Mutation Rate as a Selection Mechanism in Continuous Evolution of Bacteriophage

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Abstract

Engineering novel proteins via continuous evolution of bacteriophage currently requires the host bacteria to be transformed with two additional functions: A mutagenesis vector to provide an elevated rate of viral mutation, and a selection mechanism to increase the number of infectious progeny for the individual encoding the improved product [1]. I propose using a mutagenesis suppressor as the selection mechanism whereby the desired activity results in a higher number of clones, thus rewarding the genotype with more faithful progeny.

Introduction

Continuous evolution of bacteriophage is becomming a potent protein engineering tool [1] [4]. For example, PACE - Phage Assisted Continuous Evolution described by Esvelt,Carlson and Lui. Continuous evolution rapidly produces a viral genome containing a gene which has undergone many generations of mutation and selection for a particular property. The generality of this approach to protein engineering is limited by our ability to insert an expressable initial gene into the phage and create a selection mechanism for the desired activity.

Specifically, the PACE system requires:

- A modified viral genome replacing a crucial phage gene with a gene to be evolved.
- A transformed host with inducible mutagenesis.
- A host plasmid containing a selection mechanism to provide the crucial phage gene in proportion to the desired activity of the evolving gene.

1 M-Selection

Extremely high in vivo mutation rates are now possible [2]. With M-selection, these high rates will correspond to little or none of the desired activity, these lines of descent will be subject to catastrophic levels of mutation and low probabilities of faithful reproduction. The modulation of mutation rate within each host cell means that one of these wildly mutating strains could find an environment for faithful reproduction if it should stumble upon a promising mutation in the evolving gene.

The percentage of phage progeny which are exact copies of the parent is given by the Poisson distribution where

$$\lambda$$

is the mutation rate per virion (mutation rate/base * 6000bp/ genome). The probability of a virion with zero mutations is:

$$\mathrm{P}\,(0) = \frac{e^{-\lambda}\lambda^{0}}{0!} \tag{1}$$

Taking a phage production average of 100/hour for normal M13 infection requires us to have a clone rate of at least 2%: Two faithful copies per infection in order to avoid washout. A slower flow rate would lower this minimum fraction.

M-selection may allow for, or even require a flow rate which changes over the course of the experiment. A ramping up of the flow rate would be appropriate

PACE is designed to keep the transit time of host cells through the lagoon to be on the order of one generation. However, at very high levels of mutation it seems unlikely that multiple generations of viable E. coli mutants can be produced.

If the desired activity of the evolving protein produces a mutagenesis repressor, improvements in the desired activity will result in a higher percentage of faithful copies of the parent genome in the succeeding generation.

As the evolving protein approaches a high level of desired activity, the proportionally lowered mutation rate will ensure that more viral progeny contain exact copies of the parent genotype while a baseline mutation rate ensures that our mechanism to lower the mutation rate will not result in stagnation.

In PACE, the number of infectious progeny is proportional to the activity of the selection mechanism. In principle, if the current mutation of the gene produces a product with none of the desired activity, none of the infectivity protein will be produced by the host, and there will be no infectous progeny. This process involves a continuous flow of host cells through a reaction vessel and so any genotype producing less than two infectious virions per host will be washed out.

The benefits of M-Selection may include:

The virus is not crippled, that is, no critical genes need to be removed from the virion, and no phage genes are required in a host plasmid. This avoids the problem associated with the presence of gIII in the host and improves our ability to propagate phage prior to the evolution experiment. Of course, care must be taken to ensure that the evolving gene is not discarded before the experiment begins. However, M13 with one additional gene(domain) is exactly what has been perfected in phage display.

The nominal (high) mutation rate can be chosen to guarantee that there will be a minimum number of non-mutant copies produced for every infection. This percentage of clones will then increase as the mutation rate is reduced.

As far as we know, modulation of the mutation rate within a particular host has not been exploited in this way. We refer to this as M-selection, wherein the selection mechanism consists simply of reducing the rate of mutation.

Figure 1 shows how the combined mutagenic and selection plasmid takes the place of the AP and MP in PACE.

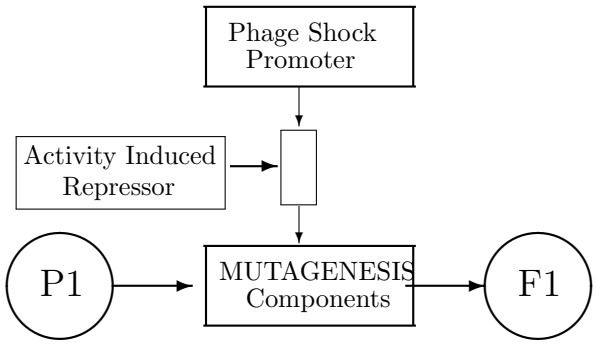

Fig 1. M-selection Plasmid

2 Comparison with Natural Selection

Selection pressure for an optimal mutation rate has its own natural history [3] although for settled phyla we see a certain natural mutation rates. Bacteriophage and multicellular eukaryote mutation rates are higher than bacteria and single cell eukaryotes (around 0.003).

A selection mechanism for lowered mutation rate would not seem to correspond to anything in the natural world. Natural selection is largely about responses to the external environment, which we control closely in laboratory evolution. Perhaps the closest parallel is found in species which can reproduce by asexual or sexual reproduction and change the rates between the alternatives as a way of accessing more diversity through sexual reproduction or producing more clones via asexual reproduction.

Materials and Methods

Population Dyamics of Bacteriophage

Clones versus Mutants

Although it may sounds like the title of a recent movie, this precisely states the selection mechanism of of mutation.

Percentage of activated genes due to Phage Shock promoter. Number of polIII- (mutagenic polymerase) transcripts times error rate due to mutagenic polymerase.

Error rate of normal polII, polIII

Number of phage particles produced np(0-6 min) = 0 (six minutes before any phage appear), then production between 75-200 per hour.

Initial single-strand (virion) DNA production occurs in non-mutagenic environment, then mutants appear along with polII- production, until suppression of polII- occurs due to binding. Binding ? anti-sense RNA transcription ? polII- repression ? reduction in mutations.

Results

Comparison with Sexual/Asexual Reproduction

Sexual reproduction is not the same as an elevated mutation rate, but it shares the characteristic that even a perfectly fit individual will not be producing faithful copied of its genome. For organisms with access to both forms, the percentage of offspring produced by asexual reproduction would be comparable to our percentage of true copies.

Yeast

Although yeast has access to both sexual and asexual reproduction, the (time) cost of sexual reproduction is high and it is generally invoked under conditions of stress, where an increase in genetic variety of offspring.

Daphnia

In Daphnia, longevity and healthy survival to the end of the season result in so called 'winter eggs' which reproduce asexually, providing a percentage of offspring which are clones of the parent. The cost differential between sexual and asexual reproduction is low and each generation represents a mix of both.

1. react
2. diffuse free particles
3. increment time by dt and go to 1

Using RNA Antisense.

The components for the broad spectrum, high level mutagenisis described in Badran and Liu [2] are variations (often single mutation variants) of the functional domains of high fidelity DNA replication and as such are not suitable targets for RNA anti-sense interference. Any such anti-sense interference would be likely to affect their normal counterparts which are needed for faithful reproduction.

RNA anti-sense of gIV could be utilized to block the primary phage shock promotor which was the primary trigger activating the high level of mutation in the lagoon.

Lowering the mutation rate can be achieved in a number of ways.

- Supress pIV production or otherwise block phage shock promotor
- Selectively block downstream components of mutagenesis
- Enhance production of wild-type high-fidelity replication components.

Discussion

Modeling needs work.

Conclusion

I think this will work.

Supporting Information

S1 Fig. Bold the title sentence. Add descriptive text after the title of the item (optional).

S1 Appendix. Algorithm. The code goes here.

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Acknowledgments

So many people to thank.

References

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