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Mini Review

Spontaneous mutation rates come into focus in *Escherichia coli*

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ABSTRACT

Although the long-term outcome of mutagenesis is evolution by natural selection, it can also have profound immediate effects even on the level of individual organisms. In humans, the accumulation of mutations can cause many types of cancer; in bacteria, mutations can lead to dangerous antibiotic resistance and other phenotypic changes; and in viruses, mutations can cause drastic changes in the pathogenesis or modes of transfer. For these reasons, among others, a thorough understanding of mutagenesis is extremely important. One of the fundamental properties of the mutagenesis is its rate—the probability of a mutation occurring within a defined time frame. Despite the lengthy history of studies on mutagenesis and mutation rates, new and exciting findings continue to emerge. This review briefly summarizes the state-of-the-art in mutation rate analysis and continues with a discussion of some recent compelling discoveries on the mutational topology of the *E. coli* chromosome.

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1. Introduction

Mutagenesis is a double-edged sword—at the same time that it provides the fuel for evolution by natural selection, it can also have deleterious effects on genome stability that can reduce fitness, promote tumorigenesis and cancer formation, or cause heritable genetic disorders. Some of these issues have long been appreciated, making mutagenesis an important research topic since early in the development of modern genetics as a discipline. In his 1928 paper "The measurement of gene mutation rate in *Drosophila*, its high variability, and its dependence upon temperature" [1], Nobel laureate Hermann Joseph Muller framed one of the central challenges in the study of spontaneous mutagenesis and the empirical measurement of mutation rates: that because mutagenesis is such a rare process under normal conditions, innovative and exceedingly sensitive techniques would be required to generate data leading to interpretable "positive knowledge" (i.e. non-negative results) above the level of experimental error. In this seminal paper, Muller went on to describe his method of "balanced lethals,"

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Table 1
Examples of spontaneous mutation rates calculated by different methods.

Mutation rate (per base pair per generation $\times 10^{-10}$)	Mutation rate (per genome per generation $\times 10^{-3}$)	Mutational target	Method	Reference
4.10	1.90	*lacI*	Fluctuation analysis	[4]
6.90	3.30	*lacI*	Fluctuation analysis	[4]
5.10	2.40	*hisGDCBHAFE*	Fluctuation analysis	[4]
0.45	0.1–0.2	–	Comparative genomics	[17]
7.90	3.70	*lacI*	Fluctuation analysis	[49]
0.89	0.41	–	Mutation accumulation with WGS	[2]
2.20	1.00	–	Mutation accumulation with WGS	[3]
0.33	0.15	Rifampicin resistance	Fluctuation analysis	[3]
0.21	0.10	Nalidixic acid resistance	Fluctuation analysis	[3]
1.99	0.92	–	Mutation accumulation with WGS	[3]

a genetic approach that allowed rare recessive mutations that accumulated over several generations to be detected. While he succeeded in experimentally calculating a spontaneous mutation frequency, even under apparently controlled laboratory conditions, repeated experimentation resulted in frequencies that varied by an order of magnitude due to unknown causes. Nevertheless, in light of the contemporary state of the art, Muller's work represented a major technical and scientific breakthrough that he rightly believed would (in his own words) "open up a new field of genetics—the quantitative study of gene mutation, as occurring throughout one or more entire chromosomes under purposely varied conditions." In the decades since the publication of this study the pursuit of accurate spontaneous mutation rates has blossomed into an important, multi-faceted, and sometimes controversial, area of research.

Many techniques have been devised to increase the accuracy and reproducibility of spontaneous mutation rate measurements in several model organisms. Because of early advances in the development of *Escherichia coli* as a model system for molecular genetic analysis, it became a favorite for studying mutagenesis and has yielded the richest collection of literature on the topic. The primary aim of this review is to summarize several notable and related recent studies from three different laboratories that provide new estimates for the *E. coli* mutation rate using current techniques [1–3]. Two of these studies, both from Patricia Foster's laboratory, have revealed especially compelling insight into the mutational topology of the *E. coli* chromosome and are discussed in further detail. To put these new studies in context, the review begins with a brief update on the standard repertoire of techniques for measuring mutation rates in *E. coli*, especially focusing on the strengths and weaknesses of each technique and their underlying assumptions.

2. Techniques for mutation rate analysis

Three approaches for measuring spontaneous mutation rates have taken particularly strong footholds in the literature: fluctuation analysis, mutation accumulation experiments, and, to a lesser extent, comparative genomics. All of these approaches have been used to measure spontaneous mutation rates in *E. coli*, but have yielded values that vary by more than an order of magnitude (see Table 1 and discussions in [1,4–6]). To begin to understand possible reasons for this wide variation, the technical and theoretical frameworks supporting each approach must be appreciated.

2.1. Estimation of mutation rates by fluctuation analysis

Salvador Luria and Max Delbrück reported results from their fluctuation experiments on the nature of mutagenesis in 1943 [7], laying not only an essential conceptual foundation for our understanding of mutagenesis in general, but also providing an effective technical methodology for estimating mutation rates. When used with mathematical approaches developed soon after by Lea and Coulson [8], a revolutionary feature of the Luria–Delbrück fluctuation experiment was that it could be used to calculate mutation rates based on extremely rare mutational events, thus providing a major leg up in overcoming this major challenge noted by Muller 15 years earlier.

While fluctuation experiments can be very laborious in practice, the basic experimental design is quite simple [9]. In a typical experiment, a small number of cells are inoculated into many parallel liquid cultures that are then grown to saturation. Under optimal experimental conditions, Kendal and Frost [10] estimated that about 40 cultures are sufficient (see [11] for further discussion of this point). Next, the cultures are plated on appropriate selective media to detect mutants that arose during the culturing and a few cultures are plated on non-selective medium to determine final number of cells. At the end of the experiment, the number of mutant cells in each culture depends on both the mutation frequency and the timing of when the original mutational event occurred during the growth of the culture—cultures in which a mutation occurred earlier will contain many more mutant cells than those with later a mutational event. The mutation rate can then be calculated by analyzing the distribution of the final number of mutant cells present in the different cultures.

The important feature of the Lea–Coulson method is that it can distinguish the number of mutational events from the number of mutants detected at the end of the experiment; however, the applicability of the underlying mathematical theories requires that several critical assumptions about the experimental conditions hold true:

i. The cells must grow exponentially.
ii. The mutation rate must be constant during the lifetime of a cell.
iii. A mutational event cannot affect the mutation rate.
iv. The growth rate of mutant cells must be the same as non-mutant cells.
v. Mutant cells must not die during the course of the experiment.
vi. Forward mutations must be the dominant mutational event and the rate of reversion mutations must be so low that it is negligible.
vii. Once the cells have been plated on a selective medium, no new mutations can arise. Although pathways leading to increased mutation rates under non-lethal selection have been described (reviewed in [4–6,12]), lethal selection methods can be used to prevent even weak proliferation on the selective media to minimize this potential problem.
viii. In experiments using batch cultures, the number of the cells in the initial inoculum must be negligible compared to the number of cells at the end of culturing. The mathematical methods used to calculate the mutation rate are valid only when the initial number of cells is no more than 1/1000 of the final number of cells [7,13].

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