Origin of Mutations Under Selection: The Adaptive Mutation Controversy

John R. Roth,[1] Elisabeth Kugelberg,[1] Andrew B. Reams,[1] Eric Kofoid,[1] and Dan I. Andersson[2]

[1]Microbiology Section, Division of Biological Sciences, University of California, Davis, California 95616; email: jrroth@ucdavis.edu, elisabeth.kugelberg@gmail.com, abreams@ucdavis.edu, eckofoid@ucdavis.edu

[2]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala SE-751 23, Sweden; email: Dan.Andersson@imbim.uu.se

Annu. Rev. Microbiol. 2006. 60:477–501

First published online as a Review in Advance on June 8, 2006

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi:
10.1146/annurev.micro.60.080805.142045

0066-4227/06/1013-0477$20.00

Key Words

natural selection, cancer, genetic adaptation, gene amplification, gene duplication, stationary-phase mutagenesis

Abstract

Growth under selection causes new genotypes to predominate in a population. It is difficult to determine whether selection stimulates formation of new mutations or merely allows faster growth of mutants that arise independent of selection. In the practice of microbial genetics, selection is used to detect and enumerate pre-existing mutants; stringent conditions prevent growth of the parent and allow only the pre-existing mutants to grow. Used in this way, selection detects rare mutations that cause large, easily observable phenotypic changes. In natural populations, selection is imposed on growing cells and can detect the more common mutations that cause small growth improvements. As slighly improved clones expand, they can acquire additional mutational improvements. Selected sequential clonal expansions have huge power to produce new genotypes and have been suggested to underlie tumor progression. We suggest that the adaptive mutation controversy has persisted because the distinction between these two uses of selection has not been appreciated.

Contents

PERSPECTIVES

Have mechanisms evolved that allow cells to mutagenize their own genomes in response to selective stress? This question has remained unresolved since the time of Darwin, who suggested that environment might stimulate increases in variability (15). We know of no compelling experimental evidence for stress-induced mutability in any system. Moreover, there are theoretical reasons to expect that temporary increases in mutation rate are unlikely to be of long-term benefit, due to their cost in deleterious mutations. Nevertheless, in some systems, selection does increase the yield of mutants. Under particular selective conditions, increases in general mutation rate can be shown experimentally and theoretically to be advantageous. We suggest that the apparent conflicts between these statements can be resolved without recourse to stress-induced mutability.

The term adaptive mutation has been defined as the process by which stresses that are not directly mutagenic activate mechanisms for causing mutations, even in nongrowing cells (stress-induced or stationary-phase mutagenesis). This definition assumes a mechanism that we think is unlikely to exist. In order to consider all explanations of the relevant phenomena, adaptive mutation is defined here as the process by which mutations arise under selective conditions, whether or not mutation rates increase or growth is required. The area discussed here has been reviewed previously from various points of view (26–28, 81, 82, 84, 85).

GENERAL INTRODUCTION TO A 150-YEAR-OLD CONTROVERSY

Darwin suggested that stress might generate the variability upon which natural selection operates (15). The classic experiments of Luria & Delbrück (63) and Lederberg (59) demonstrated that some mutations arise without the influence of selective stress. However, the lethal selections they used could not have detected mutations induced by selective conditions. Shapiro (88), Cairns et al. (13), and Hall (40, 42) pointed out this deficiency and described genetic systems in which selective conditions seemed to increase the mutation rate. The Cairns system has been analyzed in most detail and remains controversial despite this effort.

It is difficult to separate any possible effect of selective conditions on mutation rate from its known effect on relative growth rates of parents and mutants. The accepted method for measuring mutation rate (Luria-Delbrück fluctuation test) demands that the new mutants grow at the same rate as the parent during the period of the test. To circumvent this problem, the Cairns system (like others) places cells on solid medium and prevents growth by imposing a nonlethal stress; new mutants are scored as visible colonies. If the frequency of new mutants increases in a nongrowing parent population, then natural selection is eliminated as a cause and one is compelled to conclude that mutation rate has increased. If, however, even a subset of the population grows, new opportunities for mutations are provided and pre-existing variants with minimal growth ability can improve. For many of the systems described here, mutants appear in a population that is not obviously growing, but in most cases, the evidence does not exclude growth of a subpopulation and stepwise improvement of a mutant during the selection period.

Deleterious mutation: a mutation that decreases organism fitness

Adaptive mutation: any process by which fitter mutations arise under selective conditions; may or may not require mutagenesis or growth

THE CAIRNS SYSTEM REOPENS THE DEBATE

The Basic System

A system was developed by John Cairns to address the shortfalls of previous evidence for selection-independent mutability (13). In the new system, as modified by Cairns & Foster (11), Lac$^-$ cells with a revertible *lac* mutation are plated on lactose medium and selection is made for mutants that regain a Lac$^+$

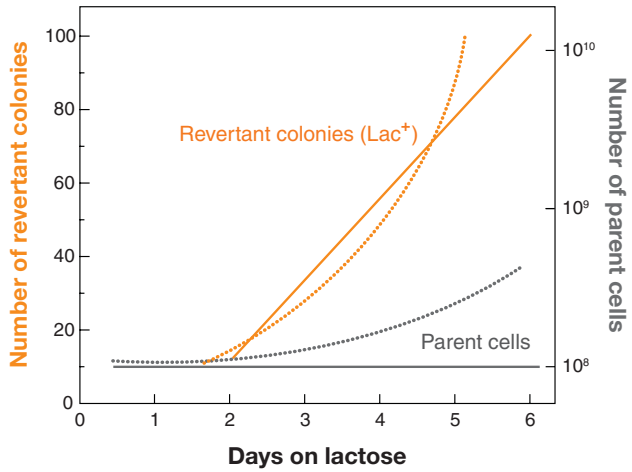

Figure 1

Diagram of a reversion experiment. While revertant accumulation is often linear, many experiments show an upward inflection (dotted line for revertants). Similarly, growth of the lawn frequently escapes inhibition (dotted line for parents), especially toward the end of the selection period. Whether or not this occurs, observed growth does not provide sufficient divisions to explain the revertant number without some gimmick.

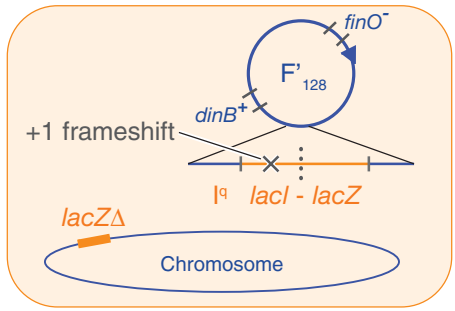

Figure 2

Genotype of the Cairns strain. The course of the reversion experiment depends on having *lac* on the F′ plasmid. The *dinB* gene is 16 kb from *lac*; associated mutagenesis, but not *lac* reversion, depends on the presence of *dinB* at this position (*cis* to *lac*).

phenotype. The selective conditions are not lethal and are adjusted to just barely prevent growth of the plated population. About 100 Lac^+ colonies accumulate above the plated lawn of 10^8 cells over 6 days. Thus the conditions appear to eliminate natural selection (growth) and reveal revertants, which accumulate at a nearly linear rate with time. That is, fully Lac^+ revertants seem to arise in a nongrowing parent population. This is diagrammed in **Figure 1**.

During unrestricted growth, the original mutant gives rise to fully Lac^+ revertants at a rate of less than 10^{-8}/cell/division (80). Under selection, the apparently nongrowing population generates over 100 revertant colonies in the course of 6 days incubation. If this whole time period allowed the entire population to divide just once, a 100-fold increase in mutation rate (to 10^{-6}) would be required to explain the revertants.

Detailed Structure of the Strain

The *lac* mutation under selection is a +1 frameshift in a chimeric gene made by fusing the *lacI* and *lacZ* coding sequences (11) (**Figure 2**). The hybrid gene is expressed from a mutant *lacI* promoter (I^q) that is not subject to regulation. The frameshift mutation is within the *lacI* portion of the gene and presents a large target (about 100 bp) within which any compensating (−1) frameshift mutation can allow expression of the distal *lacZ* portion of the gene. This is a reversion target 10- to 100-fold larger than expected for a frameshift mutation within a sequence that encodes critical regions of a protein. The mutant *lac* allele is carried by a conjugative plasmid (F_{128}) in a strain with a deletion of the chromosomal *lac* region. The transfer functions (*tra*) of this plasmid are constitutively expressed in *Escherichia coli* owing to a natural *finO* mutation in the plasmid.

The mutant *lac* allele is substantially leaky, producing 1% to 2% of the β-galactosidase level found in revertant strains (3). This residual activity is sufficient to allow the parent strain to form colonies on standard minimal lactose medium without any genetic change. To prevent its growth during a reversion experiment, the tester is plated with a 10-fold excess of scavenger cells having a *lac* deletion mutation. The scavengers consume any residual nutrients contaminating the medium and any carbon sources that leak from the tester

strain or revertant colonies (e.g., galactose, acetate, lactate, succinate). The scavenger acts as a competitor, poising the *lac* tester cells on the brink of growth. The stasis of the tester population does not reflect matched rates of growth and death (11). Growth ability can be conferred on a cell either by increasing the number of mutant gene copies or by correcting the +1 frameshift mutation.

The Reversion Experiment

Revertant colonies accumulate over the course of 6 days after the testers (10^8) are plated with scavengers (10^9) on lactose medium (**Figure 1**). The first revertant colonies to appear (before day 2) are assumed to reflect mutant cells that arose during pregrowth, and colonies that appear later are attributed to processes occurring under selection on the plate. In some experiments, revertant accumulation is linear with time, but in many other experiments there is an upward inflection.

The Lac revertants appearing late (days 5 and 6) are not slow growers, because they form colonies within 2 days in reconstruction experiments. Furthermore, the rate of accumulation of Lac^+ revertants and the final level achieved are not subject to Luria-Delbrück fluctuation when parallel independent cultures are tested. The observed, essentially Poisson, distribution of revertant number (11) is expected if reversion occurs on the selection plate. This distribution is also consistent with revertants being initiated by pre-existing cells carrying an unstable *lac* duplication (see below).

The *lac* sequence changes found in late revertants tend to be −1 frameshift mutations in homopolymeric base runs (31, 83). In the absence of selection, this type occurs but other mutations are more common (e.g., −4, +2, extended deletions or +1 frameshift mutations outside base runs). This has been taken as evidence that mutations arise under selection by a mechanism that is qualitatively different from that operating during nonselective growth. The observed shift is consistent with a fivefold increase in mutation rate if the added mutations are all −1 frameshift mutations in base runs.

Early hints of the complexity of this experiment were the following prerequisites for a full yield of Lac^+ revertants (additional requirements are discussed below).

- Residual function of the mutant *lac* allele (3).
- Recombination ability (RecA, RecBC) (11, 45).
- Location of the *lac* mutation on an F′ plasmid (35, 38, 39, 75). With *lac* in the chromosome, roughly 100-fold-fewer revertants are seen (79, 92).
- Expression of conjugative transfer functions (*tra*). Revertant yield is reduced by repression of the plasmid *tra* operon, which encodes functions needed for conjugative DNA transfer (35). Reversion is essentially blocked by elimination of TraI (32, 74), a plasmid-encoded protein that nicks DNA and initiates transfer replication. Transfer per se does not seem to be essential to reversion (32).
- A functional *dinB* gene (encoding an SOS-induced error-prone DNA polymerase) must be located close to the *lac* gene in the plasmid for a full mutant yield (66, 91).

An additional property of the system has proved the most divisive. Revertants appearing under selection show an average 20-fold increase in the probability of carrying unselected associated mutations (79, 102) and thus have been mutagenized. This mutagenesis is unevenly distributed in that 10% to 20% of Lac^+ revertant cells show a 200-fold increase, while the majority (80% to 90%) arise with no obvious mutagenesis (79). The starved nonrevertant lawn shows about a fourfold increase in associated mutations (8). All general mutagenesis is eliminated by removal of the *dinB* gene from its position near *lac* on the F′ plasmid—at least a 50-fold reduction (92); revertant yield drops only 4-fold without *dinB*

SOS: a gene system induced by DNA damage that includes enzymes involved in DNA repair

(66). Several models have been developed to explain the above observations.

Stress-induced mutagenesis: the hypothesis that organisms possess mechanisms to increase their general mutation rate in response to external stress

Hypermutable state (HMS): a model whereby stress increases the general mutation rate

MODELS THAT ASSUME STRESS-INDUCED MUTAGENESIS OF NONGROWING CELLS

Functional Direction of Mutagenesis

This early model proposes that nongrowing cells make only mutations that improve their fitness (11, 13, 29). Two mechanisms for implementing this (16, 95) were eliminated by experiment (29).

- Essence: Cells sense the problem posed by selective conditions and preferentially alter sites in their own genome that can rectify the difficulty.
- Main evidence: Under selection, the number of revertants (Lac^+) increases more than the frequency of unselected mutant types sought in the lawn of starved bacteria.

Generally Hypermutable State

A model proposed by Hall (41) suggests that stress induces a general (undirected, genome-wide) hypermutable state (HMS) in a subset (0.1%) of the nongrowing starved population. Cells in the subpopulation ultimately die of lethal mutations unless they first acquire a *lac* reversion event, which relieves the stress and terminates the HMS. Mutagenesis appears directed to *lac* because only Lac^+ revertants survive hypermutagenesis. To explain 10^2 revertants arising from 10^5 cells, the model requires a 10^5-fold increase in mutation rate for cells in the HMS (86).

- Essence: Stress induces intense genome-wide mutagenesis in a subpopulation (10^5 of 10^8 cells) and only lac^+ revertants survive.
- Main evidence: The model predicts that Lac^+ revertants should often carry associated unselected nonlethal mutations. The predicted mutations were found (102) and were fivefold more frequent among Lac^+ revertants than in the starved lawn or about 20-fold more frequent than in an unselected population (8, 79). The associated mutations are caused by the SOS-induced error-prone DinB polymerase (66).

On the basis of these findings, the model was made more explicit and now proposes that stress induces expression of the DinB polymerase, which causes the genome-wide increase in mutation rate and is responsible for the observed *lac* revertants. Mutagenesis is proposed to occur in nongrowing cells during unscheduled DNA replication initiated by recombination events (33, 46). Mutations have previously been shown to occur during recombination in yeast (77).

Positional Direction of Hypermutability

This model is a hybrid of the previous two models (12, 27). It assumes that stress induces DinB in nongrowing cells and mutagenizes any region in which recombinational replication is occurring. Mutagenesis is directed to the F′ plasmid, whose conjugation transfer functions (*tra*) produce DNA ends that stimulate intense recombination between plasmid sequences. Because only the plasmid is mutagenized, associated chromosomal mutations are not expected. The observed *lac* revertants can be explained by a 100-fold increase in mutation rate on the plasmid (rather than a global 10^5-fold increase) because the whole population (10^8 cells) is affected rather than a subpopulation (10^5). The genome-wide mutagenesis seen in a minority of revertant clones (10%) is not considered central.

- Essence: Stress in nongrowing cells induces DinB, which preferentially mutagenizes the F′ plasmid. More broadly, all recombination becomes mutagenic during stress, and the Cairns system provides a sensitive test for this because its target *lac* region experiences intense

recombination owing to its position on the F′ plasmid.

- Main evidence: During starvation for lactose, a +1 frameshift mutation in *tetA* located on the F′ plasmid produces more unselected revertants than does an identical mutation located in the chromosome (8, 25).

THE AMPLIFICATION MODEL: GROWTH AND SELECTION REPLACE MUTAGENESIS

This model assumes that mutations arise in a growing subpopulation of plated cells. It proposes that rare cells with a *lac* duplication are present in the plated population and initiate slowly growing clones on the selective plate. This is possible because tester cells are poised on the brink of growth (by scavengers) and extra *lac* copies upset this poise. Within these developing clones, selection favors common cells with further increases in mutant *lac* copy number (higher amplification); these are formed by unequal recombination between repeats (**Figure 3**).

Growth rate improves in cells of the colony that gain more copies of the mutant *lac* allele. Ultimately a reversion event (frameshift) occurs in some *lac* copy; selection now holds the revertant allele and counterselects the remaining mutant copies, which make the revertant allele subject to loss by segregation. Haploid lac^+ cells appear and overgrow the colony (**Figure 4**).

Reversion to Lac^+ is made more likely because more target *lac* alleles are added to each developing clone (more cells and more copies per cell). This process requires no stress-induced mutagenesis and appears to direct mutations to the precise base pairs that limit growth. This occurs because only the *lac* region is selectively amplified and only the revertant allele is retained in the final product.

Development of a revertant clone is described in **Figure 5**. Note that the sequence of events is basically a random walk with two boundary conditions or absorbing states.

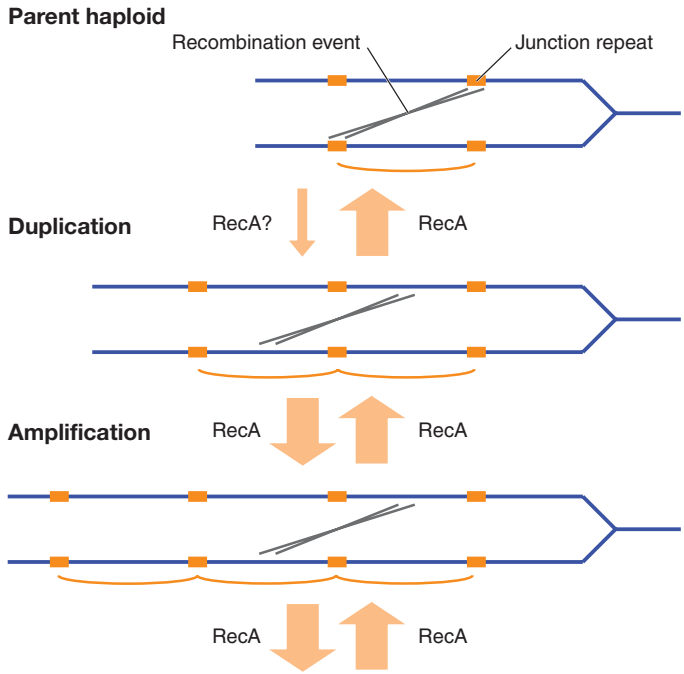

Figure 3

Recombination events involved in duplication and amplification. Duplications are formed by a rare exchange that can occur without RecA. Segregation and amplification events occur with much higher frequency and are heavily RecA dependent.

Whenever a duplication or amplification segregates to haploid, it is essentially removed from the walk. The process is initiated by a *lac* duplication, and copy number increases by further amplification. In the absence of selection, the absorbing condition at the left (haploid lac^-) limits the increase in copy number. However, under selection, copy number is driven higher when selection favors growth of cells with more mutant *lac* alleles. Once reversion occurs, all *lac* alleles (including lac^+) are subject to loss by segregation. Instability of lac^+ is minimized by removal of mutant copies until the second boundary is crossed, leaving an irreversibly lac^+ haploid cell.

In this process, selection detects common small improvements in growth rate (due to dosage increases) and divides the reversion process into many small steps, each of which initiates a clone in which the next event can occur (**Figure 6**). The process of serial mutation

Gene amplification: an increase in gene copy number, generally by accretion of tandem, head-to-tail copies of a genetic region

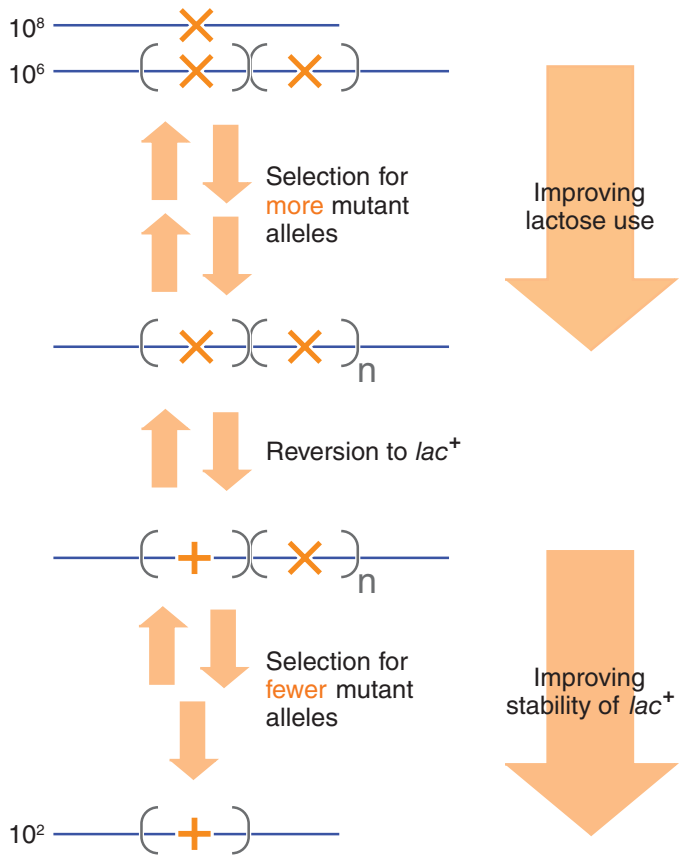

Figure 4

Amplification model. Pre-existing duplications cells grow under selection and growth is improved by further *lac* amplification. Reversion requires no mutagenesis but is made more probable by the increased number of *lac* alleles (more cells, more copies per cell).

in nested clones is a powerful way of changing genotype with small populations and low mutation rates. This does not occur in the absence of selection, when mutants grow at the same rate as the parent. Without selection, mutation frequency increases only by addition of new mutations (**Figure 6**).

The general mutagenesis seen in 10% of revertants (79) is thought to occur in clones whose *lac* amplification includes the nearby *dinB* gene (91). About 20% of revertants (close agreement to 10%, given the methods used) have a *lac* amplification that includes *dinB*, and these cells are shown to have a higher mutation rate than cells whose amplifications include only *lac*. This agrees with evidence that general mutagenesis by DinB (even of targets on an F′ plasmid) requires expression of DinB from a high-copy plasmid (52, 64) in order to overwhelm mismatch repair. General mutagenesis during reversion under selection is seen only when *dinB* is located on an F′ plasmid *cis* to *lac*, and not when *dinB* and *lac* are on different conjugative plasmids or in cells whose only *dinB* gene is in the chromosome. This position effect is expected if mutagenesis requires coamplification of *dinB* and *lac* (91).

Thus the genome-wide mutagenesis seen during the Cairns experiment (in 10% of clones) seems to be an artifact reflecting the happenstance that *dinB* is located only 16 kb from *lac* on the F'_{128} plasmid and is occasionally coamplified with *lac* to provide enough *dinB* copies to overwhelm mismatch repair. Removal of *dinB* eliminates associated mutagenesis but reduces revertant yield only about fourfold (66). In the absence of DinB, when no mutagenesis occurs, amplification alone increases revertant yield about 25-fold, based on the number of plated cells, or about 10^4-fold, based on the number of plated duplication cells. The minor increase in mutation rate caused by DinB explains the altered spectrum of revertant sequence changes, but its effect on revertant number is detectable only because it is imposed on cells with 10 to 100 copies of the target *lac* gene.

- Essence: Selection detects small progressive growth improvements due to *lac* duplication and amplification. Revertant alleles (−1 frameshifts) arise in the growing cells within developing colonies. Base changes are made more probable by an increase in the number of mutational targets (more cells and more *lac* copies per cell).
- Main evidence: (*a*) Each revertant colony includes some unstable Lac$^+$ cells (with a *lac* amplification) and some stable Lac$^+$ cells (revertant segregants) that are clonally related (49) (**Figure 7**). This fulfills a prediction of the model that each colony contains evidence of its history, including rare cells with a *lac* amplification. This evidence has

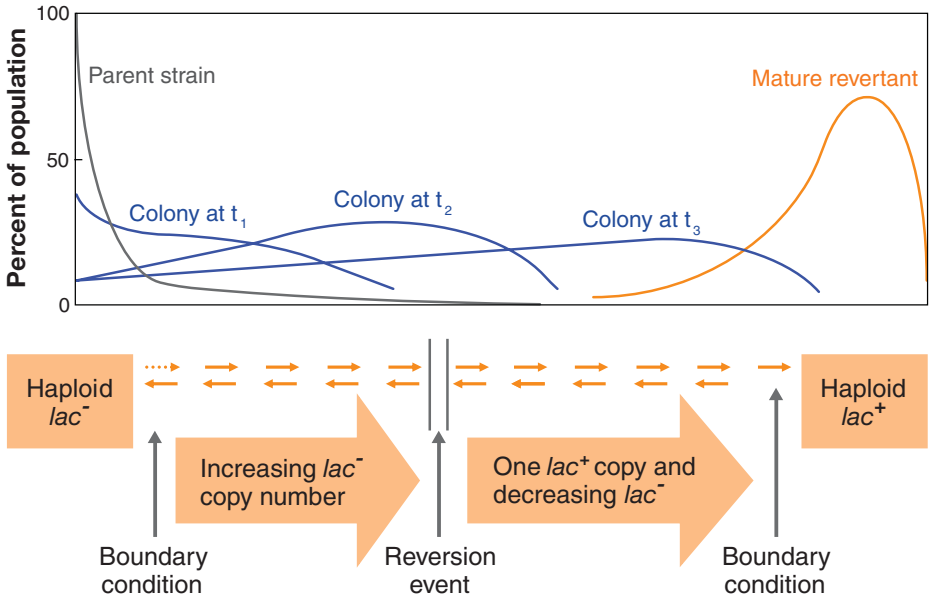

Figure 5

Population dynamics—a selection-biased random walk. The bottom of the figure presents the events as a bounded random walk with two absorbing conditions. The frequency of intermediates in this walk (*top*) is pulled to the right by selection (improved growth on lactose) and by the absorbing condition at the right, in which the Lac$^+$ phenotype becomes irreversible in haploid cells. The move to the right is opposed by spontaneous segregation and by the left-hand absorbing condition, in which cells become haploid Lac$^-$ (with low probability or forming a new duplication).

been disputed, as discussed below. (*b*) A Tn*10* element placed near *lac* on the F′ plasmid reduces Lac revertant number (49). This is attributed to inhibition of growth when the amplified region includes both *lac* and the *tetA* gene; TetA protein is known to be toxic when overexpressed (21). This experiment suggests that growth and amplification are necessary precursors of reversion. (*c*) A constructed duplication of the mutant *lac* region in the parent tester causes an increase in revertant yield (91). Selection stimulates reversion (about 25-fold) in strains that lack *dinB* (66, 91). These results suggest that amplification is sufficient to explain reversion, using only the basal (uninduced) mutation rate. (*d*) Time for a colony to mature from miniscule to full-size is highly variable as expected for a multistep stochastic process of growth improvement (49); this is not expected for one-step reversion from Lac$^-$ to Lac$^+$ as proposed by all the other models. (*e*) This process has been mathematically modeled and is numerically feasible with reasonable parameter values (73).

CHOOSING A MODEL: THE CASE FOR AMPLIFICATION

The functional direction model explains the data but has a mystical quality in requiring that the target specificity of mutation be informed by the benevolence of its outcome. Like Intelligent Design and *deus ex machina* literary devices, it solves a complex problem by invoking a mysterious ad hoc mechanism. In principle, this model could explain all of evolution without recourse to natural selection. The mechanistically more conventional

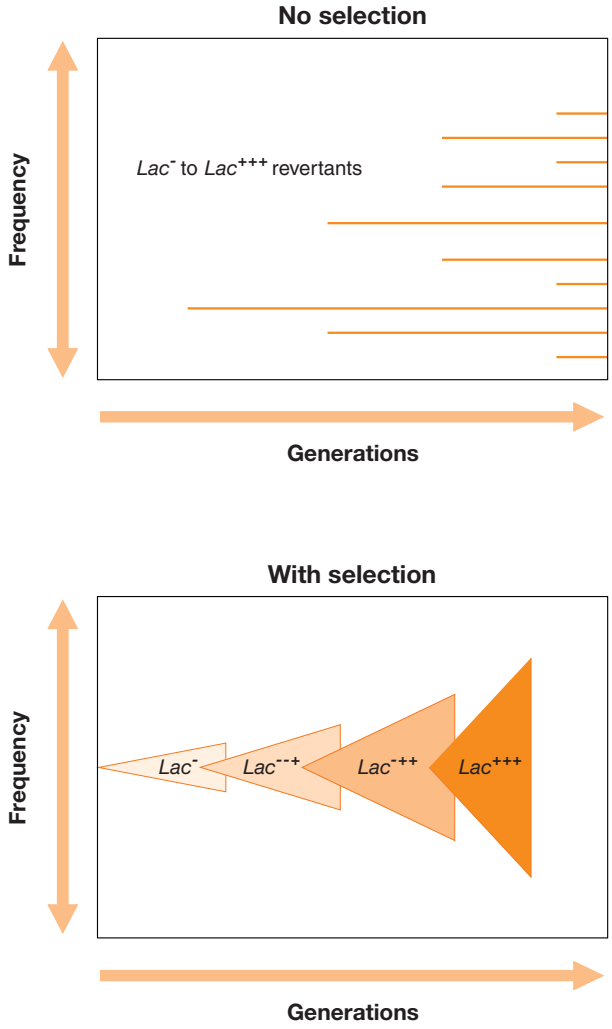

Figure 6

Expansion of nested clones under selection. Without selection (or without growth) (*top*) a population grows and new revertants (to full Lac^+) are added by rare mutations that can be detected in later tests. With selection (*bottom*), common new mutations (one shown) arise that provide a slight growth improvement and they increase in frequency because they grow faster than the parent. Within each expanded subclone another improving change can occur. Growth under selection divides the process of becoming fully Lac^+ into small steps. Individually, these small steps would be hard to detect experimentally.

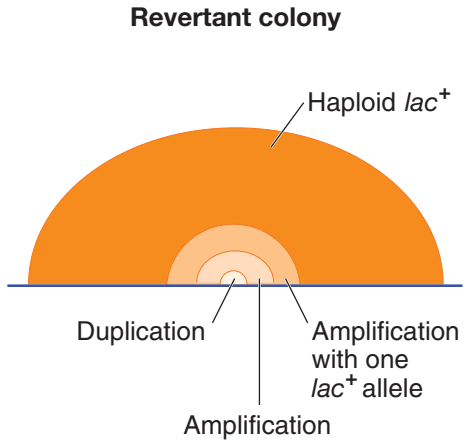

Figure 7

Revertant colony. The amplification model predicts that each colony should include representatives of each step in the history of the process, since individual improved cells grow faster but leave behind many unimproved sibs.

explanations offered by later models make the functional direction model less attractive (whether or not these alternatives prove correct).

The HMS model is clever but implausible. It proposes that 100 revertants arise from 10^5 mutagenized cells and therefore requires that stress increase the mutation rate 10^5-fold—a general mutagenesis more intense than has ever been demonstrated in resting cells (86). If realized genome wide, this mutagenesis is estimated to produce an average of five lethal mutations per cell. The observed 20-fold increase is insufficient to form even one revertant from 10^5 cells. Furthermore, 90% of revertants arise with no apparent mutagenesis (79). The observed general mutagenesis is insufficient and unnecessary to explain the revertants in the Cairns system. Its source is better explained by occasional coamplification of *dinB* with *lac*.

The positional direction model is attractive in that it explains much of the data using a plausible mechanism and does not require unrealistically intense mutagenesis. Establishment of this model would require demonstration (to the satisfaction of all) that selection causes an increase in the mutation rate (mutations per target copy) on the F′ rather than an increase in *lac* copy number by amplification. This will be difficult to demonstrate because there is no generally acceptable way to measure mutation rates under

selective conditions. The Cairns system was designed to solve this problem but so far has not eliminated growth and progressive phenotype improvement within subpopulations as suggested by the amplification model.

- Several lines of evidence suggest to us that the positional direction model is incorrect. Its main support is that during a lactose selection, a *tetA* frameshift mutation reverts more frequently when located on the F′ plasmid (25) than when it is in the chromosome (8). However, enhanced reversion of *tetA* on F′ has not been shown to require starvation for lactose. This position effect may reflect increases in *tetA* dosage due to coamplification with *lac*. In addition, the *tetA* frameshift mutation (like *lac*) is leaky and may be subject to independent selective amplification in the presence of tetracycline; this amplification (like that of *lac*) would work better on the plasmid.
- Positional direction proposes that stress mutagenizes any region in which recombination occurs. This occurs genome wide but is more easily detected on F′ where recombination is intense. Our measurements suggest that, contrary to this model, reversion rate of *lac* is not higher on the F′ than in the chromosome during nonselective growth (when rates can be measured by a fluctuation test) (E. Sano, S. Maisnier-Patin & J. R. Roth, unpublished data). Moreover, the unselected rate is not influenced by removing *recA*, by varying expression of the *tra* operon, by varying SOS induction, or by removing *dinB*—any of which might be argued to change in response to selection. Under selection on lactose, the apparent direction of mutation to the plasmid can be explained by selective increases in *lac* copy number (the mutation target) promoted by recombination and growth.
- The DinB polymerase is significantly mutagenic only when its error production exceeds the capacity of the mismatch repair system. During nonselective growth, mutagenesis requires overproduction of DinB from a high-copy-number plasmid, even when the mutational target is located on an F′ plasmid, where recombination is intense (52, 64, 103). There is no evidence that selective stress can generate an equivalently high level of DinB activity (as required by this model).
- Thus far, the mutagenic activity of DinB seems independent of RecA (103, 104), whereas reversion in the Cairns system depends on RecA.
- The Cairns experiment works when DinB is deleted (although it produces fourfold-fewer revertants). A fourfold contribution of DinB to plasmid mutation rate is far less than the 100-fold DinB-dependent increase required to explain revertants by the positional direction model.

The amplification model explains all of the available data and is supported by the evidence outlined above. Establishment of this model will require demonstrating (to the satisfaction of all) that revertant clones contain mixtures of stable haploid Lac^+ and unstable Lac^+ precursor cells (see below), that amplification is a prerequisite for reversion, and that mutation rates do not change under selective conditions.

AREAS OF DISCUSSION

An interesting feature of this controversy is that there is general agreement on most of the experimental evidence. The original system is robust and the basic reversion experiment can be repeated reliably. Supporters of the several models agree on the following observations (but disagree on their interpretation).

Leakiness of the *lac* Mutation

Starvation in the absence of any carbon source does not appear to be mutagenic

(29, 51). Residual *lac* function is required in the presence of lactose for reversion in the Cairns system (3). In the amplification model, this provides the minimal function whose amplification serves to improve growth.

In the hypermutation and the functional and positional direction models, residual *lac* function does not support growth but is needed to provide energy for plasmid replication, recombination, and mutagenesis. When lactose is provided but growth is blocked by lack of an amino acid, no reversion is seen; thus protein synthesis is also required (11). These models propose that cells use available energy and protein synthesis to support plasmid replication and mutagenesis in preference to chromosome replication and growth.

Role of Recombination

In the several stress-induced mutation models, recombination provides structures (recombination intermediates) into which DinB can load to initiate error-prone replication. Contrary to this, direct tests suggest that DinB-mediated mutagenesis does not depend on recombination (64, 103, 104).

In the amplification model, recombination functions catalyze the genetic exchanges that amplify the growth-limiting *lac* gene and remove the extra mutant copies following reversion. Thus the main role of recombination is to change *lac* copy number. Though not essential to the Cairns system, the minimal increase in mutation rate requires RecA for SOS (DinB) induction and (with other recombination functions) for coamplification of *dinB* with *lac* in a few revertant clones.

The Question of Mixed Clones and Proposal of Two Pathways of Adaptive Mutation

There is general agreement that amplification of the leaky mutant *lac* allele can allow growth (3, 47, 49, 82). In the amplification model, growth and increased *lac* copy number precede reversion and stimulate revertant yield by providing more targets. This predicts that revertant colonies should include both stable Lac^+ cells (formed following reversion, segregation, and overgrowth) and unstable Lac^+ cells (precursors that still harbor the amplification). These mixed clones were observed in *E. coli* (at a slightly higher lactose concentration than that used by others) and are more easily observed in *Salmonella*. The ratio of stable to unstable Lac^+ cells varies widely from one clone to the next and increases with age of the colony as stable types overgrow (49).

Those who do not observe mixtures report instead two types of revertant colonies—a majority type (90% at 5 days) composed entirely of stable Lac^+ cells and a minority type (10%) composed entirely of unstable Lac^+ cells; the fraction of revertants with unstable Lac^+ cells increased during the course of a reversion experiment (47). To explain this, it is proposed that cells respond to selective stress in either of two mutually exclusive ways. In some cells, stress induces only point mutations, and in others, stress induces only amplification (47, 48).

NEW ISSUES IN ADAPTIVE MUTATION

Several new aspects of work on adaptive mutation promise to shed light on the controversy, but have not yet tipped the balance either way.

Origins of Duplications and Process of Amplification

Prior to selection, *lac* duplications are carried by about 1% or 10^6 of the 10^8 cells plated (91, 92). The majority of the pre-existing duplications are large (135 kb) and flanked by direct copies of the transposable element, insertion sequence 3 (IS3) (E. Kugelberg, D. E. Andersson & J. R. Roth, unpublished data), the largest direct-order sequence repeats flanking the *lac* region on the plasmid F'_{128} (54). The number of plated duplication-bearing cells (10^6) is vastly greater than the

number of revertant clones (10^2), suggesting that the plated large duplications have a low probability of initiating a successful clone. Removal of IS3 from the plasmid reduces the revertant number two- to threefold, suggesting that IS-mediated duplication initiates about half of the revertants.

Amplifications found within revertant colonies after selection include a few with the common IS3 join points, but most (70%) include smaller regions (10 to 25 kb) and have join points with short sequence repeats (3 to 12 bp) resembling the endpoints of deletions. Duplications of the SJ type are present but rare in the preselection population; they form revertant colonies with high efficiency (E. Kugelberg, D. E. Andersson & J. R. Roth, unpublished data).

It is proposed that the common IS3 duplications are highly unstable and inherently costly (perhaps owing to included genes that are toxic when overexpressed). Therefore in the Cairns experiment, the common IS3 duplications rarely initiate a successful colony unless they are first converted to the SJ type by join point deletions, explaining their low efficiency of revertant formation. The other half of revertants may be initiated by the rare pre-existing SJ duplications that form revertants with high efficiency.

The frequency of duplications in a population is constrained by their high segregation frequency and is expected to approach steady state as the product of duplication frequency and segregation rate approaches the product of haploid cell number and formation rate. The time needed to reach this steady state (about 100 generations) is dictated by the reciprocal of the fastest rate (segregation), about 1% per generation. Nearly half of the steady-state level is achieved during growth of a cell to a full-density culture (30 generations). Thus the failure to see fluctuation in revertant yield (11) is consistent with revertants initiated by pre-existing duplication cells. Duplications are thus resistant to Luria-Delbrück fluctuation because of their reversibility.

Role of RpoS and Ppk

Recently mutations eliminating the stationary-phase sigma factor (RpoS) (57, 62) or polyphosphate kinase (Ppk) (97) have been seen to reduce the yield of revertants arising in the Cairns system. RpoS contributes to a wide variety of phenomena, many involving control of gene expression when growth is strongly limited; polyphosphate is involved in control of RpoS synthesis (50). The effects of these mutants have been interpreted as evidence that RpoS controls DinB expression in stationary-phase cells, thereby providing a connection between growth limitation and increased mutation rate that is key to the stress-induced mutability models (57, 62). However, direct tests revealed small effects of these mutations on *dinB* expression, suggesting that RpoS may act primarily at some other stage in the reversion process, e.g., recombination, mismatch repair, *tra* expression, or (consistent with the amplification model) residual growth on lactose.

Mu a bacteriophage that forms a prophage by inserting its sequence at random into the bacterial chromosome

OTHER SYSTEMS THAT SEEM TO SHOW STRESS-INDUCED MUTAGENESIS

The first two cases below are among the founding works on adaptive mutation. We outline these and several other systems that suggest mutagenesis during selection.

Deletions that Remove a Mu Prophage and Fuse the Arabinose Promoter to a *lacZ* Gene

These deletions are more frequent after extended periods of selection on lactose (88). It was ultimately shown that the detected mutants depend on events occurring prior to selection (30, 90, 92a). However, it is not clear why these deletions are detected more easily following prolonged selection or why selection appears to affect the position of deletion endpoints. We suggest that several events are necessary to complete these revertants; a

rate-limiting event prior to selection may initiate a process of phenotype improvement during growth of the colony.

Evolved β-Galactosidase

In this system, Lac^+ cells arise in a strain of *E. coli* whose *lac* operon has been deleted (43, 44). This requires two mutations affecting an operon (*ebg*) that is normally unable to support use of lactose. One mutation eliminates the repressor (allowing operon expression) and the second alters an enzyme, increasing its ability to hydrolyze lactose. The first mutation is expected to be common (on the order of 10^{-6}) and the other rare (on the order of 10^{-9}). Strains with both required mutations are expected to be present at a frequency near 10^{-15} and are not found in unselected cultures. However, they do appear within a parent colony (less than 10^9 cells) growing on rich medium containing lactose, which allows growth but still selects for improved ability to use lactose. After many days incubation, these colonies show Lac^+ papillae, which contain cells with both *ebg* mutations.

The number of total cells in each colony is far too few to assure coincidental occurrence of the two required mutations with a standard mutation rate. The result has been interpreted as evidence for directed mutation in response to stress, but the possibility has not been eliminated that the observed double mutants arise sequentially during serial expansion of nested clones resulting from stepwise improvement of growth ability. We suggest that the more common repressor mutant arises first and initiates a slowly growing clone within which the second rarer mutation type arises.

Accumulation of Mutants in Aging Colonies

Wild-type *E. coli* cells are plated on rich medium and allowed to grow for about 1 day. Some colonies are tested at this point for their frequency of Rif^R mutant cells, while others are incubated for an additional week, during which the cell population increases only about 10-fold. After one week, many of these "aged" colonies contain a greatly increased frequency of Rif^R mutants (on the order of 50-fold) (6). This phenomenon has been referred to as ROSE (resting organisms in a structured environment) (98, 99) or MAC (mutagenesis in aging colonies) (6). It is attributed to stress-induced mutagenesis of cells and depends on Crp/cAMP, RpoS, and RecA. While the induced mutagenesis is inferred to be general, the frequency of mutants resistant to other antibiotics increases marginally or not at all compared with that of Rif^R mutants.

Recently, M. Wrande & D. Hughes (unpublished data) have shown that the Rif^R frequency increases owing to clonal growth of mutants that pre-exist the aging period. For reasons that are not yet clear, some types of Rif^R mutants grow more than the parent strain under the aging conditions and give rise to about 50-fold-more descendants over one week. Results would be explained if some Rif^R mutations changed RNA polymerase so as to increase expression of genes that enhance growth in old colonies. We suggest that mutants resistant to other drugs sometimes arise during growth of Rif^R clones and hitchhike to a slightly increased frequency. Thus the phenomenon can be explained by growth under selection without any increase in mutation rate.

Loss of Fitness in Stationary-Phase Cultures

Liquid bacterial cultures are grown to full density and then incubated for extended periods, during which there is little net growth (61). Samples are removed at various times and used to initiate new cultures, whose fitness is assessed by measuring growth rate in fresh medium. Fitness appears to drop with time and the results are analyzed to infer an increased mutation rate in the growth-limited culture.

It has been suggested that this experiment involves unappreciated growth (17).

Stationary-phase cultures are known to select for extensive growth by rare individuals in the population, even when the population as a whole remains constant or decreases slightly—the GASP (growth advantage in stationary phase) phenomenon, in which a few cells grow at the expense of some dead cells (24, 106). This growth provides opportunities for mutations to arise. Selected mutations that enhance survival or growth during stationary phase may be deleterious in fresh medium. No mutagenesis is necessary to explain the results and no mutagenesis has been demonstrated by the experiments.

GASP: growth advantage in stationary phase

Selection for Loss of Kanamycin Resistance in Stationary-Phase Cells

Mutation accumulation was examined in stationary-phase colonies held under conditions that would kill any minority population that initiated growth. The lethal selection was then removed to allow detection of any mutants that arose during the period of suspended growth (5, 78). These are interesting, cleverly designed experiments, but they leave open the possibility that growth occurred in a subpopulation within the stationary-phase colony after relaxation of the lethal counterselection.

Selection for Loss of Repressor Function in Nongrowing Populations

In this interesting system (105, 105a), a normal *lac* operon is fused to the promoter of a purine biosynthetic gene (*purD*) and its expression is limited by an unlinked superrepressor mutation (*purR*^{S}). When this tester is plated on lactose as sole carbon source, Lac^{+} mutants accumulate linearly over 6 days and the tester lawn shows no residual growth. The revertants carry either *purR*$^{\text{Null}}$ mutations or *purD* operator mutations, both of which are expected to allow expression of the *lac* operon fusion. Surprisingly, in the absence of selection the frequency of operator mutations (adjacent to the *purD::lac* fusion) approaches that of null mutations in the distant *purR* gene despite the huge difference in target sizes. Under selection, the frequency of operator types is only 4% but still higher than expected. This system does not involve the F′ plasmid, and selection is made for loss-of-function mutations (in the case of *purR*) that are expected to be recessive. To our knowledge, no tests have been made for either general mutagenesis or *lac* amplification in the course of this experiment. Amplification of the *purD::lac* fusion (with its operator region) seems a possible contributing factor.

Reversion of Auxotrophs in Multiply Marked Strains of Bacteria and Yeast

Several laboratories have reported experiments involving strains of bacteria and yeast with multiple auxotrophic requirements (72, 96). Revertant frequency is assessed during starvation for individual nutrients. For any particular mutation, more revertants are found during selection for its restored function than during starvation for a different nutrient. While these results, like those for the Cairns system, suggest a stress-induced change in mutation rates, no tests have ruled out growth of a subpopulation or stepwise improvement of common partially revertant cells during development of revertant clones under selection.

THEORETICAL COSTS AND BENEFITS OF MUTATION

The intuitive appeal of stress-induced mutagenesis may wane upon closer examination.

What Selective Forces Dictate Evolution of Mutation Rates in Natural Populations?

Organism persistence depends on maintenance of genetic information. This is achieved by reducing mutation rates and by selectively removing deleterious mutations from the population. However, adaptation to new

situations requires some level of genetic variation to provide rare beneficial mutations. These forces must be balanced. The number of mutations generated in a population is determined by the population size and the mutation rate of the organism. Therefore, for any given population size, an organism is expected to develop a mutation rate that optimizes the balance between common deleterious mutations and much rarer mutations that increase long-term fitness (18, 76, 93). The optimal ratio of cost to benefit is expected to change with circumstances and lifestyle. A high mutation rate might be more costly for a well-adapted organism in a constant environment than for a poorly adapted organism in a highly variable environment.

The cost of genetic variation also will be influenced by genome size and the frequency of sexual recombination. Genome size dictates the target size for deleterious mutations (19, 20) and recombination increases the efficacy of purifying selection (23, 69). The tolerated mutation rate should increase in proportion to population size and recombination frequency and decrease in proportion to the number of genes in the genome (the target for deleterious mutations). Organisms can satisfy this relationship with various combinations of parameter values. For example, an organism such as HIV, which has a large population size, small genome, and sexual recombination, can tolerate a high mutation rate without risk of extinction. Conversely, an organism with a small population, large genome, and no sexual recombination needs to keep mutation rates low (2, 14, 23, 65, 69). However, once some suitable combination is established, these values are expected to be difficult and costly to change.

Evidence that Bacteria Minimize Mutation Rates

Most organisms invest heavily in minimizing mutation rates (e.g., exporting or destroying mutagenic compounds, proofreading by DNA polymerases, expressing DNA repair functions). These mechanisms may be as efficient as the cell can afford to make them, given pressure to replicate rapidly and use resources efficiently. In most DNA-based microbes, the substitution rate per base pair is low: 10^{-11} to 10^{-9} cells per generation. Cells of *E. coli* can accomplish 100 to 1000 replications without any error (19, 20). It would appear that these low rates provide sufficient beneficial mutations for long-term survival.

Mutators Are Demonstrably Advantageous in Some Situations

Despite the evidence that mutation rates have been minimized by selection, theoretical arguments and experiments demonstrate that cells with a high mutation rate (mutators) can be positively selected during growth in certain environments—when selection requires repeated rare mutations and the available variability is limiting (67, 100, 101). This occurs when the population is small and rare mutants provide a selective advantage (e.g., antibiotic resistance) that is larger than the fitness cost of many of the deleterious mutations (34). Here mutators win.

The difficulty with using this as evidence for beneficial effects of a high mutation rate is that the advantage of mutators persists only as long as these conditions prevail. As soon as more typical conditions resume (i.e., the drug is removed), previously accumulated deleterious mutations (34) become costly and the mutator strain is eliminated. The short-term benefit of mutagenesis is obtained at a cost of long-term extinction.

The beneficial effect of a high mutation rate was demonstrated for *E. coli* mutators during colonization of a mouse gut (36, 37). The mutator advantage was seen when the initial inoculum was a poorly adapted laboratory strain placed under strong selection to adapt to a different situation. In strains that were well-adapted to life in a mouse, mutator derivatives were strongly counterselected. Thus the mutator had a short-term advantage, but was a poor risk for long-term survival,

because it accumulated deleterious mutations and had little prospect of restoring its mutation avoidance system (60). In summary, "evolution of a disease is not the equivalent of long-term evolution of a pathogen."

Mutagenesis Is Often Unnecessary

Bacterial populations are large enough to include most beneficial mutations even with standard low basal mutation rates. Change in any particular base pair is expected to occur with a frequency of 10^{-10} cells per generation, which is within reach for most bacterial populations. If multiple mutations are required, we expect they will arise sequentially with intermediate growth of the initial mutant. Adaptation seems likely to be initiated by common small-effect mutations. In the Cairns system, these are duplications. Typically one *Salmonella* cell in a 1000 carries a duplication of any specified chromosomal gene prior to any selective enrichment (1); such mutations are likely to exist even in small populations. A fitter mutant that arises without a mutator has the advantage of competing long-term without an increased burden of deleterious mutations.

Mutagenesis Is Often Futile

Bacterial populations are estimated to spend most of their time under strong growth limitation (53, 55), and many of these stresses cannot be relieved by mutation (e.g., lack of any carbon source, exposure to extreme physical conditions). If mutagenesis were induced by such stresses, it would create deleterious mutations without hope of benefit. A mechanism for stress-induced mutagenesis might be advantageous if cells could determine (*a*) that the problem is solvable by mutation and (*b*) that death is assured if no mutation is forthcoming. Without such prescience, cells with a mechanism for stress-induced mutagenesis would be eliminated by repeated rounds of futile mutagenesis.

Temporary Increases in Mutation Rates Are Still Costly

Whether mutations are introduced slowly over time or during short periods of intense stress-induced mutagenesis, the costly deleterious mutations produced remain part of the genome and continue to reduce fitness of the cell that produced them. If a population were maintained long term under conditions that favor a high mutation rate, then it would have to readjust its whole lifestyle and find a new set of values that satisfy the relationship described above—increase recombination, increase population size, and/or reduce genome size. Even temporary disturbance of the relationship is likely to be costly.

Mutators Are Common Among Natural Isolates

Many natural isolates carry defects in their mutation avoidance systems and show high mutation rates (4, 7, 58, 71, 94). Some have been isolated from environments that are stressful (see above). However, even in the absence of strong selection, mutators are likely to be present at a high steady-state frequency because their counterselection is delayed. The steady-state level of a mutator is dictated by the combined effects of its rate of formation and its rate of removal by purifying selection. Counterselection depends on secondary accumulation of deleterious mutations. That is, a new mutator cell may show unimpaired growth for many generations, but will ultimately accumulate mutations that allow its elimination from the population. Its persistence in the population may be extended by sexual exchanges that separate it from its deleterious products. Delayed counterselection allows mutators to persist in the population for a longer time and therefore to exist at a higher steady-state level under any growth conditions. Thus mutators may be common in populations even if they never provide any beneficial mutations.

Mutators Sometimes Win in Long-Term Growth Experiments

In the classic experiments of Lenski and coworkers (22), cultures maintained under long-term cultivation are often taken over by mutators. While this could be attributed to strong selective pressures for rare mutations that improve growth in the short term (by producing beneficial mutations, as outlined above), the late appearance of mutators (after cells have already been substantially adapted to the culture conditions) argues against this.

We propose that mutators arise frequently (because many genes are involved in mutation avoidance) and are removed slowly (because of delayed counterselection). Early in the growth experiment, the frequency of mutators is kept low by periodic selection for favorable mutations that arise in the predominant nonmutator cell type. Beneficial mutations are unlikely in the small mutator population. (If a mutator has a 100-fold-higher mutation rate and is present at a frequency of 10^{-6}, then only 1 mutation in 10,000 will arise in the mutator subpopulation.) However, a common mutator will likely arise in an expanding subclone with a pre-existing beneficial mutation. (If mutators arise at 10^{-5}/cell/division, 10% of new fitter mutations should acquire a mutator by the time their subclone reaches 10^4 cells.) The frequency of this mutator will increase to 10^{-4} in the whole culture if the subclone sweeps the population. Thus the new mutator can hitchhike on a beneficial mutation that was not of its making. The proposed process should apply to any frequent mutation that is not strongly (or promptly) counterselected. By this means, a mutator could increase in frequency without generating any advantageous mutations. Once a mutator is present at 10^{-4}, 1% of new mutations will occur in the mutator subpopulation and there is a greater chance that a new advantageous mutation will allow hitchhiking to higher frequency. Counterselection of the mutator is assumed to be weak on the timescale of these experiments.

Directing Mutagenesis

In view of the cost of genome-wide mutagenesis, bacteria would be well served if they could focus their mutability on sites that are likely to contribute a fitness increase. This was proposed by the initial functional direction model for adaptive mutation. Bacteria have achieved this goal by making particular genes highly mutable. These "contingency loci" encode proteins that affect the progress of a host infection—often by dictating cell surface features (68). These genes possess base runs in which frequent frameshift mutations cause inactivation and reactivation of the gene. These base runs affect only the gene in which they occur and thus do not put the entire genome at risk. In essence, a small number of genes (about 30) are individually and stochastically turned on and off—rather like a phase-variation mechanism—generating in the population a vast array of expression patterns (2^{30}), some of which produce a cell surface that solves the particular selective problem (68). Because each inactivation is reversible, there is no long-term loss of information. This mechanism would not be useful for most genes.

Unlike contingency loci, the positional direction model for adaptive mutation does not provide low-cost mutagenesis. Positional direction proposes that all recombination becomes mutagenic under stress. This would affect the whole genome. The posited direction to the F′ plasmid occurs because this target is subject to heavy recombination for other reasons (plasmid transfer functions), not because variation here is particularly valuable to the cell.

BROADER IMPLICATIONS OF ADAPTIVE MUTATION

The special features of the Cairns system suggest that its behavior cannot be generalized easily. On the contrary, we submit that the fundamental events are general but are more easily observed because of idiosyncrasies

of the system. In the case of the amplification model, duplication, selective amplification, mutation, and segregation are expected to occur in any organism.

The principle of adapting by amplifying a rate-limiting gene and the principle of increasing mutant yield by adding target copies are general. The unique feature of the system is that it can produce detectable results within a week because recombination rates are high in the neighborhood of *lac*, and mutation rate increases slightly because the nearby *dinB* gene is occasionally coamplified with *lac*. In effect, the system does a "fast-forward" on genetic adaptation and allows a complex series of events to be completed within 20 to 30 generations (73). The system promises to reveal more principles that can be generalized broadly (e.g., selective remodeling and stabilizing of rearrangements).

THE LEGACY OF THE CAIRNS SYSTEM

We dedicate this review to John Cairns, who developed the system described here and raised fundamental issues regarding the origins of mutations under selection. Cairns has contributed heavily to thinking about the contribution of mutation to the origins of cancer (9, 10). In both the bacterial system and in the origins of malignancies, a population of cells is maintained with a strongly restricted ability to grow (shortage of lactose in bacteria, multilevel growth control mechanisms in metazoans). Each cell is under selection to escape this restriction and divide—a normal selfish response for a bacterium or a single metazoan cell viewed apart from the organism as a whole. In both cases, growing cells arise after extended time periods and are more common than can be explained given the apparent growth and ambient mutation rates. In the case of the *E. coli lac* mutation, revertant clones accumulate over 6 days of selection and exceed their predicted frequency by about 100-fold. In the case of cancer, a bigger problem is posed. If five mutations are required, each arising at 10^{-5} cells per generation, the expected likelihood of a cancer cell is about $(10^5)^5$ or 10^{-25}. Because the human body has only about 10^{14} cells, cancer is predicted to be extremely rare, but in fact it is common.

Both paradoxes might be resolved if cells under strong selection increased or directed their mutation rate, and initially this seemed to be true for the bacterial system. However, the amplification model proposes that selection does not change the rate or target specificity of mutation but rather detects small improvements in growth and initiates a series of clonal expansions—a powerful process that is already favored for explaining cancer. This process requires no dedicated mechanism and explains how rare, multiply mutant cells can arise in small populations even at low mutation rates.

The parallels between the Cairns system and current views of cancer are even closer in that amplifications are found in many tumors and may amplify functions that help cells escape growth limitation. The remodeling of rearrangements has been seen in both systems and may reduce the fitness cost of selected gene amplifications (70). Thus a bacterial system developed to give insight into origins of cancer may prove to be a good model for that disease even if the system does not work in the manner initially suggested by its designer.

SUMMARY POINTS

1. Both mutation rate (formation) and selection (relative reproductive ability of the mutant and parent) influence mutant frequency in a population, and their effects are difficult to separate.

2. Claims that stress increases mutation rate rely on the assumption that mutants arise in a nongrowing population (and must therefore be independent of selection). This assumption is unwarranted without data to exclude growth of subpopulations.
3. Models that propose an evolved mechanism for stress-induced mutagenesis disregard the long-term fitness costs associated with accumulation of deleterious mutations.
4. The behavior of the Cairns system can be explained with no change in mutation rate if one assumes that the rate-limiting gene amplifies during growth of a revertant clone. Reversion is then made more probable by an increase in number of mutation targets within the clone.
5. Coamplification of *dinB* with *lac* can explain the associated general mutagenesis seen in 10% to 15% of revertant colonies.
6. Each species has a characteristic mutation rate presumed to be optimized for congruence with long-term life-history parameters, including population size, genome size, and recombination rate.
7. Temporary increases in mutation rate do not circumvent the long-term cost of deleterious mutations.
8. The Cairns system shows many analogies to cancer development in that cell populations are under strong selection to escape growth restrictions.

ACKNOWLEDGMENTS

We thank people in our labs and collaborators who have contributed their thoughts and experiments to the effort to understand the "adaptive mutation" phenomena, including Otto Berg, Ulfar Bergthorsson, Kim Bunny, Tim Galitski, Shawn Gerum, Heather Hendrickson, Jeffrey Lawrence, Sophie Maisnier-Patin, Mats Pettersson, Emiko Sano, Semarhy Quiñones Soto, Fritz Roth, Jon Seger, Sue Slechta, and Cristina Tun-Garrido. We also thank colleagues and friends who have provided helpful suggestions and encouragement, including Sherwood Casjens, Mick Chandler, François Cornet, Maurice Fox, Glenn Herrick, Pat Higgins, Diarmaid Hughes, Jeffrey Lawrence, Frank Stahl, Beth Traxler, and Marie Wrande. We thank Phil Hastings for sharing unpublished results. Our work has been supported by NIH grant GM27068 (to JRR), grants from the Swedish Research Council (to DIA), and a fellowship from the Wenner-Gren Foundations (to EK).

LITERATURE CITED

1. Anderson P, Roth J. 1981. Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (rrn) cistrons. *Proc. Natl. Acad. Sci. USA* 78:3113–17
2. Andersson DI, Hughes D. 1996. Muller's ratchet decreases fitness of a DNA-based microbe. *Proc. Natl. Acad. Sci. USA* 93:906–7
3. **Andersson DI, Slechta ES, Roth JR. 1998. Evidence that gene amplification underlies adaptive mutability of the bacterial lac operon. *Science* 282:1133–35**

3. The first description of the amplification mutagenesis model.

4. Baquero MR, Nilsson AI, Turrientes Mdel C, Sandvang D, Galan JC, et al. 2004. Polymorphic mutation frequencies in *Escherichia coli*: emergence of weak mutators in clinical isolates. *J. Bacteriol.* 186:5538–42
5. Bharatan SM, Reddy M, Gowrishankar J. 2004. Distinct signatures for mutator sensitivity of lacZ reversions and for the spectrum of lacI/lacO forward mutations on the chromosome of nondividing *Escherichia coli*. *Genetics* 166:681–92
6. Bjedov I, Tenaillon O, Gerard B, Souza V, Denamur E, et al. 2003. Stress-induced mutagenesis in bacteria. *Science* 300:1404–9
7. Bjorkholm B, Sjolund M, Falk PG, Berg OG, Engstrand L, Andersson DI. 2001. Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*. *Proc. Natl. Acad. Sci. USA* 98:14607–12
8. Bull HJ, Lombardo MJ, Rosenberg SM. 2001. Stationary-phase mutation in the bacterial chromosome: recombination protein and DNA polymerase IV dependence. *Proc. Natl. Acad. Sci. USA* 98:8334–41
9. Cairns J. 1978. *Cancer: Science and Society*. San Francisco, CA: Freeman
10. Cairns J. 1997. *Matters of Life and Death*. Princeton, NJ: Princeton Univ. Press
11. **Cairns J, Foster PL. 1991. Adaptive reversion of a frameshift mutation in *Escherichia coli*. *Genetics* 128:695–701**
12. Cairns J, Foster PL. 2003. The risk of lethals for hypermutating bacteria in stationary phase. *Genetics* 165:2317–21
13. **Cairns J, Overbaugh J, Miller S. 1988. The origin of mutants. *Nature* 335:142–45**
14. Chao L. 1990. Fitness of RNA virus decreased by Muller's ratchet. *Nature* 348:454–55
15. Darwin C. 1872. *On the Origin of Species by Means of Natural Selection*. London: John Murray. 6th ed.
16. Davis BD. 1989. Transcriptional bias: a non-Lamarckian mechanism for substrate-induced mutations. *Proc. Natl. Acad. Sci. USA* 86:5005–9
17. de Visser JA, Rozen DE. 2004. Comment on "High deleterious genomic mutation rate in stationary phase of *Escherichia coli*". *Science* 304:518
18. de Visser JA, Rozen DE. 2005. Limits to adaptation in a sexual populations. *J. Evol. Biol.* 18:779–88
19. Drake JW. 1991. A constant rate of spontaneous mutation in DNA-based microbes. *Proc. Natl. Acad. Sci. USA* 88:7160–64
20. Drake JW. 1999. The distribution of rates of spontaneous mutation over viruses, prokaryotes, and eukaryotes. *Ann. N. Y. Acad. Sci.* 870:100–7
21. Eckert B, Beck CF. 1989. Overproduction of transposon Tn10-encoded tetracycline resistance protein results in cell death and loss of membrane potential. *J. Bacteriol.* 171:3557–59
22. Elena SF, Lenski RE. 2003. Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nat. Rev. Genet.* 4:457–69
23. Felsenstein J. 1974. The evolutionary advantage of recombination. *Genetics* 78:737–56
24. Finkel SE, Kolter R. 1999. Evolution of microbial diversity during prolonged starvation. *Proc. Natl. Acad. Sci. USA* 96:4023–27
25. Foster PL. 1997. Nonadaptive mutations occur on the F' episome during adaptive mutation conditions in *Escherichia coli*. *J. Bacteriol.* 179:1550–54
26. Foster PL. 1999. Mechanisms of stationary phase mutation: a decade of adaptive mutation. *Annu. Rev. Genet.* 33:57–88
27. Foster PL. 2004. Adaptive mutation in *Escherichia coli*. *J. Bacteriol.* 186:4846–52
28. Foster PL. 2005. Stress responses and genetic variation in bacteria. *Mutat. Res.* 569:3–11

11. Describes the modified genetic system whose characterization is the main subject of this article.

13. This paper suggested that selective conditions might increase and direct mutability.

29. Foster PL, Cairns J. 1992. Mechanisms of directed mutation. *Genetics* 131:783–89
30. Foster PL, Cairns J. 1994. The occurrence of heritable Mu excisions in starving cells of *Escherichia coli*. *EMBO J.* 13:5240–44
31. Foster PL, Trimarchi JM. 1994. Adaptive reversion of a frameshift mutation in *Escherichia coli* by simple base deletions in homopolymeric runs. *Science* 265:407–9
32. Foster PL, Trimarchi JM. 1995. Conjugation is not required for adaptive reversion of an episomal frameshift mutation in *Escherichia coli*. *J. Bacteriol.* 177:6670–71
33. Foster PL, Trimarchi JM, Maurer RA. 1996. Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. *Genetics* 142:25–37
34. Funchain P, Yeung A, Stewart JL, Lin R, Slupska MM, Miller JH. 2000. The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. *Genetics* 154:959–70

35. Galitski T, Roth JR. 1995. Evidence that F plasmid transfer replication underlies apparent adaptive mutation. *Science* 268:421–23

35. This paper and Reference 75 present the first evidence that the plasmid transfer replication origin are important for appearance of revertants under selection.

36. Giraud A, Fons M, Taddei F. 2003. Impact of mutation rate on the adaptation of gut bacteria. *J. Soc. Biol.* 197:389–96
37. Giraud A, Matic I, Tenaillon O, Clara A, Radman M, et al. 2001. Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. *Science* 291:2606–8
38. Godoy VG, Fox MS. 2000. Transposon stability and a role for conjugational transfer in adaptive mutability. *Proc. Natl. Acad. Sci. USA* 97:7393–98
39. Godoy VG, Gizatullin FS, Fox MS. 2000. Some features of the mutability of bacteria during nonlethal selection. *Genetics* 154:49–59
40. Hall BG. 1990. Directed evolution of a bacterial operon. *Bioessays* 12:551–58
41. Hall BG. 1990. Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* 126:5–16
42. Hall BG. 1992. Selection-induced mutations. *Curr. Opin. Genet. Dev.* 2:943–46
43. Hall BG. 1997. On the specificity of adaptive mutations. *Genetics* 145:39–44
44. Hall BG. 1998. Adaptive mutagenesis: a process that generates almost exclusively beneficial mutations. *Genetica* 102–103:109–25
45. Harris RS, Longerich S, Rosenberg SM. 1994. Recombination in adaptive mutation. *Science* 264:258–60
46. Harris RS, Ross KJ, Rosenberg SM. 1996. Opposing roles of the Holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. *Genetics* 142:681–91
47. Hastings PJ, Bull HJ, Klump JR, Rosenberg SM. 2000. Adaptive amplification: an inducible chromosomal instability mechanism. *Cell* 103:723–31
48. Hastings PJ, Slack A, Petrosino JF, Rosenberg SM. 2004. Adaptive amplification and point mutation are independent mechanisms: evidence for various stress-inducible mutation mechanisms. *PLoS Biol.* 2:e399

49. Hendrickson H, Slechta ES, Bergthorsson U, Andersson DI, Roth JR. 2002. Amplification-mutagenesis: Evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. *Proc. Natl. Acad. Sci. USA* 99:2164–69

49. Provides evidence that amplification is a necessary precursor of reversion under selection.

50. Hengge-Aronis R. 2002. Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. *Microbiol. Mol. Biol. Rev.* 66:373–95
51. Hughes D, Andersson DI. 1997. Carbon starvation of *Salmonella typhimurium* does not cause a general increase of mutation rates. *J. Bacteriol.* 179:6688–91

52. Kim SR, Matsui K, Yamada M, Gruz P, Nohmi T. 2001. Roles of chromosomal and episomal dinB genes encoding DNA Pol IV in targeted and untargeted mutagenesis in *Escherichia coli*. *Mol. Genet. Genomics* 266:207–15
53. Koch AL. 1997. Microbial physiology and ecology of slow growth. *Microbiol. Mol. Biol. Rev.* 61:305–18
54. Kofoid E, Bergthorsson U, Slechta ES, Roth JR. 2003. Formation of an F' plasmid by recombination between imperfectly repeated chromosomal Rep sequences: a closer look at an old friend (F'(128) pro lac). *J. Bacteriol.* 185:660–63
55. Konopka A. 2000. Microbial physiological state at low growth rate in natural and engineered ecosystems. *Curr. Opin. Microbiol.* 3:244–47
56. Deleted in proof
57. Layton JC, Foster PL. 2003. Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli*. *Mol. Microbiol.* 50:549–61
58. LeClerc JE, Li B, Payne WL, Cebula TA. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* 274:1208–11
59. Lederberg J, Lederberg EM. 1952. Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* 63:399–406
60. Levin BR, Bull JJ. 1994. Short-sighted evolution and the virulence of pathogenic microorganisms. *Trends Microbiol.* 2:76–81
61. Loewe L, Textor V, Scherer S. 2003. High deleterious genomic mutation rate in stationary phase of *Escherichia coli*. *Science* 302:1558–60
62. Lombardo MJ, Aponyi I, Rosenberg SM. 2004. General stress response regulator RpoS in adaptive mutation and amplification in *Escherichia coli*. *Genetics* 166:669–80
63. Luria SE, Delbruck M. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 28:491–11
64. Maisnier-Patin S, Roth JR, Fredriksson A, Nystrom T, Berg OG, Andersson DI. 2005. Genomic buffering mitigates the effects of deleterious mutations in bacteria. *Nat. Genet.* 37:1376–79
65. Mao EF, Lane L, Lee J, Miller JH. 1997. Proliferation of mutators in a cell population. *J. Bacteriol.* 179:417–22
66. McKenzie G, Lee P, Lombardo MJ, Hastings P, Rosenberg S. 2001. SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol. Cell* 7:571–79
67. Miller JH, Suthar A, Tai J, Yeung A, Truong C, Stewart JL. 1999. Direct selection for mutators in *Escherichia coli*. *J. Bacteriol.* 181:1576–84
68. Moxon ER, Rainey PB, Nowak MA, Lenski RE. 1994. Adaptive evolution of highly mutable loci in pathogenic bacteria. *Curr. Biol.* 4:24–33
69. Muller HJ. 1964. The relation of recombination to mutational advance. *Mutat. Res.* 106:2–9
70. Nigg EA. 2006. *Genome Instability in Cancer Development*. Berlin: Springer
71. Oliver A, Canton R, Campo P, Baquero F, Blazquez J. 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 288:1251–54
72. Pedraza-Reyes M, Yasbin RE. 2004. Contribution of the mismatch DNA repair system to the generation of stationary-phase-induced mutants of *Bacillus subtilis*. *J. Bacteriol.* 186:6485–91
73. Pettersson ME, Andersson DI, Roth JR, Berg OG. 2005. The amplification model for adaptive mutation: simulations and analysis. *Genetics* 169:1105–15

54. Shows the position of *lac* vis-à-vis nearby Rep elements and the *dinB* gene.

74. Ponder RG, Fonville NC, Rosenberg SM. 2005. A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation. *Mol. Cell* 19:791–804
75. Radicella JP, Park PU, Fox MS. 1995. Adaptive mutation in *Escherichia coli*: a role for conjugation. *Science* 268:418–20
76. Rainey PB. 1999. Evolutionary genetics: the economics of mutation. *Curr. Biol.* 9:R371–73
77. Rattray AJ, Strathern JN. 2003. Error-prone DNA polymerases: when making a mistake is the only way to get ahead. *Annu. Rev. Genet.* 37:31–66
78. Reddy M, Gowrishankar J. 1997. A genetic strategy to demonstrate the occurrence of spontaneous mutations in nondividing cells within colonies of *Escherichia coli*. *Genetics* 147:991–1001
79. Rosche WA, Foster PL. 1999. The role of transient hypermutators in adaptive mutation in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 96:6862–67
80. Rosche WA, Foster PL. 2000. Determining mutation rates in bacterial populations. *Methods* 20:4–17
81. Rosenberg S. 2001. Evolving responsively: adaptive mutation. *Nat. Rev. Genet.* 2:504–14
82. Rosenberg SM, Hastings PJ. 2004. Adaptive point mutation and adaptive amplification pathways in the *Escherichia coli* Lac system: stress responses producing genetic change. *J. Bacteriol.* 186:4838–43
83. Rosenberg SM, Longerich S, Gee P, Harris RS. 1994. Adaptive mutation by deletions in small mononucleotide repeats. *Science* 265:405–7
84. Roth JR, Andersson DI. 2004. Adaptive mutation: how growth under selection stimulates Lac(+) reversion by increasing target copy number. *J. Bacteriol.* 186:4855–60
85. Roth JR, Andersson DI. 2004. Amplification-mutagenesis—how growth under selection contributes to the origin of genetic diversity and explains the phenomenon of adaptive mutation. *Res. Microbiol.* 155:342–51
86. Roth JR, Kofoid E, Roth FP, Berg OG, Seger J, Andersson DI. 2003. Regulating general mutation rates: examination of the hypermutable state model for Cairnsian adaptive mutation. *Genetics* 163:1483–96
87. Deleted in proof
88. Shapiro JA. 1984. Observations on the formation of clones containing araB-lacZ cistron fusions. *Mol. Gen. Genet.* 194:79–90
89. Deleted in proof
90. Shapiro JA. 1995. Adaptive mutation: Who's really in the garden? *Science* 268:373–74
91. Slechta ES, Bunny K, Kugelberg E, Kofoid E, Andersson DI, Roth JR. 2003. Adaptive mutation: general mutagenesis is not a programmed response to stress but results from rare coamplification of dinB with lac. *Proc. Natl. Acad. Sci. USA* 100:12847–52
92. Slechta ES, Liu J, Andersson DI, Roth JR. 2002. Evidence that selected amplification of a bacterial *lac* frameshift allele stimulates Lac^+ reversion (adaptive mutation) with or without general hypermutability. *Genetics* 161:945–56
92a. Sniegowski PD. 1995. A test of the directed mutation hypothesis in *Escherichia coli* MCS2 using replica printing. *J. Bacteriol.* 177:1119–20
93. Sniegowski PD, Gerrish PJ, Johnson T, Shaver A. 2000. The evolution of mutation rates: separating causes from consequences. *Bioessays* 22:1057–66
94. Sniegowski PD, Gerrish PJ, Lenski RE. 1997. Evolution of high mutation rates in experimental populations of *E. coli*. *Nature* 387:703–5

79. A key paper providing evidence that about 90% of revertants arise without general mutagenesis.

86. Presents theoretical reasons that stress-induced general mutagenesis is unlikely to explain the Cairns system or to evolve under natural selection.

91. Shows that general mutagenesis observed under selection requires coamplification of *dinB* with *lac*.

95. Stahl FW. 1988. News and views: a unicorn in the garden. *Nature* 355:112–13
96. Steele DF, Jinks-Robertson S. 1992. An examination of adaptive reversion in *Saccharomyces cerevisiae*. *Genetics* 132:9–21
97. Stumpf JD, Foster PL. 2005. Polyphosphate kinase regulates error-prone replication by DNA polymerase IV in *Escherichia coli*. *Mol. Microbiol.* 57:751–61
98. Taddei F, Halliday JA, Matic I, Radman M. 1997. Genetic analysis of mutagenesis in aging *Escherichia coli* colonies. *Mol. Gen. Genet.* 256:277–81
99. Taddei F, Matic I, Radman M. 1995. cAMP-dependent SOS induction and mutagenesis in resting bacterial populations. *Proc. Natl. Acad. Sci. USA* 92:11736–40
100. Taddei F, Radman M, Maynard-Smith J, Toupance B, Gouyon PH, Godelle B. 1997. Role of mutator alleles in adaptive evolution. *Nature* 387:700–2
101. Tanaka MM, Bergstrom CT, Levin BR. 2003. The evolution of mutator genes in bacterial populations: the roles of environmental change and timing. *Genetics* 164:843–54
102. Torkelson J, Harris RS, Lombardo MJ, Nagendran J, Thulin C, Rosenberg SM. 1997. Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* 16:3303–11
103. Wagner J, Nohmi T. 2000. *Escherichia coli* DNA polymerase IV mutator activity: genetic requirements and mutational specificity. *J. Bacteriol.* 182:4587–95
104. Yang H, Wolff E, Kim M, Diep A, Miller JH. 2004. Identification of mutator genes and mutational pathways in *Escherichia coli* using a multicopy cloning approach. *Mol. Microbiol.* 53:283–95
105. Yang Z, Lu Z, Wang A. 2001. Study of adaptive mutations in *Salmonella typhimurium* by using a super-repressing mutant of a *trans* regulatory gene purR. *Mutat. Res.* 484:95–102
105a. Yang Z, Lu Z, Wang A. 2006. Adaptive mutators in *Salmonella typhimurium* phenotypic of *purR* super-repression. *Mutat. Res.* 595:107–16
106. Zambrano MM, Kolter R. 1996. GASPing for life in stationary phase. *Cell* 86:181–84

102. First evidence that general mutagenesis (albeit weak) is associated with reversion under selection.