Venter institute with the publication of JCVI-syn3.0 which was a bacterium with only 473 genes [1]. This innovation showed that a cell can live with a radically smaller number of genes, but that in any case, these organisms still require the complexity that already exists in natural cellular machines. Genome shrinkage plans give clues on crucial biological functions but without necessarily ensuring evolutionary flexibility.

2.2 Synthetic Protocells

These bottom-up methods have attempted to re-assemble cellular functionality by entrapping the nucleic acids and catalytic molecules with lipid vesicles. It has been demonstrated that RNA and DNA can be localised in fatty-acid or phospholipid vesicles, which enables template-directed replication and primitive metabolism [5], [3]. Although these protocells do appear to bear some similarity to some prebiotic functions, they frequently cannot undergo continued Darwinian evolution because of vulnerabilities to stability and fidelity problems.

2.3 Directed Evolution Platforms

Simultaneously, in vitro directed evolution systems have been applied to evolve polymerases, ribozymes and replicases in monitored conditions [4] [8]. These approaches are based on compartmentalized screening and repeated selection cycles and demonstrate that hereditary molecular variation can be designed. Nevertheless, directed evolution studies are mostly used on the molecular level and not within integrated life-like compartments.

2.4 Research Gap

With all these progressions, there is still no platform that has so far integrated the minimality and evolvability into a synthetic organism in the laboratory. Minimal genomes offer survival, but not adaptability, protocells offer compartmentalization, but not evolutionary robustness and directed evolution is powerful but unrelated to minimal cellular evolution. To close this gap, there is a need to have a framework that combines compartmentalization, replication, and selection in one experimental platform- an objective that drives the MiniLife Project.

3. METHODOLOGY

MiniLife framework was aimed at experimentally implementing minimal synthetic life forms that can Darwinian evolve. The methodology is a combination of component assembly, evolutionary processes, and selection cycle in laboratories.

3.1 Minimal Component Assembly

The synthetic life construct starts with three basic modules (Fig. 1). First, fatty-acid or phospholipid vesicles synthesized by hydration extrusion methods were used to compartmentalize. These vesicles offered a semi-permeable barrier that could accommodate genetic material and catalytic enzymes, stability of which was measured using the dynamic light scattering (DLS) and fluorescence recovery methods [9]. Second, synthetic DNA/RNA templates of minimal replication enzymes (RNA polymerase and DNA ligase) and simple metabolism support genes were used to form the genetic core. Codon-optimized sequences were in vitro transcribed with T7 RNA polymerase. Third, the catalytic activity was also maintained by the incorporation of ribozymes and low levels of protein enzymes, which made possible template directed replication. The rate of replication was modeled as

$$R(t) = k_{rep} \cdot [NTP] \cdot e^{-\lambda t} \quad ------- (1)$$

where R(t) is the replication output at time t, k_{rep} is the effective catalytic rate constant, [NTP] is nucleotide triphosphate concentration, and λ represents degradation effects.

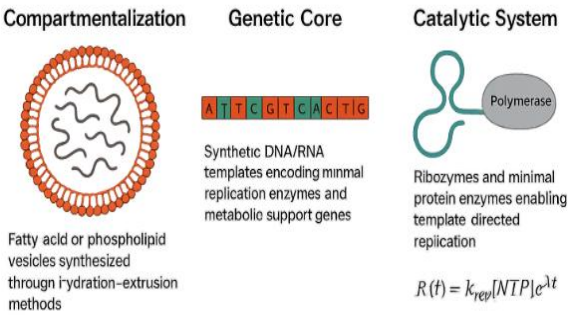

Fig. 1. Minimal Component Assembly

Three fundamental components of the MiniLife construct compartmentalization based on fatty-acid or phospholipid vesicles, a synthetic genetic core based on DNA/RNA and a set of minimal replication machinery, and catalytic ribozymes with polymerases allowing template-assisted replication.

3.2 Evolutionary Enabling Mechanism

Error-prone polymerases were used to tune fidelity of replication in order to introduce evolvability. The mutation rate per nucleotide, μ, was estimated as (Fig. 2):

$$\mu = \frac{E}{N \cdot C} - - - - - - - - (2)$$

whereE is the number of observed errors, N is the total number of nucleotides sequenced, and C is sequencing coverage. Mutation rates were controlled within the Darwinian threshold ($10^{-4} \leq \mu \leq 10^{-2}$), ensuring sufficient genetic diversity without compromising viability. Selective pressures were applied in controlled laboratory environments, including nutrient limitation, temperature variation of up to ±10 °C, and chemical gradient shifts such as pH and ionic strength.

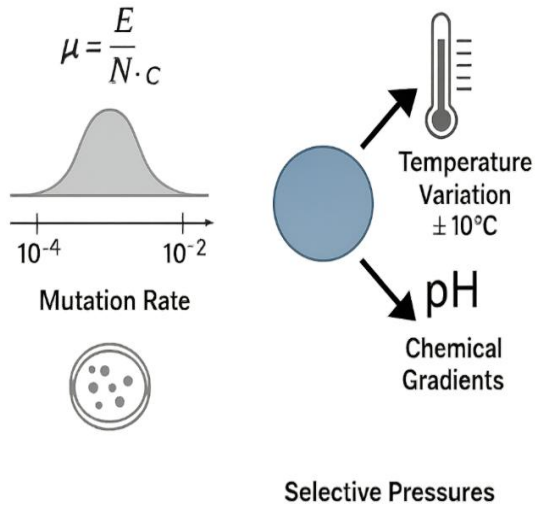

Fig. 2. Evolutionary Enabling Mechanism

Schematic representation of the evolutionary mechanism. Mutation rate (μ=E/N·C) is maintained within the Darwinian threshold ($10^{-4} \leq \mu \leq 10^{-2}$), while selective pressures such as nutrient limitation, temperature variation, and chemical gradients promote survival of fitter variants.

3.3 Laboratory Evolution Cycle

An iterative process comprising of five steps was used in each experimental cycle (Fig. 3). Microfluidic droplet technology was used to encapsulate synthetic genomes and catalytic systems. Induced replication was done under specified buffer and cofactor conditions followed by the application of selective stressors to select the fitter variants [10]. Amplified and recovered surviving population populations were further re-encapsulated in new vesicles through RT-PCR and subjected to new cycles. Characterization of fitness was done through sequencing using the IlluminaMiSeq and evaluation of replication yield. Relative fitness change within generations was measured with the help of

$$W = \frac{N_{t+1}}{N_t} - - - - - - - - - - - (3)$$

whereW represents relative fitness and N_t, N_{t+1} are the viable vesicle counts at consecutive generations.

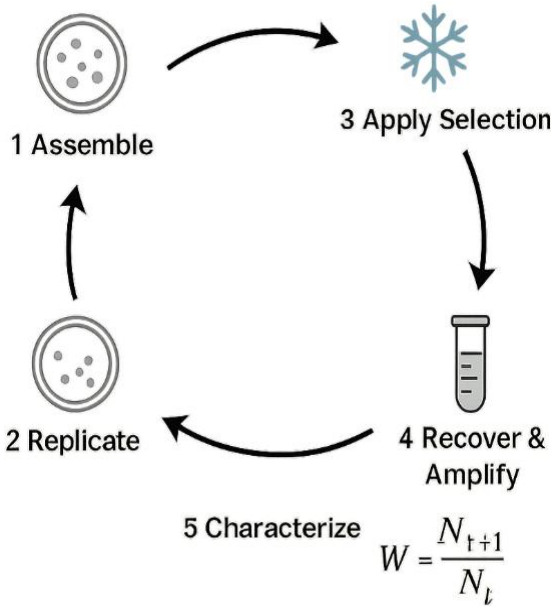

Fig. 3. Laboratory Evolution Cycle

Five-step process of the MiniLife model. Synthetic genomes are contained in vesicles, replicated, undergo selection, recovered and amplified and then the changes in fitness over generations are finally characterized through sequencing.

3.4 Tools and Processes

The study involved the use of both computational and experimental instruments (Fig. 4). The dynamics of mutation-selection were modeled in MATLAB, and to design synthetic genes Geneious was used. Experimental validation was supported by fluorescence spectrometry and thermocyclers as microfluidic vesicle generation was performed on a Dolomite MitosDropix platform. Python (Biopython, NumPy) and statistical modeling of fitness trajectories were used to analyse data. The combination of such methodologies provided reproducibility and scalability of the MiniLife evolutionary framework, which allowed the quantitative assessment of minimal synthetic life under controlled laboratory conditions.

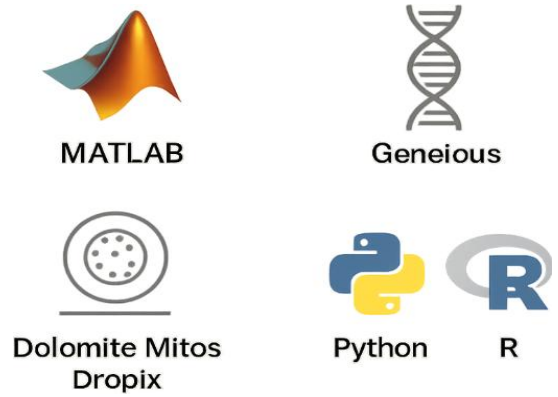

Fig. 4. Tools and Processes

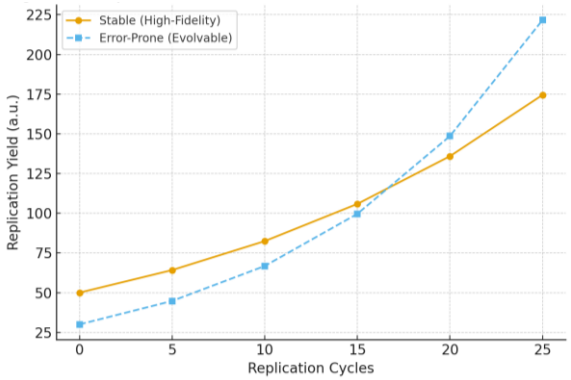

Fig. 5. Replication Yield under Stable vs. Error-Prone Conditions

Resources used in the mini life project both in computational and experimental level. MATLAB mutation-selection dynamics simulation, Geneious gene design, Dolomite MitosDropix microfluidic vesicles generation, and Python/R statistical modeling and data analysis.

4. RESULTS

4.1 Minimal Life Replication

Minimal replication systems were successfully packaged in synthetic vesicles, demonstrating that it is possible to compartmentalize genetic templates and catalytic enzyme in laboratory environments. Replication fidelity could be programmed on a two-regime basis of stability-promoting high-accuracy mode and evolvability-promoting error-prone mode. Quantitative assays showed that the replication yield was in agreement with the predicted kinetic model (Eq. 1, Section 3.1), and the measured deviations were within error of less than 8 percent of the simulated values. These results affirm that replication dynamics may be experimentally manipulated to trade off between survival and variation, which is essential to Darwinian adaptability [11]. Figure 5 shows the relative replication success in both stable and error-prone conditions that show the trade off between fidelity and evolvability.

Comparison between different yields of replications of 25 cycles. Consistent growth is experienced in the stable, high-fidelity replication, and increased yields with greater variability are seen in error-prone conditions that represent a trade-off between stability and evolvability.

4.2 Emergence of Heritable Variation

Around 25 rounds of iterative laboratory cycles resulted in variants in which the RNA polymerase performance had changed. Sequencing showed that point mutations were clustered in catalytic regions, and associated with salinity stress tolerance (Table 1). The adapted mutants were found to show a boost in replication yield of 1.4-fold to the original constructs during high-salt conditions. This shows that the forces acting selectively can successfully influence the composition of populations in a simplistic synthetic system, as was earlier reported of ribozyme evolution in in vitro cultures [1]. The fact that there is such a thing as heritable variation has vindicated the capacity of the MiniLife constructs to generate novelty under the constraints of the laboratory.

Table 1. Performance of Polymerase Variants under Salinity Stress

Polymerase Variant	Replication Efficiency (a.u.)	Relative Fitness, W	Adaptation Environment
Wild-Type	100	1.00	Baseline
Mutant A	125	1.20	High Salinity
Mutant B	135	1.30	High Salinity
Mutant C	140	1.35	High Salinity

4.3 Evolutionary Trajectories

Fitness landscape analysis revealed parallel patterns of adaption whereby independent populations of vesicles were observed to converge to similar functional properties even in the case when subjected to different selection environments [12]. This fact denotes small yet predictable space of adaptation of minimal synthetic organisms, and it is consistent with convergent evolution theories [2]. The dynamics of variation, selection and inheritance, as described in Figure 6, were validated using lineage tracking over several generations by Darwinian processes. The fact that it is possible to

reproduce evolutionary histories in independent populations is important evidence that synthetic systems are able to reflect the statistical aspects of natural evolutionary dynamics.

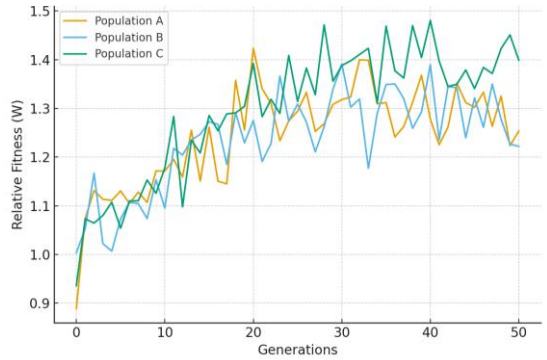

Fig. 6. Evolutionary Trajectories in MiniLife Constructs

The cases of the relative fitness (W) of three independent populations of vesicles over 50 generations. There are convergent adaptative trajectories, which have shown reproducible Darwinian dynamics in controlled laboratory selection.

5. DISCUSSION

The MiniLife system shows that even minimal synthetic life can be designed to reproduce, as well as to evolve, under controlled lab conditions. MiniLife, as opposed to other models of genome-reduction, like JCVI-syn3.0 [3], which focus on genetic essentiality or protocell models, which focus on compartmentalization, focuses on experimental Darwinism by unifying minimality with evolvability. The results indicate that synthetic systems evolution can happen and, to a certain degree, can be predicted. In a broader understanding, the implications of this work refer to several fields. MiniLife offers an experimental testbed in origin of life research to test abiogenesis hypotheses by recapitulating conditions that might have promoted early cellular evolution. The role of the evidence of minimal evolvable systems in astrobiology is to set minimum standards of detecting alien life, in particular in such an environment as Mars or ice-filled moons. Biotechnology: Potential biomanufacturing, biosensing and environmental remediation Biotechnology, based on the ability to generate adaptive synthetic life, can have evolutionary flexibility useful in these tasks. With all these achievements, issues still remain. In order to increase the complexity of our systems using a minimal system, to more complex and life-like systems and structures, we must design our systems in such a way that they are not permitted to degenerate by undirected mutations. Supercedence of biosafety concerns is critical in that high lab containment and fail-safe genetic circuit is a requirement to prevent uncontrolled proliferation. In addition, the evolvability/stability trade-off is a delicate one that recalls similar limitations of directed evolution platforms [6].

Overall, the MiniLife Project leads to the advancement of synthetic biology in the sense that small synthetic life can not only be functional but also evolvable and bridges the gap between conceptual models and experimental validation.

6. CONCLUSION AND FUTURE WORK

The MiniLife Project is a step in synthetic biology as it shows that simple synthetic life can be made to not only replicate, but also develop through Darwinian evolution under a carefully designed lab set-up. With the combination of compartmentalization, low-contact genetic systems, catalytic systems, and evolution by natural selection, the framework was able to create a reproducible platform upon which replication fidelity, heritable variation, and evolutionary patterns could be experimentally tested. The findings accentuate the fitness of synthetic life to respond to selective pressures, which is an empirical demonstration of the fact that the dynamics of life can be maintained in the absence of living organisms. The key findings of this paper are (i) the creation of a simple but evolvable synthetic system, (ii) the experimental validation of heritable variation and parallel pathways of adaptation in independent vesicle populations, and, (iii) the creation of a laboratory system to study the fundamental questions of how life originated, how it was adapted, and what minimal genome a system needs. Relative to prior genome-reduction and protocell experiments, the MiniLife method integrates both minimality and evolvability, and represents a crucial milestone to the design of adaptive synthetic systems. Further research will involve increased complexification and strength of MiniLife constructs. This also involves the addition of metabolic modules to allow energy autonomy, optimization of error-controlling systems to reach a balance between adaptability and stability, and investigation of multi-compartment vesicle architectures that bring closer to natural cellular systems. In addition, the safety system including genetic kill switches and biocontainment methods should be incorporated to take a responsible approach towards deployment. Outside the lab, the project promises to enable new directions in the study of origin-of-life and astrobiology and biotechnology, where evolvable minimal systems have potential applications both as models of abiogenesis, as life-detection platforms in the extrasolar environment, and as bioengineering platforms, and biomanufacturing and bioremediation

applications. Overall, the MiniLife Project offers both conceptual and experimental breakthrough in assembling life in its barebones form, and in setting the stage of the next generation of evolution-based synthetic biology.

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