Transient Mutators: A Semiquantitative Analysis of the Influence of Translation and Transcription Errors on Mutation Rates

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ABSTRACT

A population of bacteria growing in a nonlimiting medium includes mutator bacteria and *transient mutators* defined as wild-type bacteria which, due to occasional transcription or translation errors, display a mutator phenotype. A semiquantitative theoretical analysis of the steady-state composition of an *Escherichia coli* population suggests that true strong genotypic mutators produce about 3×10^{-3} of the single mutations arising in the population, while transient mutators produce at least 10% of the single mutations and more than 95% of the simultaneous double mutations. Numbers of mismatch repair proteins inherited by the offspring, proportions of lethal mutations and mortality rates are among the main parameters that influence the steady-state composition of the population. These results have implications for the experimental manipulation of mutation rates and the evolutionary fixation of frequent but nearly neutral mutations (*e.g.*, synonymous codon substitutions).

WHEN a bacterial mutant arises and is selected, did it originate from a standard wild-type bacterium or did it arise in a subpopulation with special properties? This question has been brought to the forefront of evolutionary thinking by the spectacular results of CAIRNS, OVERBAUGH and MILLER (1988) and HALL (1990) demonstrating that some mutations are "more frequent when advantageous than when neutral" (HALL 1990). My purpose here is not to present alternatives to published models (*e.g.*, STAHL 1988) but to clarify some quantitative aspects of mutagenesis, and in particular to examine whether point mutations are usually contributed by standard bacteria or by a special class of error-prone bacteria.

The bacterial types considered here are wild-type and genetically mutator bacteria as well as *transient* (or phenotypic) mutators: bacteria that are genetically wild type but, due to transcriptional or translational errors, have a reduced accuracy of replication or repair for one or two generations.

STEADY STATE BETWEEN WILD-TYPE AND MUTATOR BACTERIA

Let us consider a large population containing wild-type bacteria that produce mutations at a standard frequency f. The average number of mutations per replication of the entire genome is constant for a wide range of microorganisms, the best estimate being $f = 3 \times 10^{-3}$ (DRAKE 1991). Estimates of this and other parameters used in this study are shown in Table 1. The population may also contain antimutator bacteria (which we neglect), transient mutators (not considered until the next section) and true mutators, producing mutations at a frequency kf. Let y and $(1 - y)$ represent the fractions of mutator and wild-type bacteria in the population.

Mutators are constantly generated from wild-type bacteria. They are easily obtained, for instance, through mutations that inactivate key proteins in DNA repair systems. The key genes controlling the mutator genotypes occupy at least 10^{-3} of the genome, the MutH, MutL and MutS proteins alone having a cumulative molecular mass of 190 kDa corresponding to about 5000 nucleotides (LAHUE, AU and MODRICH 1989). In the standard genetic code, 3 out of 64 codons are nonsense codons. Therefore, nonsense mutations represent roughly 5% of all base pair substitutions. In addition, some amino acid substitutions may also be fatal to protein function. This fraction may vary from a few per cent in the case of β-galactosidase (LANGRIDGE 1974) to about 40% in the case of the β subunit of RNA polymerase (GLASS, NENE and HUNTER 1982). If we assume that 5% of amino acid substitutions inactivate a DNA repair protein, then a fraction $d = 10^{-4}$ of all base pair substitution mutations lead to the mutator genotype. In addition, other disruptive mutations, including frameshifts, deletions and insertions may generate mutators. In some cases, such mutations may represent more than half of the spontaneous mutations in a gene (SCHAAPER, DANFORTH and GLICKMAN 1986). On average, I believe they will represent about 30% of all mutations. In what follows, I shall for simplicity reason as though all mutations were base pair substitutions and take the $d = 10^{-4}$ to represent the fraction of all mutations leading to a mutator genotype.

TABLE 1

Estimated magnitudes of parameters

Symbol	Description	Approximate magnitude
d	Proportion of mutations to the mutator genotype	10^{-4}
e	DNA replication error rate per nucleotide before repair	10^{-5}
f	Mutations per genome replication in wild-type *E. coli*	3×10^{-3}
h	Fraction of lethal mutations	10^{-1}–10^{-2}
k	Enhancement of mutation rate in true or transient mutator	10^{2}–10^{4}
r	Proportion of mutations from mutator to wild-type	2×10^{-7}
y	Fraction of genotypically mutator cells	10^{-5}
None	Transcription error rate per nucleotide	10^{-5}
None	Translation error rate per amino acid	10^{-4}
None	Number of copies of limiting mismatch repair protein transmitted to daughter cells	5–7
None	Fraction of cells lacking mRNA suitable for the synthesis of a rare protein	10^{-2}
None	Proportion of wild-type *E. coli* without descent	10^{-3}

Suppressor mutations that restore the wild-type mutation frequency in mutator bacteria should be quite rare: most reversions will be true reversions. Therefore, the fraction r of mutations that restore the wild-type mutation frequency must be of the same magnitude as the reciprocal of the genome size: $r \approx 1/(5 \times 10^6)$.

In the absence of selection for or against mutators, the equilibrium between wild-type and mutator subpopulations implies that the fluxes from each subpopulation to the other are equal; hence

$$(1 - y)df = yrkf \quad (1)$$

$$y = d/(rk + d). \quad (2)$$

This equation cannot be satisfied with realistic values of the parameters. When a population is enriched in the mutator genotype, its mutation rate easily increases by a factor >100 (Cox 1976), implying that the mutator subpopulation was initially small ($y < 1/100$). With $d = 10^{-4}$ and $r = 1/(5 \times 10^6)$, we would have $k > 5 \times 10^4$. Each mutator bacterium would accumulate $fk > 150$ mutations per replication and there would be enormous lethality among mutators, contradicting the initial assumption of no selection against mutators.

Thus, the equilibrium between wild-type and strong mutators cannot be understood without explicit reference to the differential mortality of the subpopulations. Let us define h (in honor of Haldane) as the fraction of lethal mutations. We now view the steady-state level of mutator bacteria as dominated by two fluxes: the production of mutators from wild-type and the loss of mutators due to increased mortality by lethal mutations. Equating the two fluxes, we have

$$df(1 - y) = h(k - 1)fy. \quad (3)$$

Because $k - 1 \approx k$ and $d << hk$ (see below), one gets

$$yk \approx d/h. \quad (4)$$

This is a particularly powerful relationship: the fraction of mutations arising in a bacterial population contributed by its mutator subpopulation is equal to yk, hence to d/h. One can then formulate an elegant rule of thumb:

If, in a wild-type population, a fraction d of mutations leads to a strong mutator genotype, and a fraction h of mutations is lethal, then the steady state between the mutator and wild-type subpopulations is such that the fraction of mutations contributed by the mutators is of the order of d/h.

Let us try to estimate h. In a culture of *E. coli* cells growing without nutritional limitation, about one cell in a thousand has no progeny (Gallant and Palmer 1979). The standard mutation rate being 3×10^{-3}, h must be less than 1/3 and is probably considerably less. Knowing that, on average, more than 0.1 of the mutations occurring in a gene are likely to abolish its function, but that not all genes are essential, I consider 10^{-1} to be a reasonable estimate for h. However, even lower values have been proposed. In his theoretical study on the population dynamics of mutator and wild-type bacteria, Painter (1975) considers that $h = 10^{-2}$ is consistent with both experimental results and theoretical simulations. Chao and Cox (1983) estimated the loss of fitness to be about 2×10^{-3} in a mutator strain for which $k = 100$ (hence $fk = 0.3$ mutation/genome replication). This again agrees with $h = 10^{-2}$. To compromise between my own prejudice and the accepted value, I take $h = 3 \times 10^{-2}$. Then $d/h \approx 3 \times 10^{-3}$. Equation 4 implies that in the absence of selection for a particular mutation, the mutators normally present in a population (considered to be wild type) contribute $yk \approx 3 \times 10^{-3}$ of the mutations arising in the population (Table 2). With a lower value of h (10^{-2}), the mutator contribution would rise to 10^{-2} of the total.

For a more sophisticated treatment, one could introduce mutator subtypes with different k values. However, y and k intervene by their product in Equation 4, so that the conclusions are not very sensitive to the spread of k values. The DNA replication error rate e prior to mismatch repair is about 2×10^{-7} per nucleotide (Fersht and Knill-Jones 1983), corresponding to one error per DNA strand prior to mismatch repair. Then, if all post-replicative mismatch repair systems were disabled, mutations should increase by a factor $k = 300$. Disabling just one system would give $k < 300$. However, mutators with $k > 300$

TABLE 2

Proportions of mutations contributed by genotypic or transient mutators

Parameter	Wild type	Genotypic mutator ($k = 300$)	Transient mutator ($k = 300$)
Frequency of mutator	1	10^{-5}	5×10^{-4}
Relative contribution to single mutations	1	3×10^{-3}	0.15
Relative contribution to simultaneous double mutations	1	1	50
Relative contribution to successive double mutations	1	1	0.15

are known, and they are mostly due to error-prone replication. In *mutD5* strains deficient in *Pol*III proofreading, mutation rates are increased by a factor ranging from about 10^2 in poor medium to about 10^4 in rich medium (FOWLER, DEGNEN and COX 1974). The highest mutation rates are due to a secondary effect, saturation of the mismatch repair system (SCHAAPER and RADMAN 1989). Error rates are also increased by a factor >100 in the *dnaQ49* mutator (MARUYAMA *et al.* 1983). For simplicity, I shall use a standard value $k = 300$ for all mutators in the subsequent calculations. Then $yk \approx 3 \times 10^{-3}$ implies that $y = 10^{-5}$ and $yk^2 = 1$. Unselected double mutants would be produced in roughly equal numbers by the wild-type and the mutator subpopulations (Table 2).

About 1% of natural isolates of *E. coli* are strong mutators (JYSSUM 1960; GROSS and SIEGEL 1981; see also TRÖBNER and PIECHOCKI 1984). In the absence of selection, I have estimated above that mutators produce 3×10^{-3} of the mutations. After a round of strong selection for one particular mutation, I therefore expect to find 0.3% of mutator clones. Thus, the observed frequency of 1% suggests that the bacteria were recently selected for one mutation, and were perhaps under selective pressure for a second mutation. Alternatively, I may have underestimated d/h.

THE INCIDENCE OF TRANSLATION AND TRANSCRIPTION ERRORS

In vivo determinations of transcription errors converge around a value of 10^{-5} per nucleotide (ROSENBERGER and HILTON 1983; BLANK *et al.* 1986) producing, after translation, a background of amino acid incorporation errors around 2×10^{-5}. The translation error rate, reviewed by PARKER (1989), is thought to be 10^{-6} to 10^{-4} per codon (EDELMAN and GALLANT 1977) and could reach 10^{-3} at some loci (BOUADLOUN, DONNER and KURLAND 1983). A standard error rate of 10^{-4} per codon corresponds to an error rate of about 3% per polypeptide chain of 300 amino acid residues. A higher error rate of, say, 10% per protein is unlikely considering the quality of resolution achieved in two-dimensional protein gels (O'FARRELL 1978). A lower error rate is also unlikely: the existence of general high-fidelity ribosomal mutants (STRIGINI and GORINI 1970; BISWAS and GORINI 1972) indicates that translation accuracy is not limited by transcription accuracy, so that the frequency of true translation errors must be substantially higher than the background of 2×10^{-5} contributed by transcription errors.

The DNA-synthesis-error pathway: Normally in *E. coli* a single copy of *Pol*III replicates a complete DNA strand while another copy, associated with the first, replicates the other strand (*e.g.*, MCHENRY 1988). The proofreading activity of *Pol*III is carried by the ϵ subunit, which is about 200 amino acids long (SCHEUERMANN and ECHOLS 1984). Erroneous ϵ subunits may not always be incorporated into the holoenzyme, so let us assume that 1% of the *Pol*III holoenzymes carry an erroneous epsilon subunit. Let us further consider that a fraction between 10^{-2} and 10^{-1} (say 3×10^{-2}) of the erroneous but functional molecules of *Pol*III are strongly error-prone. Then the bacterial population contains 3×10^{-4} of transient mutators due to defective *Pol*III proofreading.

With $k = 300$, the contribution of such transient mutators to single mutations would be small (around 10%), but they would contribute 30 times more (simultaneous) double mutations than would the wild-type subpopulation. This DNA-synthesis-error pathway is mainly caused by translation errors but transcription errors also contribute inasmuch as they may be responsible for the (~20%) background of amino acid incorporation errors.

The DNA-repair-error pathway: Consider here the conjunction of two independent improbable events. First, due to defective transcription, the cell does not produce an mRNA molecule suitable for the synthesis of a rare DNA mismatch repair protein. Second, it does not inherit copies of that protein from the mother cell. I estimate the probability of each of these events to be around 1%, leading to a transient mutator subpopulation of about 10^{-4} compared to the wild type.

The *mutHLS* DNA repair system in *E. coli* is easily saturated, as though it could not repair more than a small (unknown) number of mismatches. MutL and to a lesser extent MutH are thought to be the limiting components (SCHAAPER and RADMAN 1989). Such a limited capacity had been envisaged following two theoretical arguments concerning mechanistic aspects of DNA scanning by the repair system and the risk of generating new errors after excising a nonmutant patch of DNA (NINIO 1987).

The mismatch repair system will usually detect the one or two errors per genome replication made by the polymerase and correct these, but it may also attack a number (m) of unmutated sequences and

excise a DNA patch of length l. Errors may be generated during resynthesis, to a level eml. The number of DNA repair components may be increased as long as eml remains substantially lower than f. Let us write $eml = f/3$. Then, for an average repair patch of 500 nucleotides, $m = 10$. There would be 10 unwarranted excision events for one mismatch correction, and the former would account for 1/3 of the errors remaining after mismatch correction. Having used about 10 components, the cell still has a few available components that may be transmitted to the offspring. The number, estimated by another optimization argument (not detailed here), could be around 5–7. Therefore, a daughter cell will fail to receive any copy of the limiting component with a probability around $2^{-6} \approx 2\%$. The number of copies of limiting repair proteins estimated here is consistent with preliminary experimental estimates of 10–30 (DAMAGNEZ, DOUTRIAUX and RADMAN 1989).

The absence of functional mRNA may be due to two causes. If just one mRNA copy is made, it may correspond to a nonfunctional protein due to a transcription error (probability ~0.5%). Alternatively, if a few mRNA copies are made following a Poisson distribution, there is a chance of making no copy. With an average copy number of 4, the Poisson probability is 2% that no copy is made.

Taking a 1% probability for the first event and a 1% probability for the second, we would have a transient DNA-repair-error mutator subpopulation of 10^{-4}, comparable in size to the DNA-synthesis-error mutator sub-population of 3×10^{-4}. This defective-DNA-repair pathway is mostly determined by transcription errors, but it also depends upon factors that influence the number of functional repair proteins and therefore upon growth rates and translation accuracy.

Because there are a few other routes by which translation and transcription errors may produce transient mutators, an aggregate size of 5×10^{-4} is by no means exaggerated.

DISCUSSION

One general difficulty encountered in this study lies in the fact that the values of some relevant parameters may vary by orders of magnitude from one experimental system to another. Each choice may be criticized. However, the selected parameters form a consistent set (Table 1) describing an ideal population of bacteria.

Under conditions of stress the values of many parameters may change, various error rates may rise due to substrate imbalances, and the transient mutator subpopulations might increase out of control (HOLLIDAY and ROSENBERGER 1988). However, I favor the view that error levels are connected by regulatory circuits. Strategies in molecular evolution may be conceived, in which translation and replication error rates are inversely related (NINIO 1986). There is experimental evidence for genetic linkage between genes affecting translation accuracy and those affecting DNA repair (CAILLET and DROOGMANS 1988; CONNOLLY and WINKLER 1989).

While high-accuracy translation mutants are easily found, high-accuracy replication mutants ("general antimutators") seem to be almost impossible to isolate (DRAKE 1990). One reason could be that mutation frequencies have a lower limit due to the background contributed by transient mutators. According to Table 2, there would still be some room for general antimutators. The difficulty in finding them suggests that our estimates rather lie on the conservative side. Within the range of known experimental uncertainties, our estimates for transcription and translation error rates may be revised upward by a factor of 2, but not much beyond that. Perhaps the construction of general antimutators will be easier in bacteria with high fidelities of translation, or will have to await the discovery of high-fidelity transcription mutants.

The predicted simultaneous double-mutation frequencies of Table 2 may be checked with mutants with reduced accuracies of translation (ROSSET and GORINI 1969) or transcription (BLANK *et al.* 1986; LIBBY and GALLANT 1991). Transcription errors mainly influence the DNA-repair-error pathway, generating transient mutators with moderate k values (<300). The effect of transcription errors may best be measured in artificial situations placing increased demand on repair systems, such as the presence of a nucleotide-analog mutagen.

Two lines of experimental studies would be particularly useful in the context of this discussion. One is related to the problem of how the cell regulates the production of proteins that are present in small numbers. Fluctuations in protein numbers do produce observable phenotypes (SPUDICH and KOSHLAND 1976) and sometimes create pseudo-genotypes (NOVICK and WEINER 1975); see BERG (1978) for theoretical estimates. Is randomness in numbers and randomness in partitioning among daughter cells the only rule? The second line of experiments is related to the precise definition and quantification of cell death. Translation errors must be an important cause of mortality because a moderate increase in translation errors (by a factor of 10 to 20) produces a similar increase in mortality (GALLANT and PALMER 1979). Beyond that, due to secondary effects, mortality grows faster than translation error rates (TAI, WALLACE and DAVIS 1978).

My analysis suggests that while single mutations are mostly contributed by the wild-type subpopulation, most simultaneous double mutations are provided by

transient mutators (Table 2). To be more rigorous, because there are cases where a unique replication mishap produces a pair of neighboring errors (DE BOER and RIPLEY 1984), I mean here by double mutations the occurrence of two independent mutational events. The double events are suggested to occur at a frequency substantially higher than the second power of the single mutation frequency. The simultaneous occurrence of two very improbable mutations, each occurring at a frequency of 10^{-10}, must be a rare event, requiring tons of bacteria. If, on the other hand, we are concerned with a class of events that can occur in many ways (for instance, the replacement of a codon by a synonymous codon), the standard single-mutation frequency is around $10^{-2} \times (3 \times 10^{-3}) = 3 \times 10^{-5}$. In a transient mutator, the frequency would rise to 10^{-2}. One gram of bacteria contains about 10^{12} cells and therefore about 5×10^{8} transient mutators (and, in the steady state, 50-fold fewer genotypic mutators). This population size is sufficient to generate cells that have simultaneously changed four codons into more advantageous synonymous codons. Such quadruple changes might confer a significant selective advantage.

Under conditions of intense selection, simultaneous double mutants can occur 10^{8}-fold more frequently than expected but not mediated by mutator mutations (HALL 1991). However, the accelerating mechanism remains unknown.

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