Emergence of RNA Editing in an Evolution Experiment

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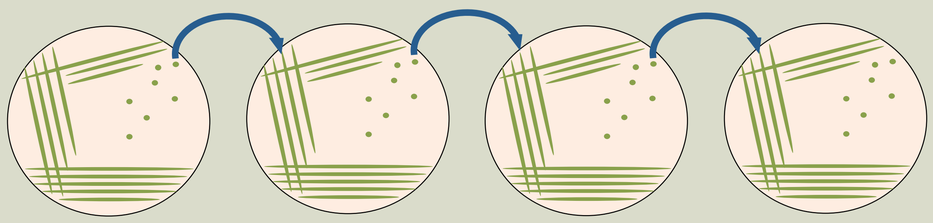
RNA editing has evolved multiple times independently, and some have suggested that it may have been a feature of early low fidelity biological systems. Covello and Gray[1] proposed a 3-step model for the evolution of RNA editing. However, this model has yet to be tested experimentally. In this model, RNA editing activity pre-exists but there is no substrate for it to act upon. Subsequently, mutation creates editable nucleotide sites, which may be fixed by genetic drift. RNA editing is now required for function, and thus becomes indispensable for gene expression. We sought to test this model by asking whether slippage-type editing can evolve under lab conditions favoring genetic drift. Our previous work on *Buchnera*, an endosymbiont of aphids, showed that RNA polymerase slips upon encountering poly(A/T) tracts, leading to stochastic incorporation or removal of As or Us in the nascent messenger RNA. This results in a heterogeneous population of mRNAs[2]. Slippage-type editing can restore the open reading frame at the mRNA level where frameshift mutations have been acquired at the DNA level. This in turn leads to the expression of functional proteins but may also reduce expression efficiency of in-frame genes[2]. To establish whether slippage-type editing emerges under conditions of genetic drift, we propagated 10 *E. coli* lines through daily single-cell bottlenecks. After 50 days, one line showed a decrease in growth rate and genome sequencing revealed the emergence of 38 frameshift mutations that, appear as pseudogenes. To our knowledge, this is the first demonstration of the emergence of RNA editing process under laboratory conditions. Our results are consistent with the constructive neutral evolution model of Covello & Gray. Furthermore, our results indicate that under conditions favouring genetic drift, editing may readily emerge. This in turn suggests that RNA editing could well have been a feature of early biological systems.

Question

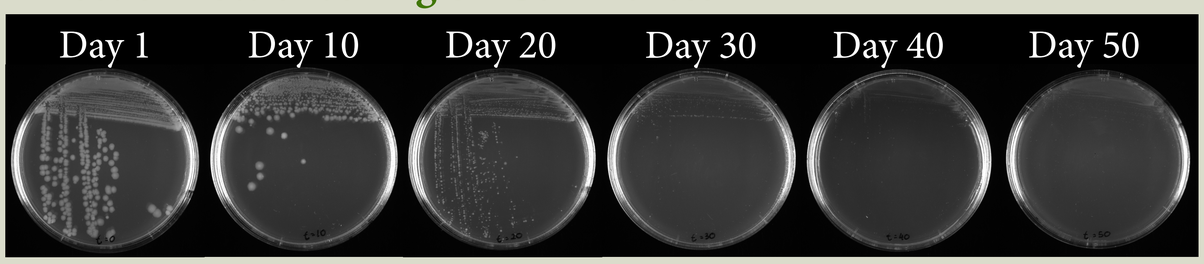
Does slippage-type editing evolve under lab conditions favoring genetic drift?

Single-Cell Bottleneck

Ten independent *E. coli* lines bearing a *mutD5* mutator allele were subjected to daily single-cell bottlenecks



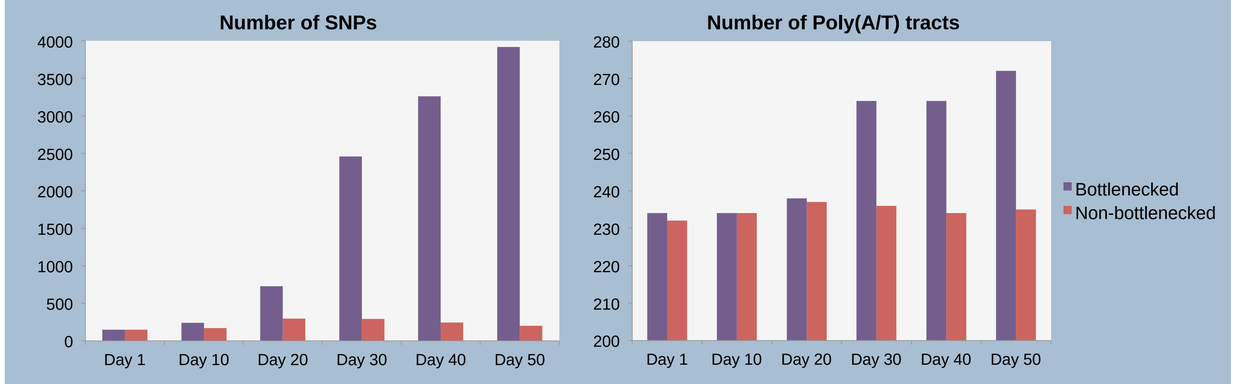
Effect of Bottlenecking on Cell Fitness



- One of the 10 independent lines subjected to continual bottlenecking showed a severe decrease in fitness
- A decrease in fitness is one of the effects of Muller's ratchet; an irreversible process of the accumulation of slightly deleterious mutations[3]

Whole Genome Sequencing

Whole genome sequencing (Illumina's HiSeq) was carried out at 10-day intervals for a single lineage that showed a marked decrease in growth rate

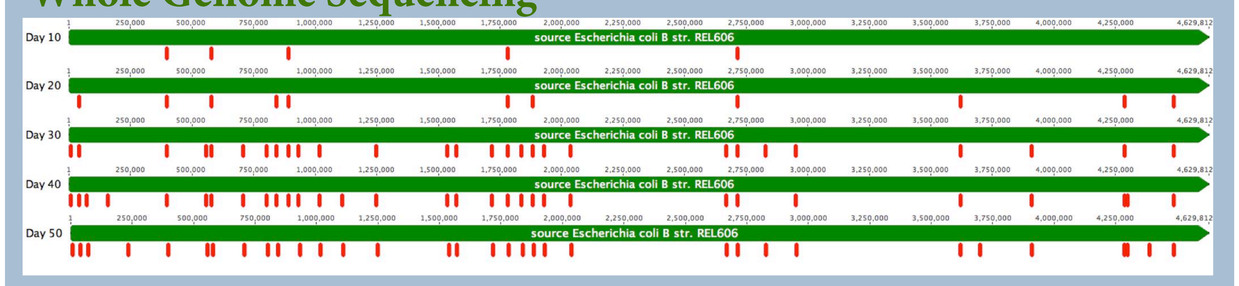


- Increase in number of SNPs and poly (A/T) tracts in the bottlenecked population

References

[1] Covello, P.S. & Gray, M. W. (1993) On the evolution of RNA editing. *Trends Genet.*, 9(8), 265-268. **[2]** Tamas, I. *et al.* (2008) Endosymbiont gene functions impaired and rescued by polymerase infidelity at poly(A) tracts. *Proc. Natl. Acad. Sci.*, 105**,** 14934-9. **[3]** Andersson, D. I. & Hughes, D. Muller's ratchet decreases fitness of a DNA-based microbe. *Proc. Natl. Acad. Sci. U. S. A.* 93**,** 906–907 (1996).

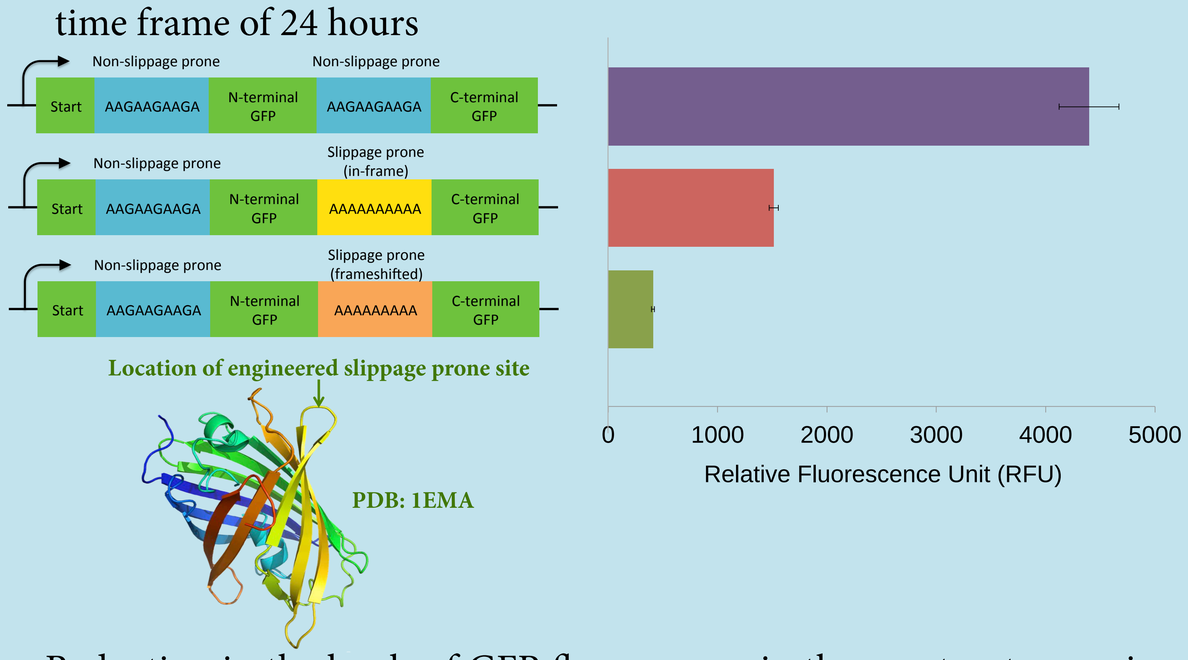
Whole Genome Sequencing



- Emergence of frameshifted poly(A/T) tracts was observed in one of the ten lines after 50 days of bottlenecking, as indicated above by the vertical red lines relative to position in REL606 genome

Using a GFP Reporter to Quantify the Effect of Slippage on Protein Synthesis

- Slippage-prone poly(A) tracts were inserted into the GFP gene at a point corresponding to a loop in the protein
- The levels of relative fluorescence unit (RFU) were measured across a time frame of 24 hours



- Reduction in the levels of GFP fluorescence in the constructs carrying slippage-prone poly(A) tracts

Conclusions

1. Emergence of slippage-type editing machinery	*Ancestral (no slippage)*	AAA AAG AAA AGT T K K K S
2. Mutation at the editable site and fixation by ***genetic drift***	*G deletion (slippage site created)*	AAA AAA AAA GTT K K K V
3. Slippage-type editing is ***indispensible*** and maintained by selection	*Slippage-type editing (A addition)*	AAA AAA AAA AGT T K K K S

- Emergence of slippage-type editing is consistent with Covello and Gray's model for the origin of RNA editing[1]
- Preliminary results show that frameshifted poly(A/T) tracts require slippage-type editing to produce full length proteins (in progress)
- GFP expression is reduced, but is not completely eliminated