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Stress-Induced Mutagenesis in Bacteria

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Abstract

Bacteria spend their lives buffeted by changing environmental conditions. To adapt to and survive these stresses, bacteria have global response systems that result in sweeping changes in gene expression and cellular metabolism. These responses are controlled by master regulators, which include: alternative sigma factors, such as RpoS and RpoH; small molecule effectors, such as ppGpp; gene repressors such as LexA; and, inorganic molecules, such as polyphosphate. The response pathways extensively overlap and are induced to various extents by the same environmental stresses. These stresses include nutritional deprivation, DNA damage, temperature shift, and exposure to antibiotics. All of these global stress responses include functions that can increase genetic variability. In particular, up-regulation and activation of error-prone DNA polymerases, down-regulation of error-correcting enzymes, and movement of mobile genetic elements are common features of several stress responses. The result is that under a variety of stressful conditions, bacteria are induced for genetic change. This transient mutator state may be important for adaptive evolution.

Keywords

adaptive mutation; stationary-phase mutation; SOS response; general stress response; error-prone polymerase; polyphosphate

INTRODUCTION

In a stimulating but controversial paper published in 1988, Cairns *et al.* (1988) described experiments suggesting that a population under selection had access to a process that could direct mutagenic change to the very genes that would relieve the selective pressure. Such a process, of course, would be a great boon to adaptive evolution (Fitch, 1982). After nearly 20 years of research, evidence now suggests that various types of stresses induce responses that have mutagenic consequences, and that sometimes this essentially random process can appear to be directed. Originally it was also thought that there was just one mechanism for all cases of adaptive mutation. This idea also proved to be wrong–it seems there are many mechanisms by which genetic change can be produced when organisms are under stress.

This review concentrates on stress-induced mutagenesis in *Escherichia coli* and related bacteria with a few examples from other organisms. The mutagenic phenomena are variously called adaptive, stationary-phase, stress-induced, or starvation-induced mutagenesis (see Table 1). In the first section, I describe some of the global responses to stress in bacteria, and how each can result in increased mutation rates. In the second section, I give some specific examples of stress-induced mutagenesis (see Table 2), and then discuss in greater detail what we have learned from studying a particular case, adaptive mutation to Lac^+ in *E. coli*. I conclude with a

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discussion of the potential impact that stress-induced mutagenesis may have on survival and adaptation.

GLOBAL STRESS RESPONSES

The SOS Response

The stress that most obviously and directly results in genetic change is exposure to DNA damaging agents. *E. coli* and other bacteria have an extensive and highly effective response to DNA damage, the SOS response, that minimizes the lethal and mutagenic consequences of such exposure (for reviews, see Friedberg *et al.*, 2006; Kelley, 2006). When the cell's DNA is damaged, regions of single-stranded DNA arise, either directly as a result of the damage or indirectly when the DNA is repaired or when DNA replication is stalled. The ssDNA is recognized and bound by RecA, the bacterial recombinase, forming nucleoprotein complexes. These complexes stimulate the self-cleavage of the LexA repressor. The destruction of LexA results in the derepression of the SOS genes, many of which encode enzymes that promote DNA repair, recombination, and DNA synthesis past lesions that block replication. Approximately 30 genes are known to be repressed by LexA in *E. coli* (Fernandez de Henestrosa *et al.*, 2000; Wade *et al.*, 2005); *Bacillus subtilis* has a similar number, but only eight are homologs of the *E. coli* genes (Au *et al.*, 2005).

Three of the genes induced as part of the SOS response in *E. coli* encode DNA polymerases that have the ability to replicate damaged DNA (for reviews, see Fuchs *et al.*, 2004; Lehmann, 2006; Nohmi, 2006; Tippin *et al.*, 2004). These polymerases temporarily take the place of the replicative polymerase (Pol III) and synthesize past blocking lesions in the DNA template. One of the SOS polymerases is Pol II, a conventional polymerase that under most circumstances is accurate and processive. Pol II can bypass some DNA lesions and can extend synthesis when lesions are bypassed by other polymerases; its major role may be to restart stalled replication forks. Two of the SOS polymerases belong to the widely distributed Y-family of specialized polymerases. Members of this family are characterized not only by their ability to replicate damaged DNA, but also by their lack of processivity and low fidelity when copying undamaged template. Pol V, encoded by the *umuDC* genes, can replicate past many types of DNA lesions, and is error-prone on both damaged and undamaged DNA. Pol IV, encoded by the *dinB* gene, is also error-prone, but the repertoire of lesions that it can bypass is limited. Pol IV readily extends DNA synthesis from a mispaired or misaligned primer-template terminus, and thus, like Pol II, may restart stalled replication forks and extend replication after lesion-bypass by another polymerase. In such situations, Pol IV and Pol II may be in competition.

In normally growing, undamaged cells neither Pol IV (Kuban *et al.*, 2004; McKenzie *et al.*, 2003; Strauss *et al.*, 2000; Wolff *et al.*, 2004) nor Pol V (Kuban *et al.*, 2006) contributes much to spontaneous mutation rates. Without SOS induction the cellular levels of Pol V are undetectable (Woodgate *et al.*, 1991), so it is not surprising that its loss has no phenotype. In contrast, uninduced cells have about 250 molecules of Pol IV (Kim *et al.*, 2001), a fairly large number for a polymerase (for example, there are 10 to 20 copies of Pol III holoenzyme; Wu *et al.*, 1984). With that many copies it is not clear why, under most conditions, Pol IV has so little mutational effect. However, Pol IV does contribute to spontaneous mutagenesis of genes on the large conjugal plasmid, F (Kuban *et al.*, 2004) and on the bacteriophage lambda chromosome (Brotcorne-Lannoye *et al.*, 1986). In addition, Pol IV is active in nongrowing cells (discussed below).

When induced *via* the SOS response or other pathways (see below), both Pol IV and Pol V increase the cell's spontaneous mutation rate even in the absence of DNA damage. This phenotype can be observed in the laboratory using mutant strains with a constitutively activated SOS response. In such strains Pol IV accounts for about a 3-fold, and Pol V accounts for about

a 10-fold, increase in mutation frequencies (Kuban *et al.*, 2006). The mutations are presumably due to errors made by the polymerases replicating undamaged DNA (*i.e.*, they are "untargeted" mutations), although spontaneous DNA damage may also contribute. In the absence of SOS induction, overproduction of Pol IV is also a powerful mutator–simply supplying the *dinB* gene on a plasmid increases mutation frequencies as much as 100-fold (Kim *et al.*, 1997; Wagner *et al.*, 2000). Pol V cannot be overproduced so simply because to be active, UmuC (the polymerase subunit) must associate with two UmuD subunits that have undergone a RecA-facilitated autocleavage reaction; RecA is additionally required to stimulate Pol V activity (Schlacher *et al.*, 2006). However, Pol V homologues on many naturally-occurring plasmids are not so stringent. For example, the RumAB protein, originally found on a conjugal R (antibiotic resistance) plasmid, increases the spontaneous mutation frequency of an uninduced *E. coli* strain four-fold when on a low-copy plasmid; in a strain constitutively expressing SOS, it has 50-fold effect (Mead *et al.*, 2007). Many of these Pol V homologues are not as active when in their native locations, suggesting that there are additional functions on plasmids in nature that regulate their mutagenic activity (Koch *et al.*, 2000).

Exposure to exogenous DNA damaging agents is not the only way that the SOS response can be induced. DNA damage from metabolic intermediates or mistakes in replication, recombination, and chromosome segregation can be powerful SOS stimuli if not repaired (O'Reilly *et al.*, 2004). The SOS response will also be induced, at least partially, whenever the levels of active LexA decline. For example, LexA is inactivated *in vitro* under slightly alkaline conditions (Little, 1991), and *in vivo* in aging colonies (Taddei *et al.*, 1995) and when cells reach saturation in rich medium (Dri *et al.*, 1994). Other, seemingly unrelated, stresses can activate the SOS response; these include exposure to antibiotics (see below) and high pressure (1000-fold higher than atmospheric) (Aertsen *et al.*, 2006; Bjedov *et al.*, 2003). Interestingly, high-pressure SOS-induction is triggered by double-strand breaks induced by one of *E. coli's* restriction endonucleases acting on its own DNA (Aertsen *et al.*, 2005).

The General Stress Response

When *E. coli* and its relatives enter stationary phase or experience nutrient limitation they induce the "general stress response" (for a review see Hengge-Aronis, 2002). The master controller of this response is the sigma factor RpoS, also called σ^{38}, σ^{S}, or KatF. Sigma factors are the subunits of RNA polymerase (RNAP) that direct transcription to particular classes of promoters. During exponential growth under aerobic conditions, most genes are transcribed by RNAP complexed with the vegetative sigma factor, σ^{70}, also called RpoD (the holoenzymes are designated as Eσ^{70} or Eσ^{38}). As cells enter stationary phase the amount of active RpoS dramatically increases, resulting in the formation of Eσ^{38} that transcribes genes required for survival during starvation and other stresses. In *E. coli*, over 200 genes are, directly or indirectly, under RpoS control (Patten *et al.*, 2004; Weber *et al.*, 2005). In addition to nutrient limitation and starvation, the general stress response is induced by growth-rate reduction, high osmotic pressure, low pH, and extreme shifts in temperature. The induction and activation of RpoS is controlled at several levels by a variety of *cis*- and *trans*-acting regulatory elements; in addition, the RpoS regulon overlaps extensively with other global response networks. This complex regulation ensures that the stressed cells are prepared for a variety of contingencies.

There are two ways currently known by which the RpoS-dependent general stress response can increase the spontaneous mutation rate of stressed cells. The first is by up-regulating the error-prone DNA polymerase, Pol IV. About 10 hours after *E. coli* enters stationary phase, the cellular levels of Pol IV increase about three-fold. Surprisingly, this increase is independent of the SOS response but is, instead, RpoS-dependent. In the absence of RpoS Pol IV is rapidly lost, so that after 48 hours in stationary phase, RpoS-proficient cells have 30 to 50 times the amount of Pol IV as do RpoS-deficient cells (Layton *et al.*, 2003). The Pol IV protein is

unstable, and its instability is not affected by RpoS (as measured by quantitative immunoblots after protein synthesis inhibition; Storvick *et al.*, 2007); therefore, continuous transcription of the *dinB* gene under RpoS control is required to maintain the high levels of Pol IV during stationary phase. This RpoS-dependent induction of Pol IV is required for Lac^+ adaptive mutation (see below). In addition, maintenance of Pol IV transcription is required for survival and for adaptive changes in *E. coli* populations during long-term stationary phase (Yeiser *et al.*, 2002).

The second way that RpoS enhances spontaneous mutation rates during stress is by down-regulating enzymes that are responsible for mismatch repair (MMR). In *E. coli* and other bacteria, the MMR complex recognizes base mismatches in the DNA and excises the newly synthesized DNA strand; the old strand then serves as template for new synthesis. *E. coli* normally discriminates between the newly synthesized (presumably error-containing) strand and the template (correct) strand by their methylation state–only newly-synthesized DNA is unmethylated (for a review, see Kunkel *et al.*, 2005). Although MMR remains active in stationary-phase cells (Foster, 1999a; Reddy *et al.*, 1997), the levels of two of the MMR proteins, MutS and MutH, are down-regulated in an RpoS-dependent manner (Feng *et al.*, 1996; Tsui *et al.*, 1997). Two types of evidence indicate that this down-regulation can, at least under some circumstances, increase mutation rates: (1) the frequency of mutation is decreased if one or more of the MMR proteins is overproduced (Bjedov *et al.*, 2003; Foster *et al.*, 1995a; Harris *et al.*, 1999; Zhao *et al.*, 2000); and (2) the frequency of mutation is not increased if MMR is genetically eliminated (Bjedov *et al.*, 2003). Which MMR protein becomes limiting in stationary phase differs among experiments, experimenters, and (perhaps) genetic backgrounds (Bjedov *et al.*, 2003; Foster, 1999a; Harris *et al.*, 1999; Zhao *et al.*, 2000). The contribution that MMR decline makes to stationary-phase mutation is also debated. As discussed below, in nutrient-limited populations mutations do arise in MMR proficient cells (Rosche *et al.*, 1999). In addition, loss of the MMR system cannot produce mutations *de novo* but can only preserve errors made during previous DNA synthesis.

RpoS has also been implicated in stress-induced mutation in ways that are less well understood than the above examples. Epistasis tests show that in addition to up-regulating Pol IV, RpoS has a second role in Lac^+ adaptive mutation (Layton *et al.*, 2003). RpoS is also required for amplification of the *lac* region during lactose selection (Lombardo *et al.*, 2004). RpoS is required for base substitution mutations in aging *E. coli* colonies (Bjedov *et al.*, 2003), frameshift mutations in starved *Pseudomonas putida* cells (Saumaa *et al.*, 2006), and transposition and transposon-induced rearrangements in starving *E. coli* (Gomez-Gomez *et al.*, 1997; Lamrani *et al.*, 1999) and *P. putida* (Ilves *et al.*, 2001). Although the exact pathways by which RpoS exerts its effects may not be the same in all of these examples, these various mutagenic events are consequences of the RpoS-dependent general stress response.

Polyphosphate-Mediated Nutrient-Limitation Response

Polyphosphate (polyP) consists of chains of phosphates, tens to hundreds of residues long, linked together by high energy phosphoanhydride bonds. Although polyP is found in organisms from all domains life, in most cases its function remains a mystery (for reviews, see Kornberg *et al.*, 1999; Kulaev *et al.*, 2000; Shiba *et al.*, 2000). PolyP is made by polyphosphate kinase (Ppk), which transfers the gamma phosphate of ATP onto the growing phosphate chain. PolyP accumulates when cells enter stationary phase or undergo nutritional deprivation. In *E. coli*, a large part of this accumulation is due to the inhibition of the major enzyme that degrades polyP, exopolyphosphatase (Ppx), by (p)ppGpp, which is induced by the stringent response (discussed below) (Kuroda *et al.*, 1997). Since polyP has profound effects on cell physiology, loss of Ppk or overproduction of Ppx has pleiotropic effects. Of particular relevance here, polyP is required

for the transcriptional induction of RpoS in stationary phase (Shiba *et al.*, 1997), and of RecA after DNA damage (Tsutsumi *et al.*, 2000).

Some of the effects of PolyP may be due to the fact that it acts as a DNA mimic and binds to DNA binding proteins. PolyP can inhibit the *in vitro* reactions of restriction enzymes, ligase, and Taq polymerase (Rodriguez, 1993). However, polyP-binding may also serve a regulatory function. For example, RNA polymerase isolated from *E. coli* cells in stationary phase, but not from cells in exponential phase, was found to be complexed with polyP (Kusano *et al.*, 1997). Kusano *et al.* (1997) further showed that at low salt concentrations polyP inhibited the *in vitro* transcriptional activity of both Eσ^{70} and Eσ^{S}; but, at high salt concentrations polyP stimulated transcription by Eσ^{S} and not Eσ^{70}. These results suggest that, at least at high osmolarity, polyP helps RNA polymerase to distinguish between vegetative and stationary-phase promoters (Kusano *et al.*, 1997). Lon, which is a major ATP-dependent protease, is also a DNA-binding protein. PolyP binds to purified Lon and stimulates it to degrade ribosomal proteins, which also bind polyP (Kuroda *et al.*, 2001).

Recently we discovered that polyP regulates the activity of *E. coli*'s Y-family polymerases (Stumpf *et al.*, 2005). Pol IV-dependent Lac^{+} adaptive mutation is reduced by 65 to 80% in a *ppk* mutant strain. Overproduction of Pol IV in exponentially growing cells is profoundly mutagenic, and loss of Ppk inhibits this mutagenesis as well. Yet, in both of these cases the amount of Pol IV is not affected, but only its activity. The pathway by which Ppk activates Pol IV is independent of Pol IV's regulation by RpoS or RecA. Pol V-dependent UV-induced mutations are also reduced in a *ppk* mutant strain (Stumpf *et al.*, 2005); likewise, Pol IV's lesion-bypass activity is lost in a *ppk* mutant strain (Stumpf *et al.*, 2007a). Although we favor the hypothesis that polyP is the active agent, we have not ruled out the possibility that it is the Ppk enzyme itself that is exerting these effects. Taken together, however, these results suggest that the build-up of polyP in stationary phase and during nutritional deprivation stimulates the mutagenic and lesion bypass activities of the Y-family polymerases.

The Stringent Response

When *E. coli* and other bacteria are starved for amino acids or experience nutritional down-shift, the synthesis of stable RNA species (tRNA and rRNA) is rapidly down-regulated. This reaction, called the "stringent response," is mediated by the unusual guanosine nucleotides pppGpp and ppGpp, together known as (p)ppGpp. Decades of research has revealed the complex genetic control of the stringent response and much of the physiological effects of (p)ppGpp accumulation. The stringent response is global, encompassing not only down-regulation of stable RNA, but also down-regulation of protein synthesis, up-regulation of protein turnover, and activation of amino acid biosynthesis (for recent reviews, see Braeken *et al.*, 2006; Magnusson *et al.*, 2005).

(p)ppGpp (together with the co-effector DskA) regulates transcription by binding to RNAP and changing the distribution of its transcriptional activity at various promoters (for a review see Gourse *et al.*, 2006). One promoter that is positively, but probably indirectly, regulated by (p)ppGpp is RpoS; in addition, many RpoS-dependent genes require (p)ppGpp for their induction (Kvint *et al.*, 2000). Thus, the RpoS-dependent general stress response, discussed above, and the ppGpp-dependent stringent response are co-induced and overlapping (Chang *et al.*, 2002; Traxler *et al.*, 2006). (p)ppGpp also positively regulates a large number of genes encoding enzymes for the biosynthesis and uptake of amino acids (Cashel *et al.*, 1996; Paul *et al.*, 2005). Because amino acid biosynthetic genes tend to be regulated by end-product repression, they will be transcriptionally activated both by the stringent response and by derepression during starvation for amino acids.

Transcription creates a bubble of single-stranded DNA and single-stranded DNA is more susceptible than double-stranded DNA to a variety of spontaneous damage. For example, cytosine deaminates to uracil 100 times faster in single stranded DNA than in double stranded DNA (although the half-life of a cytosine in single stranded DNA is still 200 years) (Frederico *et al*., 1990). Since actively transcribed genes have more single-stranded DNA, they would be expected to sustain more potentially mutagenic damage. In addition, DNA repair is recruited to transcribed genes (for a review, see Mellon, 2005); while this transcription-coupled repair would be expected to reduce mutations, the resulting more frequent DNA synthesis could also create mutations. If transcription is intrinsically mutagenic, during nutrient deprivation mutations would be "directed" to actively transcribed genes that could be the very ones that, when mutated, might relieve the stress (Davis, 1989; Fitch, 1982; Wright, 2004). For example, the transcriptional activation of amino acid biosynthetic genes would make them potentially more vulnerable to mutation during amino acid starvation (Wright *et al.*, 1999).

Active transcription of a gene has been reported to increase its spontaneous mutation rate in *E. coli* (Beletskii *et al.*, 1996; Klapacz *et al.*, 2002; Klapacz *et al.*, 2005; Wright *et al.*, 1999), *B. subtilis* (Rudner *et al.*, 1999), and in the yeast, *Saccharomyces cerevisiae* (Datta *et al.*, 1995). In some experiments, the increase in mutation was dependent on the bacteria being competent for the stringent response (Reimers *et al.*, 2004; Rudner *et al.*, 1999). Interestingly, stationary-phase mutation in *B. subtilis* cells is dependent on Mfd, the factor that couples DNA repair to transcription (Ross *et al.*, 2006) (although apparently this is not the case in *E. coli*; Bridges, 1995b). This last result suggests that transcription-coupled DNA repair may, at least in some circumstances, stimulate error-prone DNA synthesis.

While the results referenced above indicate that transcription can be mutagenic, in other cases it has been shown not to be. For example, gratuitous induction of the *lac* operon in the absence lactose did not increase the number of Lac^+ revertants subsequently recovered (Foster *et al.*, 1992). Revertants of a histidine auxotrophy did not accumulate when *Salmonella typhimurium* cells were incubated in the absence of histidine (Hughes *et al.*, 1997). In one case, the number of Trp^+ revertants increased during starvation of *E. coli* for tryptophan in the absence of transcription of the gene (Barionovi *et al.*, 2003). Clearly more research is needed before the impact of transcription on mutation rates is understood.

Induction of the stringent response causes DNA replication to halt, presumably to await the restoration of nutrients (Gourse *et al.*, 2007). However, the way in which DNA replication is arrested appears to differ among bacteria. In *E. coli*, (p)ppGpp inhibits replication initiation (Schreiber *et al.*, 1995), whereas in *B. subtilis* (p)ppGpp inhibits replication elongation (Autret *et al.*, 1999). Recently it was found that in *B. subtilis*, (p)ppGpp binds to RNA primase, inhibiting its activity and thus halting the replication fork (Wang *et al.*, 2007). The forks are not disrupted, at least in the short term–they do not recruit RecA and are thus not perceived by the cells as needing repair. However, after a prolonged arrest replication might be restarted and, if the process involved error-prone polymerases, be a source of mutations (Wang *et al.*, 2007).

What is Nutritional Stress?

The hallmark of adaptive mutation is that mutations occur not when the bacteria are merely starving, but only when they are incubated under a specific nutritional selection, *e.g.*, Lac^- cells incubated on lactose medium. Indeed, several studies have shown that mutations do not usually accumulate when cells are incubated without any nutrients at all (Cairns *et al.*, 1988; Cairns *et al.*, 1991; Foster, 1997; Hughes *et al.*, 1997) (although this is not true of movement of IS elements; see below). In some cases of starvation-induced mutagenesis, the starving population may be dynamic, with some cells dying and others slowly proliferating (Saumaa *et al.*, 2006). Even when cells are incubated for long periods in spent medium, they are not

without nutrients–mutant bacteria able to survive on the waste products of their neighbors periodically appear and sweep the population (Finkel, 2006).

The failure to observe mutations in the absence of nutritional selection suggests that some small amount of energy is required for the mutational process. For example, in the case of adaptive mutation to Lac^+ the mutant *lac* allele is slightly leaky, making about two units of β-galactosidase, an amount that is not sufficient for cell proliferation. As discussed below, our model for adaptive mutation postulates that a double-strand break causes a replication fork to collapse, initiating the recombination that leads to Lac^+ mutation. We propose that the role of lactose is simply to supply a slow influx of energy that allows occasional DNA replication (Stumpf *et al.*, 2007b). In support of this hypothesis, lactose is not required if double-strand breaks are artificially induced (Ponder *et al.*, 2005).

When cells are not starving but growing in nutrient-limited chemostats they express what has been called “the hunger response” (for a review, see Ferenci, 2001). This response is characterized by up-regulation of scavenging functions, and is distinguished from response to starvation by its independence of RpoS. Indeed, the hunger response is antagonized by RpoS, probably because RpoS competes with RpoD or other sigma factors required for transcription of the scavenging pathway genes (Ferenci, 2001; Notley-McRobb *et al.*, 2002). In contrast, starvation-induced or adaptive mutation usually requires RpoS function (see below), suggesting that “hungry” bacteria do not indulge in such mutagenesis.

Since natural populations of *E. coli* most likely experience frequent periods of nutrient scarcity, their periodic requirement for scavenging functions may explain why *E. coli* populations are heterogenic for *rpoS* alleles. Attenuation of RpoS function is frequently selected in long-term stationary-phase cultures, during which cells evolve heightened abilities to scavenge various micronutrients (Finkel, 2006). But the fact that null alleles of RpoS are not selected suggests that additional RpoS-regulated functions that are dispensable in nutrient-limited chemostats are useful to cells during long-term stationary phase.

The Heat-Shock Response

When subjected to a rapid increase in external temperature, all organisms respond by inducing proteins that increase their thermo-tolerance. In *E. coli* and other bacteria, the heat-shock response is mainly under control of the sigma factor RpoH (σ^{32}); another sigma factor, RpoE (σ^{24}), also participates in response to extreme temperature elevation and envelope stress (for reviews see Gruber *et al.*, 2003; Rosen *et al.*, 2002; Yura *et al.*, 2000). The RpoH-regulon is induced, in whole or in part, by conditions that result in unfolded proteins and cytoplasmic stress. In addition to heat, these include DNA damage, oxidative stress, exposure to antibiotics or heavy metals, phage infection, and starvation for carbon source or amino acids. Thus, induction of the RpoH-regulon is a general stress response.

The heat-shock proteins include molecular chaperones that aid in protein folding and proteases that degrade unwanted or damaged proteins. The molecular chaperone GroE (*E. coli*’s HSP60) interacts with at least 250 *E. coli* proteins; 85 of these, including 13 essential proteins, require GroE for activity (Kerner *et al.*, 2005). Thus, GroE is essential at all temperatures. UV mutagenesis is GroE-dependent because GroE interacts with UmuC, the polymerase subunit of Pol V, and protects it from degradation (Donnelly *et al.*, 1989; Liu *et al.*, 1990). GroE is likely also to interact with Pol IV. Cells that are deficient for GroE have only 10% of Pol IV compared with GroE proficient cells, and GroE is required for Pol IV-dependent adaptive mutation (Layton *et al.*, 2005). Presumably GroE protects Pol IV from degradation, although there may be an additional Pol IV-independent role for GroE in adaptive mutation (Layton *et al.*, 2005). Thus, during stress conditions that induce the RpoH-regulon, both Y-family DNA

polymerases, Pol IV and Pol V, will be protected from degradation by GroE, and thus able to perform their mutagenic functions.

Exposure to Antibiotics

Antibiotics of the quinolone class, such as ciprofloxacin and nalidixic acid, target gyrase and topoisomerase IV, enzymes that coil and uncoil DNA. These antibiotics 'trap' the topoisomerase-DNA complex after the DNA strands have been cleaved but before they are rejoined. Thus a double-strand break is produced that, after RecBCD produces a region of single-stranded DNA, induces the SOS response (for a review, see Drlica *et al.*, 1997). Mutations in the genes that encode gyrase and topo IV can convey resistance to the antibiotics. The quinolone antibiotics are clearly mutagenic (for example, see Mamber *et al.*, 1993), so it would not be surprising if resistance to the antibiotics were induced by exposure to them. Indeed, recently it was shown using a mouse-infection model that pathogenic *E. coli* became resistant to ciprofloxacin after exposure to it (Cirz *et al.*, 2005). In a laboratory reconstruction, all three SOS-induced DNA polymerases (Pol II, IV, and V) were involved in this mutational response (Cirz *et al.*, 2005). Interestingly, when *E. coli* cells were plated on medium containing a bacteriostatic concentration of ciprofloxacin, resistant mutants appeared over the course of 2 weeks–conditions very similar to those used in adaptive mutation experiments (see below). In addition to simply inducing mutations, exposure to quinolone antibiotics can produce antibiotic resistances in other ways. For example, exposure of *Vibrio cholerae* to ciprofloxacin can induce the movement of integrating conjugative elements (ICEs), some of which carry genes that confer antibiotic resistances (Beaber *et al.*, 2004).

Toxin-antitoxin systems are often encoded on large, low copy plasmids to ensure plasmid transmission. Typically the toxin is long-lived and the antitoxin is short-lived, so a cell that does not inherit the plasmid will die. Several of the toxins, such as microcin B17 and CcdB, are topoisomerase inhibitors with activities similar to the quinolones, and these toxins similarly induce the SOS response (Couturier *et al.*, 1998; Drlica *et al.*, 1997). Recently it was reported that transcription of CcdB increases as cells enter stationary phase, and that CcdB enhances adaptive mutation to Lac^+. Thus, plasmids carrying such toxins may increase the genetic diversity of their host cells during times of nutritional deprivation (Aguirre-Ramirez *et al.*, 2006).

β–lactam antibiotics, such as penicillin, also induce the SOS response but do so by a completely different pathway (Miller *et al.*, 2004). These antibiotics act by binding to the penicillin binding proteins (PBPs) and disrupting cell wall synthesis. Thus, cells that are not actively dividing are resistant to killing by *β*-lactams. Inactivation of PBP3, either by exposure to *β*-lactam antibiotics or genetically, induces the *dpiBA* operon, which encodes a response-effector two-component system (for a review of two-component systems, see Mascher *et al.*, 2006). DpiA, the effector, binds to the chromosomal replication origin, inhibiting replication and inducing the SOS response (Miller *et al.*, 2003). One of the SOS genes induced, *sulA* (also known as *sfiA*), encodes an inhibitor of cell division, and inhibition of cell division protects the cell, at least temporarily, from killing by the *β*-lactam antibiotic (Miller *et al.*, 2004). While this protection is likely the selective advantage that shaped the *dpiBA* response, a consequence of inducing the SOS response is an increase in genetic variability (Miller *et al.*, 2004). Exposure to *β*-lactam antibiotics also induces the *dinB* gene via a SOS-independent pathway (Perez-Capilla *et al.*, 2005). Thus, exposure to both *β*-lactam and quinolone antibiotics appears to produce a state of global genetic instability in bacteria.

Sublethal doses of streptomycin produce a mutator state that is independent of SOS mutagenic functions (Balashov *et al.*, 2002). Streptomycin and other amino-glycoside antibiotics interfere with ribosome function and inhibit translation. Exposure to sublethal doses of streptomycin has long been known to result in inaccurate translation (Balashov *et al.*, 2003; Rosset *et al.*,

1969). As first proposed by Ninio (1991), mistranslation of DNA repair and replication proteins can create transient mutator states. For example, mutations that result in certain mutant tRNAs have mutator phenotypes because the proofreading subunit of the DNA polymerase III holoenzyme is mistranslated, making replication error-prone (Al Mamun *et al.*, 2002; Slupska *et al.*, 1998). The mutator phenotype induced by exposure to streptomycin is likely to have a similar cause.

STRESS-INDUCED MUTAGENESIS

In the sections below, I describe examples of what appear to be stress-induced mutagenesis (summarized in Table 2). This survey is not intended to be comprehensive, but instead to be an exploration of the ways that stress can increase genetic variability. Hence, I have chosen examples from *E. coli* and other bacteria that reveal the genetic factors coupling stress to mutagenic mechanisms. In the last part of this section I discuss the well studied Lac^+adaptive mutation in *E. coli*.

Starvation-Induced Mutation on Solid Surfaces

Bacteria living on solid surfaces display some phenotypes that are not exhibited by planktonic bacteria (Ben-Jacob *et al.*, 1998; DiLuzio *et al.*, 2005; Kolter *et al.*, 2006; Shapiro, 1995). Taddei *et al.* (1995; 1997a) discovered that the SOS response was induced in colonies of *E. coli* as they aged for a week on rich agar medium; concomitantly, the frequency of rifampicin resistant (Rif^R) mutants within the colonies increased10-fold. Calling the phenomenon ROSE, for “resting organisms in a structured environment”, Taddei *et al.* (1995) found that cyclic AMP was required for both SOS induction and ROSE mutagenesis, suggesting that declining energy reserves signal the response. However ROSE was independent of RpoS. ROSE mutagenesis was completely dependent on RecA, UvrB (encoding a component of the nucleotide excision repair complex), and DNA polymerase I (Pol I) but independent of Pol V and only slightly dependent on RecBCD. ROSE mutagenesis required the expression of at least one LexA-repressed gene in addition to RecA (which could be UvrB). These results suggested that ROSE mutagenesis results from error-prone DNA synthesis, perhaps by Pol I, stimulated by excision repair (Taddei *et al.*, 1997a).

Because the frequency of Rif^R mutants did not increase when *E. coli* was incubated for a week in liquid rich medium (Taddei *et al.*, 1997a), even though the SOS response is induced under such conditions (Taddei *et al.*, 1995), ROSE mutagenesis suggests that the special conditions inside a colony result in DNA damage. A number of studies have found that inactivation of the enzymes for repair of oxidative and alkylation damage has severe mutagenic consequences in nondividing cells on agar surfaces (Benov *et al.*, 1996; Bharatan *et al.*, 2004; Bridges, 1995a; Bridges *et al.*, 1998; Bridges *et al.*, 2001; Foster *et al.*, 1992; Mackay *et al.*, 1994; Rebeck *et al.*, 1991). Thus, DNA damage by endogenous oxidative and alkylating agents appears to be rampant during prolonged incubation on solid surfaces.

Most studies of stress-induced mutagenesis use laboratory strains of bacteria that may have diverged substantially from their free-living ancestors. Bjedov *et al.* (2003) collected 787 *E. coli* isolates from various environments around the world and assayed them for mutagenesis using the ROSE method. Because the mutagenic phenomenon appeared to have different genetic requirements than ROSE (see Table 2), they named it MAC for “mutagenesis in aging colonies.” Most isolates showed an increase in the frequency of Rif^R mutants within their colonies during 7 days of incubation; 40% had increases that were 10 times the median and 13% had increases that were 100 times the median. About 3% of the isolates were constitutive mutators (*i.e.* a high frequency of Rif^R mutants on day one). Interestingly, there was a negative correlation between an isolate’s frequency of Rif^R mutants on day one and its fold-increase in this frequency by day 7, suggesting that bacteria have either high constitutive mutation rates,

or high inducible mutation rates, but not both. In a sample of ten isolates, MAC proved to be dependent on carbon source starvation, aerobic conditions, and incubation on a solid substrate. The genetic requirements for MAC of one isolate were explored. In this isolate, MAC was dependent on RpoS, cyclic-AMP, the cAMP receptor protein (required for catabolite repression and activation), and RecA; MAC was independent of LexA inactivation. Overproduction of MutS inhibited MAC, suggesting that MMR declines in the aging cells. In contrast to ROSE (see above), MAC was not dependent on nucleotide excision repair or DNA polymerase I, but was dependent on DNA polymerase II.

This study by Bjedov *et al.* (2003) is by far the most far-reaching analysis of stress-induced mutagenesis in bacteria to date. Since the isolates were from various environments and hosts, and included both commensal and pathogenic strains, it is clear that the phenomenon is widespread. There was a tendency for pathogenic isolates to have high constitutive mutation frequencies and for nonpathogenic isolates to have high MAC frequencies, suggesting that pathogens experience rapidly changing conditions, whereas commensals experience rather static environments punctuated by periods of stress.

Mutagenesis Due to Spontaneous DNA Damage

As mentioned above, exogenous and endogenous agents may cause extensive DNA damage in nongrowing cells. The mutations that result will be dependent not only on the nature of the damage, but also on how active the enzymes that repair the damage are in nongrowing cells. Although some types of damage, such as alkyl-lesions, may cause mutations directly, most damage-induced mutations depend on induction of the SOS response.

Many alleles of the *E. coli trp* genes revert only *via* specific mutations (Yanofsky *et al.*, 1966; Yanofsky *et al.*, 1987) and are useful for mutational studies (for examples, see Eisenstadt, 1987). Some of these mutant alleles revert to Trp^+ continuously during several days of selection for tryptophan prototrophy. Bhamre *et al.* (2001) found that the revertants of *trpA23* that accumulate during ten days of tryptophan selection were predominantly AT base pair transversions at the *trpA23* site. This accumulation was dependent on SOS induction of Pol V, and was enhanced if the strain had a plasmid carrying the MucAB homologs of Pol V. In contrast, Timms *et al.* (1999) found that in a *mutY* mutant strain, Pol V-dependent GC to CG mutations that reverted *trpA23* accumulated during selection (in addition there was a substantial level of SOS- and Pol V- independent reversion during these experiments). Because MutY encodes a glycosylase that removes A paired with 8-oxoG, Timms *et al.* (1999) concluded that 8-oxoG was a major spontaneous mutagenic lesion in nutritionally deprived cells. However, this may only be true in a *mutY* mutant strain, as the mutations at AT base pairs found by Bhamre *et al.* (2001) in MutYproficient cells are not easily explained by 8-oxoG lesions. Nonetheless, oxidative damage has been implicated in other cases of mutation in nutritionally deprived cells (Benov *et al.*, 1996; Bharatan *et al.*, 2004; Bridges, 1995a; Bridges *et al.*, 1998; Bridges *et al.*, 2001). Using a system designed to distinguish between mutations occurring in nongrowing and growing cells, Bhratan *et al.* (2004) found that MutY, MutT (which hydrolyzes 8-oxoGTP), and glycosylases that remove oxidized pyrimidines, were active in preventing mutations in nongrowing cells. Given the large contribution that oxidative damage makes to spontaneous mutation in growing cells (Bhamre *et al.*, 2001; Miller, 1996; Sakai *et al.*, 2006), and the oxygen-induced damage that dormant cells suffer (Eisenstark *et al.*, 1992), it is not surprising that oxidative damage produces major mutagenic damage in nongrowing cells.

DNA alkylation by endogenous alkylating agents is an important source of spontaneous DNA damage, and cells have several repair activities to cope with this damage (for a review, see Sedgwick *et al.*, 2007). Two enzymes, Ada and Ogt, remove alkyl groups from the O^6 position of guanine and the O^4 position of thymine. Loss of either or both of these enzymes dramatically

increases spontaneous mutation in nongrowing cells (Bharatan *et al.*, 2004; Foster *et al.*, 1992; Mackay *et al.*, 1994; Rebeck *et al.*, 1991). The mutagenic lesions are likely to be produced by methylation agents created by the enzymatic nitrosation of amides (Taverna *et al.*, 1996). Ogt is constitutively expressed, but Ada is induced by alkylation damage and, in stationary phase, under control of RpoS (Taverna *et al.*, 1996). In nongrowing cells, the levels of Ada are 20-fold higher than in rapidly growing cells, suggesting that aging cells are particularly subject to alkylation damage (Taverna *et al.*, 1996).

Stress-induced Mutation in Other Bacteria

This review focuses on *E. coli* and its close relatives, but studies of other bacteria and microorganisms have revealed that the phenomenon of stress-induced or stationary-phase mutagenesis is general. Below I describe experiments characterizing mutation in nongrowing cells of *Pseudomonas putida* and *Bacillus subtilis*.

P. putida cells carrying plasmid-encoded promoterless *pheAB* genes cannot grow on phenol, but Phe^+ mutations accumulate during 10 days incubation on plates with phenol as the only carbon source. The Phe^+ phenotype is due to mutations that activate transcription by creating a new promoter sequence (Kasak *et al.*, 1997). During incubation on the phenol plates, the number of viable cells detected by plating was constant, but vital staining revealed that many cells had died; presumably surviving cells had proliferated on nutrients from the dead ones (Saumaa *et al.*, 2006). Thus, the population on the phenol selective plates appears to be dynamic, resembling the conditions that prevail in long-term stationary phase experiments (*e.g.*, Finkel *et al.*, 1999). These population dynamics probably explain why the accumulation of Phe^+ mutations over time is not linear.

Several types of mutations, including base substitutions, small deletions, and the insertion of transposable elements, can create a good promoter sequence for the *pheAB* genes. During phenol selection all of these different types of mutations occurred, but by different mechanisms (Saumaa *et al.*, 2002). For example, the Phe^+ mutations that appeared early during phenol selection (three to four days after plating) were dominated by a particular C to A base substitution; in growing cells MutY prevents these mutations from occurring, but the levels of MutY declined early during selection. Later during selection, deletions creating the Phe^+ phenotype appeared, and these required RpoS (Saumaa *et al.*, 2002). Transposition of a transposon into the promoter region also depended on RpoS (Ilves *et al.*, 2001). The occurrence of single base-pair deletions, but not base substitutions, was dependent on Pol IV but not on RecA (Tegova *et al.*, 2004). In *P. putida*, Pol IV is poorly induced by DNA damage, and although transcription of Pol IV increases in stationary phase, it is not under RpoS control (Tegova *et al.*, 2004). The appearance of Phe^+ mutations during phenol selection was not due to a decline in mismatch repair because the spectrum of stationary-phase mutations changed if MMR was defective (Saumaa *et al.*, 2006).

When an auxotrophic strain of *B. subtilis* is incubated without its required amino acid, prototrophic mutants appear at a constant rate for about 9 days (Sung *et al.*, 2002). The authors called these stationary-phase mutations. The appearance of these mutations did not depend on RecA or *B. subtilis'* RpoS analogue. Interestingly, the stationary-phase mutagenesis depended, at least partially, on the Com factors that regulate the development of natural competence (Sung *et al.*, 2002). In addition, some, but not all, of the stationary-phase mutations depended on a Pol IV homologue, YdjH (Sung *et al.*, 2003). Loss of MMR increased and overexpression of MutS reduced stationary-phase mutagenesis, suggesting that MMR was deficient in some, but not all, of the cells (Pedraza-Reyes *et al.*, 2004). As was found in *E. coli* (see below), about 1% of the revertants carried second, nonselected mutations. This result, plus the involvement of the Com factors, suggests that the population under selection included a hypermutating subpopulation (Sung *et al.*, 2002).

Mfd, also known as transcription repair coupling factor (TRCF), is required for the repair of DNA that is being actively transcribed. Mfd recognizes a stalled RNAP, dislodges it, and recruits DNA repair enzymes to the site (for a review see Saxowsky *et al.*, 2006). As mentioned above, in *B. subtilis* loss of Mfd reduced stationary-phase mutation (Ross *et al.*, 2006). In addition, loss of Mfd increased the proportion of revertants that were due to suppressor tRNAs relative to true revertants. This result suggested that Mfd targeted the mutational process to genes that were being actively transcribed, which under most conditions would be those that were under selection. Thus, Mfd would be an agent for "directed mutagenesis." Ross *et al.* (2006) hypothesized that Mfd could promote this mutagenesis by facilitating RNAP's ability to synthesize pass a lesion in the DNA template, producing a mutant transcript. The mutant transcript might be translated into a protein active enough to allow some metabolism, and the cell would then replicate its DNA without repairing the damage, immortalizing the mutation. This scheme, called by some "retromutagenesis' (Saxowsky *et al.*, 2006), has been proposed before (Bridges, 1994; Davis, 1989; Fitch, 1982) and is a variation of that suggested by Cairns *et al.* (1988) for producing directed mutations. In support of this hypothesis, RNAP can bypass lesions and produce mutant transcripts; however, Mfd does not facilitate, but opposes this bypass (Bregeon *et al.*, 2003; Saxowsky *et al.*, 2006). Thus a more likely explanation for the role of Mfd in stationary-phase mutagenesis is that the DNA synthesis associated with transcription-coupled repair is error-prone.

Movement of Mobile Genetic Elements

The various mobile genetic elements found in bacteria–insertion elements, transposons, integrating bateriophage, integrating conjugative elements, self-splicing group II introns, retrons, integrons–constitute a flexible gene pool that can be exchanged among individuals, and even between species, by horizontal gene transfer (Hacker *et al.*, 2001). Given the selfish nature of the elements, it is not surprising that their mobility would be linked to bacterial stress responses. In addition, given that the movement of mobile elements can induce DNA breaks and rearrangement, it is not surprising that hosts would attempt to regulate the movements of their resident elements. The dynamic nature of mobile element evolution and host responses predicts a plethora of stress-induced phenomena.

As mentioned above, induction of the SOS response in *Vibrio cholerae* causes integrating conjugative elements (ICEs) to move (Beaber *et al.*, 2004; Quinones *et al.*, 2005). ICEs are related to bateriophage, and their induction resembles the well-known UV-induced induction of prophages. The SOS response has been reported to both stimulate (Kuan *et al.*, 1991) and inhibit (Weinreich *et al.*, 1991) transposition of transposons. Recently, a random search for mutations affecting transposition revealed that *dinD*, a SOS gene in *E. coli*, enhanced transposition of insertion element IS*903* and transposon Tn*10*; however, transposition of other elements was not stimulated (Twiss *et al.*, 2005). Thus SOS induction appears to affect different classes of mobile genetic elements in different ways.

Transposition requires the formation of protein-DNA complexes, and can be regulated by host factors that interact with the chromosome and modify its structure. The amounts and activities of two of these factors, IHF and H-NS, are modulated by stress responses (Haniford, 2006; Twiss *et al.*, 2005). That various types of nutritional stress cause small insertion elements (ISs) to move is well documented. For example, a strain of *E. coli* stored for 30 years in an agar stab was found to have experienced both constant and episodic IS movement (Naas *et al.*, 1994). The cryptic *bgl* operon in *E. coli* can be activated by IS movement into the control region, allowing growth on the β-glucosides salicin or arbutin (Reynolds *et al.*, 1981); such Bgl^+ mutants appear at increasing frequencies over several days when the cells have arbutin as their only carbon source (Hall, 1998a). Incubation of a Phe^- strain of *Pseudomonas putida* with phenol as the only carbon source stimulates activation of the phenol-utilization operon by

insertion of a transposon into the promoter region (Ilves *et al.*, 2001). In a particular strain of *E. coli*, excision of a Mu element produces an active *lacZ* gene by fusing the *araB* regulatory region to the *lacZ* and *lacY* genes; fusion is stimulated by incubating the Lac^- parent cells with lactose as the only carbon source and arabinose as inducer (Cairns *et al.*, 1988; Shapiro, 1984), or by prolonged aerobic incubation without a carbon source (Foster *et al.*, 1994a; Maenhaut-Michel *et al.*, 1994; Mittler *et al.*, 1990; Sniegowski, 1995). Interestingly, the structures of the *ara-lac* fusions that are induced by incubation on lactose-arabinose or by aerobic starvation are different (Maenhaut-Michel *et al.*, 1994; Maenhaut-Michel *et al.*, 1997). Because fusion formation is a function of bateriophage Mu induction and transposition, the genetic requirements are complex (Gomez-Gomez *et al.*, 1997; Lamrani *et al.*, 1999). However, the link to environmental stress is obvious, as fusion formation requires RpoS and is inhibited by H-NS (Gomez-Gomez *et al.*, 1997; Lamrani *et al.*, 1999).

Adaptive Mutation in *E. coli* strain FC40

For over a decade adaptive mutation in FC40 has been the paradigm for mutagenesis in nutritionally stressed bacteria. Nonetheless, there is still controversy about the mechanism by which the adaptive mutations are produced. Below, I describe the phenomenology and genetics of adaptive mutation in this particular strain of *E. coli*, and then discuss the various competing models that attempt to explain the phenomenon.

The Characteristics of E. coli Strain FC40—The lineage of strain FC40 dates back to Jacob and Monod. Its immediate parent, P90C, also known as CSH142 (Miller, 1992), has a chromosomal deletion, originally called $\Delta(lac\text{-}proB)_{XIII}$ but now known as $\Delta(gpt\text{-}lac)5$, that removes approximately 2.5 minutes of the chromosome including the *lac* and *proAB* operons (but not the *dinB* gene). The F'_{128} episome is a large conjugal plasmid that carries about 3 minutes of the *E. coli* chromosome including the *lac* and *proAB* operons (and the *dinB* gene). F'_{128} can be mated into and stably maintained in P90C and its derivatives by selecting for proline prototrophy. P90C carrying the F'_{128} episome with an up-promoter mutation ($lacI^Q$) in the *lacI* gene promoter, and a down-promoter mutation (L8) in the *lac* operon promoter, is known as GM1 (Coulondre *et al.*, 1977), or CSH100 (Miller, 1992); this strain has figured prominently in the extensive mutational studies by Miller and colleagues.

The immediate parent of FC40 is C36, which is a spontaneous Rif^R mutant of P90C. The version of F'_{128} in FC40 has a fusion of the *lacI* gene to the *lacZ* gene, $\Phi(lacI\text{-}lacZ)$, originally isolated by Müller-Hill and colleagues (Brake *et al.*, 1978). The fusion eliminates all of the *lac* regulatory region, the last four residues of *lacI* and the first 23 residues of *lacZ*. Transcription of the fusion initiates at the $lacI^Q$ promoter and is constitutive. The particular allele of the fusion that we use, $\Phi(lacI33\text{-}lacZ)$, has a +1 frameshift at the 320th codon of *lacI*, changing CCC to CCCC (Calos *et al.*, 1981). This and similar alleles have been used by Miller and colleagues to study various mutational mechanisms (Miller, 1985; Tlsty *et al.*, 1984). The F'_{128} episome carrying $\Phi(lacI33\text{-}lacZ)$ (obtained from J.H. Miller) was mated into C36 to give FC40 (Cairns *et al.*, 1991). FC40 is Lac^- but the allele is slightly leaky, probably due to ribosomal frameshifting, and makes about 2 Miller units of β-galactosidase. The *lacI33* mutation is polar on *lacY*, so FC40 is also defective for lactose permease. When fully reverted, FC40 makes about 200 Miller units of β-galactosidase, which is enough to make it Lac^+, but much less than the 1000 units made by fully-induced wild-type Lac^+ bacteria (Miller, 1992).

Lac^- FC40 cannot grow on lactose. When incubated in liquid minimum lactose medium, the Lac^- population is completely static for at least 4 days (Foster, 1994) (Figure 1). However, when plated on minimal lactose plates, FC40 can double several times over the course of a few days. This growth is due to the impurities in the agar or, possibly, breakdown products of

lactose, and can be prevented by adding an excess of Lac$^-$ "scavenger" cells to the plates. The scavengers, FC29 (Cairns *et al.*, 1991), are P90C carrying an F'_{128} episome that is deleted for *lacZ*, and so cannot revert to Lac$^+$ (obtained from J. Beckwith). In a typical adaptive mutation experiment, 10^8 FC40 cells are mixed with 10^9 FC29 cells, plated on minimal plates with lactose as the only carbon source, and incubated for five days. Because of the low amount of β-galactosidase made by Lac$^+$ revertants of FC40, Lac$^+$ colonies take 2 days to become visible. Therefore, most of the colonies that appear on day two are due to mutations that occurred during growth prior to plating. Thereafter, from day 3 to day 5, new Lac$^+$ colonies appear at a constant rate. These late-arriving revertants are due to mutations that occurred after the cells were on lactose, and are called adaptive mutations.

The Phenomenology of Adaptive Mutation in E. Coli Strain FC40—The basic phenomenology of adaptive mutation in FC40 is as follows:

1. During growth under non-selective conditions, Lac$^+$ mutations occur at the rate of about one in 10^9 cells per generation. During lactose selection, Lac$^+$ mutations appear at the rate of 20 to 50 per 10^8 cells per day (Cairns *et al.*, 1991; Foster, 1994).
2. Lac$^-$ cells can be incubated on lactose minimal medium without growth or death for several days (Cairns *et al.*, 1991; Foster, 1994; Rosche *et al.*, 2001). During this time, the rate at which Lac$^+$ mutations appear remains constant.
3. Lac$^+$ adaptive mutation is not restricted to agar plates, but, as shown in Figure 1, occurs at the same rate in liquid medium as on plates (Foster, 1994). Thus, adaptive mutation may occur equally well in structured and non-structured environments (with the caveat that the cells in the liquid medium were not agitated and settled to the bottom.)
4. Lac$^+$ mutations do not accumulate when FC40 is incubated without a carbon source, but immediately begin to appear when lactose is added to the starving cells. Thus mere starvation in the absence of selection does not induce Lac$^+$ mutations–lactose is required (Cairns *et al.*, 1991; Foster, 1994).
5. While incubating on lactose, Lac$^-$ cells accumulate nonselected mutations. The rate at which these appear is high for genes on the episome (Foster, 1997) and low for genes on the chromosome (Bull *et al.*, 2000a; Foster, 1994). Thus mutations are not "directed" to *lac*.
6. While incubating on lactose, Lac$^+$ revertants accumulate nonselected mutations at frequencies 20-to 100-fold higher than the frequencies at which these mutations appear among the Lac$^-$ population (Rosche *et al.*, 1999; Torkelson *et al.*, 1997). Since after isolation the Lac$^+$ revertants with second mutations do not have a higher than normal mutation rate, some proportion of the cells must experience a transient hypermutation during lactose selection.
7. About 2% of the Lac$^+$ colonies that appear after 5 days on lactose plates are composed of cells that have amplified the Lac$^-$ allele (Foster, 1994; Foster *et al.*, 1995a). After 10 days on lactose plates, this proportion increases to 60% (Hastings *et al.*, 2000; Powell *et al.*, 2001). These colonies appear late because they are slow to develop.

Genetic Requirements for Adaptive Mutation in FC40—Adaptive mutation in FC40 has genetic requirements that distinguish it from mutation during normal growth. These are:

1. The mutations that give rise to Lac$^+$ revertants during selection are different than those that give rise to Lac$^+$ revertants during nonselected growth. The mutations that occur during lactose selection are almost all one base-pair deletions in runs of the same base,

whereas the mutations that occur during growth include deletions, duplications, and larger frameshifts (Foster *et al.*, 1994b; Rosenberg *et al.*, 1994).

The −1 frameshifts produced during lactose selection are exactly the mutations that would be expected to be made by DNA polymerases in general, and Pol IV in particular (Kim *et al.*, 1997; Wagner *et al.*, 2000). During nonselected growth, most of these potential −1 frameshifts mutations are corrected by MMR (Longerich *et al.*, 1995). Thus, it has been argued that the difference in the spectrum of Lac^+ mutations during growth and during lactose selection is due a failure of MMR in the latter case (Longerich *et al.*, 1995). However, it is equally as likely that because Pol IV is active in the nondividing cells, there are simply more −1 frameshifts being made.

2. The Lac^+ mutations that appear during lactose selection occur by a different mechanism than the Lac^+ mutations that appear during growth. Enzymes for the recombinational repair of double-strand breaks, namely RecA, RecBCD, and RuvABC, are required for Lac^+ mutations during lactose selection but not during growth (Cairns *et al.*, 1991; Foster, 1993; Foster *et al.*, 1996; Harris *et al.*, 1994; Harris *et al.*, 1996).

3. The high level of adaptive reversion to Lac^+ seen in FC40 requires that the *lac* allele be on the episome. When the *lac* allele is in its normal place on the chromosome, the rate of reversion to Lac^+ during lactose selection falls 100-fold and it is no longer dependent on recombination functions (Foster *et al.*, 1995b; Radicella *et al.*, 1995).

4. The high level of adaptive reversion to Lac^+ also requires that the episome's conjugal functions be expressed. If conjugal functions are defective, the rate of adaptive mutation falls 10-fold, but it is still largely dependent on recombination functions (Foster *et al.*, 1995b).

   The most likely role for the conjugal functions is to initiate recombination by producing a nick at the conjugal origin (Ponder *et al.*, 2005; Rodriguez *et al.*, 2002). However, defects in several of the conjugal *tra* genes, not just *traI*, the gene that encodes the nicking enzyme, have the same inhibitory effect on adaptive mutation (Foster *et al.*, 1995b; Rodriguez *et al.*, 2002). This is likely because the Tra proteins form a multi-protein complex (Lawley *et al.*, 2003), and defects in one protein can disrupt the function of the whole complex.

5. Fifty to 80% of adaptive Lac^+ mutations are due to DNA polymerase IV (Foster, 2000; McKenzie *et al.*, 2001). Pol II is also active, but does not contribute to adaptive mutations (Foster *et al.*, 1995a); in fact, Pol II appears to compete with and limit the mutational potential of Pol IV (Foster, 2000). Pol V is not involved (Cairns *et al.*, 1991; McKenzie *et al.*, 2000).

   In the absence of Pol IV, the remaining Lac^+ mutations are most likely produced by Pol III (Foster, 2000; McKenzie *et al.*, 2001). Pol IV is also required for transient hypermutation (see below) (Tompkins *et al.*, 2003).

6. Adaptive mutation to Lac^+ is reduced about 10-fold in the absence of RpoS (Layton *et al.*, 2003; Lombardo *et al.*, 2004). Most of this reduction is due to failure to induce Pol IV (discussed above), but RpoS also has a small Pol IV-independent role in adaptive mutation (Layton *et al.*, 2003; Lombardo *et al.*, 2004).

7. Adaptive mutation to Lac^+ is reduced 10- to 20-fold if GroE is deficient (Layton *et al.*, 2005). The amount of Pol IV declines 10-fold in GroE deficient cells (discussed above), and this is sufficient to explain the reduction in adaptive mutation in wild-type cells. However, in *recG* mutant cells GroE has a small effect on adaptive mutation that is independent of Pol IV (Layton *et al.*, 2005).

8. Adaptive mutation to Lac^+ is reduced about five-fold in the absence of polyphosphate kinase, Ppk (Stumpf *et al.*, 2005). As discussed above, PolyP (or Ppk itself) is required for the maximum mutagenic and lesion bypass activity of both Pol IV and Pol V (Stumpf *et al.*, 2005, 2007a). In addition, Ppk has a small Pol IV-independent role in adaptive mutation (Stumpf *et al.*, 2005).

9. The rate of adaptive mutation to Lac^+ is increased up to 100-fold by loss of RecG (Foster *et al.*, 1996; Harris *et al.*, 1996) or DNA Pol II (Foster *et al.*, 1995a). In strains defective for *recG* or *polB* (encoding Pol II), the levels of Pol IV are increased 5- to 30- fold (Layton *et al.*, 2005), which may account for the increase in mutation rate.

   recG mutant cells are partially induced for the SOS response (Lloyd *et al.*, 1991; McCool *et al.*, 2004), and a similar induction may explain the elevated amounts of Pol IV in *polB* mutant cells. In addition, the errors leading to Lac^+ adaptive mutations in *recG* mutant cells appear to be poorly corrected by MMR, possibly because the MMR system is overwhelmed (Foster *et al.*, 1996). Whether these two effects are sufficient to explain the large increases in adaptive mutation in *recG* and *polB* mutants remains to be seen.

10. The number of Lac^+ revertants appearing during lactose selection is increased 10- to 30-fold in a *recD* mutant strain (Harris *et al.*, 1994). However, most, if not all, of this increase is due to the accumulation of extra copies of the episome and, consequently, of the *lac* allele (Foster *et al.*, 1999).

    RecD is the exonuclease subunit of RecBCD and *recD* mutants have a hyper-recombination phenotype. But, loss of RecD also causes plasmids to over-replicate (for example, see Seelke *et al.*, 1987). *recD* mutants of FC40 have extra copies of the episome and, because of their extra *lac* alleles, can proliferate on lactose. The combination of over-replication and cell-growth accounts for an ever increasing accumulation of Lac^+ revertants during lactose selection (Foster *et al.*, 1999). This nonlinear accumulation, which is expected when the number of *lac* alleles increases, is not characteristic of adaptive mutation to Lac^+ in wild-type strains (Foster, 1994).

11. Lac^+ adaptive mutation is increased about 100-fold if MMR is defective. MMR deficiency can be produced by inactivating one of the MMR proteins (Foster *et al.*, 1992), or by overproducing the Dam methylase, which eliminates strand-discrimination (Foster *et al.*, 1996). These results show that, during lactose selection, MMR is active and the DNA is methylated.

    MMR could be inactive during lactose selection in a subpopulation of cells. This hypothesis is supported by the fact that overproduction of the MMR enzymes MutS, MutL, or both, reduces Lac^+ adaptive mutation (Foster *et al.*, 1995a; Foster, 1999a; Harris *et al.*, 1997). However, overproduction of the MMR enzymes can also reduce mutations in growing cells, suggesting that some components of MMR are always limiting (Foster *et al.*, 1995a; Foster, 1999a). Disagreement over the meaning of these various results is still unresolved (Foster, 1999a; Harris *et al.*, 1999). As discuss below, loss of MMR does not increase the frequency at which other, nonselected mutations appear in Lac^+ cells, indicating that MMR is inactive in those cells that are hypermutators (Rosche *et al.*, 1999).

Models for Adaptive Mutation in FC40—Currently there are two alternative models for how Lac^+ mutations arise during lactose selection–recombination-dependent mutation (RDM) and amplification-dependent mutation (ADM), each of which is described below. While these models are not mutually exclusive, what animates the disagreement about the models is that they incorporate different beliefs about the basic process by which mutations are produced.

The RDM model proposes that a special mutagenic mechanism is at work in cells during selection, whereas the ADM model proposes that mutations occur by an ordinary mechanism among a growing subpopulation. More detailed discussions of the models, the evidence supporting each, and personal opinions *pro* and *con*, can be found in references Foster, 2004; Rosenberg *et al.*, 2004; and, Roth *et al.*, 2004.

The initiating event for RDM is production of a double-strand break in the DNA, which occurs at a high frequency on the episome because the conjugal origin is subjected to continuous nicking even in the absence of active conjugation. While the Lac^- cells are on lactose, the slow influx of energy produced by the leaky *lac* allele allows replication to be occasionally initiated from one of the episome's vegetative origins (Figure 2A). When the moving replication fork encounters the persistent nick at *oriT*, a double strand end is produced (Figure 2B) and this initiates double-strand break repair. This RecA-and RecBCD- dependent repair proceeds via strand invasion of either the homologous duplicated portion of the same episome or of another copy of the episome (Figure 2D). DNA synthesis primed by the 3′ end of the invading strand is initiated by the PriA-dependent primosome (Figure 2E). The new DNA is synthesized by either Pol II, in which case it is mostly error-free, or by Pol IV, in which case it has a high frequency of errors. Mistakes can also be made by Pol III, which is recruited to continue replication (Figure 2F). The Holiday junction produced during recombination is then resolved by the RuvAB/RuvC enzymes. If the region of new DNA includes the *lac* region, then Lac^+ mutations can arise; if other regions of the episome are included, then the genes in those regions can have mutations. While the recombination-dependent mechanism occurs at a high rate on the episome, it can also produce mutations on any replicon, including the chromosome, whenever the replication fork encounters a nick, or when a double-strand break is produced in some other way.

The initiating event for ADM is a duplication of a region of DNA that includes the *lac* region. These duplications arise during nonselective growth before lactose selection at a frequency of about one in every 1000 cells. On lactose medium, cells with *lac* duplications have enough energy to slowly proliferate and further amplify their *lac* regions *via* a RecA, RecBCD-dependent mechanism. Eventually a true Lac^+ revertant arises among the amplified arrays, allowing the cell with the Lac^+ copy to proliferate. Within this clone the amplified arrays deamplify, leaving the single Lac^+ copy, and these Lac^+ cells overgrow the remaining slower-growing precursors.

The disagreements about which of these models is correct reflect real disagreements among researchers about the actual phenomenology of the experiment. Is there a slow-growing subpopulation (Hendrickson *et al.*, 2002), or not (Foster, 1994)? Does every Lac^+ colony contain cells that are unstably Lac^+ (Hendrickson *et al.*, 2002), or not (Slack *et al.*, 2006)? Are all sectored colonies the result of amplification (Andersson *et al.*, 1998), or not (Slack *et al.*, 2006)? Can the amplification model describe the dynamics of Lac^+ mutant appearance (Pettersson *et al.*, 2005), or not (Stumpf *et al.*, 2007b)? Does the amplification model account for all of the genetic requirements (Roth *et al.*, 2004), or not (Hastings *et al.*, 2004)?

While all researchers agree that both true reversion and pseudo-reversion by amplification occur while FC40 is under lactose selection, it remains contentious whether amplification is a separate pathway or a necessary precursor for true reversion. The question could be settled by inhibiting amplification and determining if Lac^+ reversion is reduced. This was attempted by Hendrickson *et al.* (2002), who placed a transposon encoding tetracycline resistance (Tet^R) on the episome at various distances from the *lac* region. Although the Tet^R genes normally make cells resistant to tetracycline, if they are in multiple copy cells become sensitive to tetracycline because overproduction of the efflux transporter encoded by *tetA* is toxic (Chopra *et al.*, 1981; Eckert *et al.*, 1989). Thus, if the *lac* region is amplified during lactose selection, Tet^R

genes close to the *lac* region should be co-amplified and kill the amplifying cells. If amplification is a necessary precursor to Lac^+ reversion, the closer the Tet^R genes are to *lac*, the more the rate of Lac^+ reversion should be reduced. While it was true that the rate of adaptive mutation to Lac^+ was low in the presence of tetracycline when the Tet^R genes were to close to *lac* (2002), further analysis showed that this was also true when tetracycline was not present, *i.e.*, when the transposons were close to *lac* the rate of *lac* reversion was low for some reason other than toxicity (Stumpf *et al.*, 2007b). In addition, a similar effect was seen when the transposon *tetA* genes were mutant. The rate at which the mutant *tetA* genes reverted to Tet^R during lactose selection was independent of the distance between *tetA* and *lac*; indeed, Tet^R reversion occurred even when *tetA* and *lac* were on different replicons. Thus reversion of a gene during lactose selection does not require that it is co-amplified with the *lac* region (Stumpf *et al.*, 2007b).

In addition, the ADM model simply does not account for the fact that during lactose selection the appearance of Lac^+ revertants is nearly linear with time (Figure 1). Like other "growth of an intermediate" models (Lenski *et al.*, 1989), the ADM model predicts that the rate at which Lac^+ revertants appear should increase with time. The ADM model further predicts a rapid increase in the appearance of Lac^+ colonies when the number of Lac^- alleles reaches a critical threshold. In confirmation, computer simulations and laboratory reconstructions of ADM show that nearly all the Lac^+ revertants arise almost simultaneously after five days on lactose (Pettersson *et al.*, 2005;Slechta *et al.*, 2003). Yet, in actual adaptive mutation experiments, hundreds of Lac^+ colonies appear at a constant rate from day 2 to 5.

Transient Hypermutation—As mentioned above, a significant fraction of the Lac^+ revertants that appear during lactose selection also carry mutations that were not being selected (Rosche *et al.*, 1999; Slechta *et al.*, 2002; Torkelson *et al.*, 1997). When isolated and tested, these Lac^+ revertants do not have elevated mutation rates, meaning that they are not constitutive mutators. Therefore, they must have experienced a transient increase in their mutation rate during lactose selection. This phenomenon is called hypermutation, and was originally proposed by Hall (1990) to explain why mutations appear to be directed. While all researchers agree that hypermutation is a feature of adaptive mutation in FC40, they disagree about its causes and consequences.

The percentage of Lac^+ revertants that have a second mutant phenotype increases linearly with the time that the population is under lactose selection. For example, over 5 days the proportion of the Lac^+ revertants that is also defective for motility (Mot^-) increases from 0% to 2%. This increase indicates either that the hypermutating cells do not die, and thus continue to accumulate mutations, or that the proportion of cells that are progressing into the hypermutator state increases with time (Rosche *et al.*, 1999). Second mutations appear at a much lower frequency among Lac^+ revertants if Pol IV is defective, suggesting that both the Lac^+ and the nonselected mutations are due to the activity of Pol IV (Slechta *et al.*, 2003; Tompkins *et al.*, 2003). Loss of MMR increases the rate at which adaptive Lac^+ revertants appear about 100-fold, but only increases the frequency of second mutations in the Lac^+ revertants by about 70%, meaning that the hypermutating cells are wholly or partially MMR defective (Rosche *et al.*, 1999).

The high mutation rate of hypermutators cannot be due solely to loss of MMR. Lac^+ revertants with a second mutation must be derived from the hypermutating population, and the frequency of a third mutation among these Lac^+ double mutants is about 10-fold higher than the frequency of a second mutation among MMR^- Lac^+ cells (Rosche *et al.*, 1999). This means that loss of MMR is not sufficient to account for hypermutation. A likely additional factor is overexpression of Pol IV, which could occur, for example, in cells expressing both the RpoS-dependent and SOS responses. Loss of MMR and hyper-induction of Pol IV could be

independent, or MMR could be saturated by errors due to very high levels of Pol IV activity in a subpopulation of cells (McKenzie *et al.*, 2001; Tompkins *et al.*, 2003).

An alternative hypothesis for hypermutation is that it occurs only in the subpopulation of cells that have amplified the *dinB* gene along with the *lac* region (Slechta *et al.*, 2003). The original recombination event that created F'_{128} placed *dinB* much closer to *lac* than it is on the chromosome, and *dinB* could be included when the *lac* region is amplified (Kofoid *et al.*, 2003). However, in a strain with Φ(*lacI33-lacZ*) in its normal place on the chromosome, 2% of the chromosomal Lac^+ revertants had second, nonselected mutations (Rosche *et al.*, 1999); since adaptive mutation in this case is not recombination-dependent (Foster *et al.*, 1995b), hypermutation is unlikely to be due to co-amplification of *dinB.*

Although it has been proposed that all Lac^+ revertants occur in hypermutating cells (Torkelson *et al.*, 1997), simple logic dictates that there are at least two mutating populations. All available data indicate that the frequency of second mutations in Lac^+ single mutants is only a tenth of the frequency of third mutations among Lac^+ double mutants (Rosche *et al.*, 1999; Torkelson *et al.*, 1997). Since the Lac^+ double mutants arose in hypermutators, some fraction of the Lac^+ single mutants must have arisen in a population that has a lower mutation rate than the hypermutators (Cairns, 2000; Foster, 2004).

Based on ratios of the frequencies of single, double, and triple mutants, Rosche *et al.* (1999) estimated that about 0.1% of the Lac^- population were hypermutators and their mutation rate was 200-fold higher than the rest of the population. If so, then they account for only 10% of the adaptive Lac^+ mutants (but virtually all of the double and triple mutants), and 90% the Lac^+ mutations are produced in cells that do not have an extraordinary increase in mutation rate (although there could, in fact, be a continuum of mutation rates within the population) (Foster, 2004). Note, this conclusion does not mean that the mechanism by which mutations are produced must differ among the populations. Rosenberg and coworkers also estimated the hypermutating subpopulation to be 0.01 to 0.1% of the total, but they hypothesize that all Lac^+ revertants come from this population, and so the mutation rate must be much higher (Bull *et al.*, 2000b; Torkelson *et al.*, 1997). However, Roth *et al.* (2003) argue that to produce all the observed number of Lac^+ mutants the mutation rate within this tiny population would have to be so high (more than 10^5-fold higher than normal) that the hypermutators would be unlikely to survive. This problem mostly disappears if the mutation rate on the episome, which carries the *lac* allele in FC40, is higher than on the chromosome, where the genes essential for life reside (Cairns *et al.*, 2003).

The calculations of the size of the hypermutating subpopulation and its contribution to Lac^+ adaptive mutations depend on the frequencies of single, double, and triple mutants, and the number of triple mutants is very small (Rosche *et al.*, 1999). In addition, the calculations also depend on the frequency of the same nonselected mutations in Lac^- cells, and this frequency is low and may not be due to mutations that occurred during selection. So the magnitude of the contribution of hypermutators to adaptive mutation to Lac^+ is still open. The theoretical relationship among the population of the Lac^- cells that are hypermutators (p), their increase in mutation rate (M), and the proportion of Lac^+ revertants that occur in the hypermutators (R), is plotted in Figure 3.

Are There Any Directed Mutations?

As pointed out by Hall (1998b), there are two ways that mutations could be directed–selective generation or selective capture. The only examples of selective generation appear to be transcription-induced mutations in genes that are highly induced by the selective conditions (Wright, 2004) (see above). The fact that F′ has a higher mutation rate than the chromosome gives the appearance of selective generation, but nonselected mutations on the episome readily

occur during selective conditions (Foster, 1997). Thus it seems that organisms only occasionally have access to a selective generation process.

Selective capture, on the other hand, may be a constant feature of mutation during selection. Indeed, it may be unavoidable. The idea is as follows: Cells under selective pressure experience potentially mutagenic alterations to their DNA from a variety of causes. If a sequence change does not have a useful result, it is repaired or discarded. However, if a sequence change gives rise to an immediately expressed useful phenotype, the cell will begin to proliferate, fail to repair the damage, and immortalize the mutation. Of course, other premutations existing at the moment the cell begins to grow may also be immortalized, but the population at large will not accumulate unwanted mutations. Variations of this "trial and error" idea have been discussed for years (Boe, 1990; Cairns *et al.*, 1988; Stahl, 1988, 1992). Hall's original "hypermutator state" model, which proposed that hypermutating cells die if a useful mutation does not occur (Hall, 1990), can be considered an extreme form of "trial and error." However, evidence strongly suggesting a trial and error mechanism has only recently been generated. When $LacI^+$ cells are given phenyl-galactoside (Pgal) as a carbon source, the mutations that allow them to grow are those that inactivate the *lacI* gene or destroy the ability of the *lac* operator to bind LacI. $lacI^-$ mutations can be recessive or dominant whereas the operator mutations, designated $lacO^c$, are always dominant. Bharatan *et al.* (2004) found that when cells were placed under Pgal selection, early appearing mutations were almost all recessive, but the proportion of dominant mutations increased with time; after 10 days, almost all the mutations that appeared were dominant. They proposed that this bias reflected a need for "instantaneous gratification" in order to capture the mutagenic sequence change. The "instantaneous gratification" mechanism may prove to be universal, but previously overlooked because most mutations that have been studied in nongrowing cells are dominant.

CONCLUDING REMARKS

It has become almost *de rigueur* to begin reviews of mutagenesis in natural populations by declaring that bacteria have a "feast and famine" lifestyle (Koch, 1971). Since without genetic variation there is no evolution, the ability to mutate yourself out of famine and into feast would be adaptive. In recent years, it has become apparent that among isolates of bacteria from natural environments, several percent have constitutive mutation rates high enough to qualify them as mutators compared to laboratory strains (Matic *et al.*, 1997). Laboratory experiments have demonstrated the ease at which constitutive mutators can be enriched by selecting for novel phenotypes (Mao *et al.*, 1997; Miller *et al.*, 1999) or competitive fitness (Cox *et al.*, 1974; Sniegowski *et al.*, 1997). Modeling and simulations have confirmed that mutator alleles can contribute to adaptive evolution in changing environments (Leigh, 1973; Taddei *et al.*, 1997b). However, the mutator allele is always doomed–when a successful mutation is achieved, individuals that continue to mutate are at a disadvantage. Thus, continuous feast-full conditions should select for low mutation rates, and they apparently do (Trobner *et al.*, 1984).

However, if mutation rates increased only when mutations would be advantageous, and then returned to normal when success was achieved, the gene that conferred a transient mutator phenotype would not be strongly selected against. The individual that achieved a successful mutation would be burdened only with the extra mutations that occurred during the mutator state. Simulations have shown that if a population is undergoing periodic stress, stress-inducible mutator genes can be fixed more readily than constitutive mutator genes; and, importantly, under these conditions stress-inducible genes can speed up adaptive evolution (Bjedov *et al.*, 2003).

Stress-induced mutagenesis is neither a theoretical construct nor a laboratory artifact. Among natural isolates of *E. coli*, 40% had at least a 10-fold increase in mutant frequency, and 13%

had at least a 100-fold increase in mutant frequency, when they were incubated on agar plates for 7 days (Bjedov *et al.*, 2003). Among the same group of isolates, only 3% and 1% had equivalently increased constitutive mutant frequencies, respectively (Bjedov *et al.*, 2003). These numbers suggest that inducible mutators are widespread, and that they make a greater contribution to natural variation within a population than do constitutive mutators.

No matter how adaptive the consequences, a question remains–have inducible mutator genes been retained to achieve an increase in variation during stressful times, or is the mutator state simply an unavoidable side effect of cellular activities that have been selected for other purposes? For example, all three SOS-inducible DNA polymerases contribute to competitive survival in long-term stationary phase cultures (Yeiser *et al.*, 2002). The conservative interpretation of this result is that the three polymerases improve fitness by allowing damaged DNA to be replicated. However, they may also be advantageous because they create mutations, allowing the evolution of the “growth advantage in stationary phase” (GASP) phenotype (Finkel, 2006). Likewise, since the MMR system sacrifices a piece of newly synthesized DNA thousands of bases long to repair one mismatch, down-regulation of the MMS repair system in stationary phase may simply be a way to save energy. Nonetheless, the result of MMR decline is that any errors made by DNA polymerases in stationary phase may not be efficiently corrected. What may be more important, since MMR is normally a barrier to recombination between non identical DNA, down-regulation of MMR could enhance variation by allowing genetic exchange of diverged alleles (Rayssiguier *et al.*, 1989). Up-regulation of error-producing DNA polymerases and down-regulation of error-correcting systems may have evolved for other purposes, but yet may be retained in the population because they increase diversity when it is advantageous to do so.

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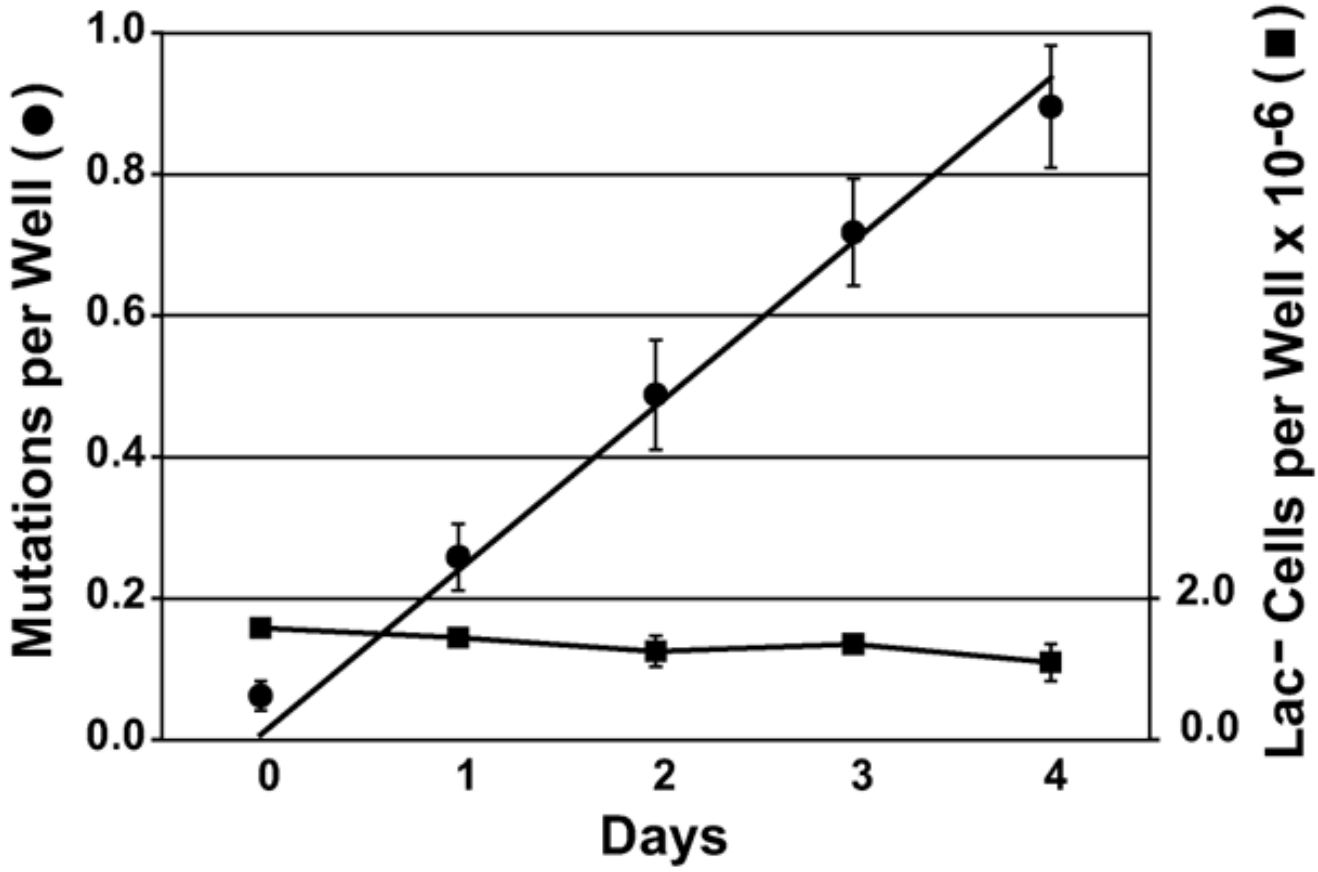

FIGURE 1.

The accumulation of Lac^+ mutations in liquid minimum lactose medium. Eight independent cultures of FC40 were diluted into minimum lactose medium and 100 μl aliquots containing approximately 10^6 cells were dispensed into 726 microtiter dish wells. Turbid wells were counted daily. The total number of Lac^- cells was determined in cultures incubated in parallel. The minimum lactose medium had been scavenged of non-lactose carbon sources by incubating it with 10^9 Δ(*lac*) cells per mL for three hours; these cells were removed by centrifugation followed by filtration. Because it takes 2 days for a well to become turbid after a Lac^+ mutation occurs, the mutations per well have been shifted two days to the left. (Reprinted with permission from Foster, 1994. *Genetics.* 138:256. The Genetics Society of America. www.genetics.org.)

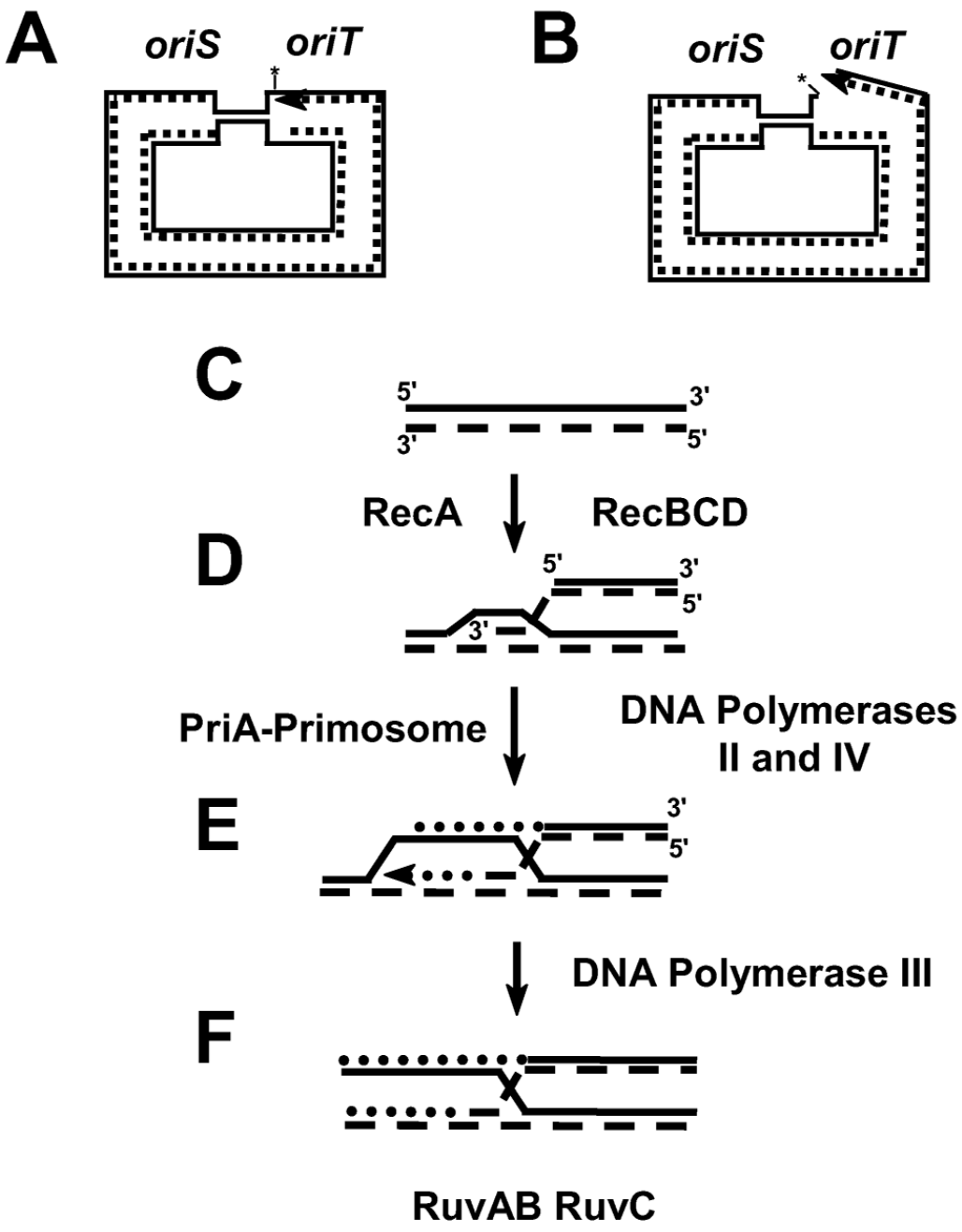

FIGURE 2.

A model for recombination-dependent mutation. *A* and *B*: the replication fork initiated at *oriS* collapses when it encounters the nick at *oriT*, creating a double-strand end. *C* and *D*: RecA and RecBCD catalyze invasion of the broken arm into the homologous region of the sister chromosome or a different episome copy. *E*: the PriA-primosome initiates DNA synthesis from the invading 3′ end, using either Pol IV or Pol II; the four-stranded Holliday junction is formed. *F*: the replication fork is re-established with Pol III. Not shown: the Holliday junction is resolved by RuvABC. Dashed lines are newly synthesized DNA; dotted lines are DNA synthesized after recombination. The arrow head indicates the 3′ end of the leading DNA

strand; * at *oriT* indicates TraI. (Reprinted, with permission, from Foster 1999, *Ann Rev Gene* 33:62. Annual Reviews. www.annualreviews.org)

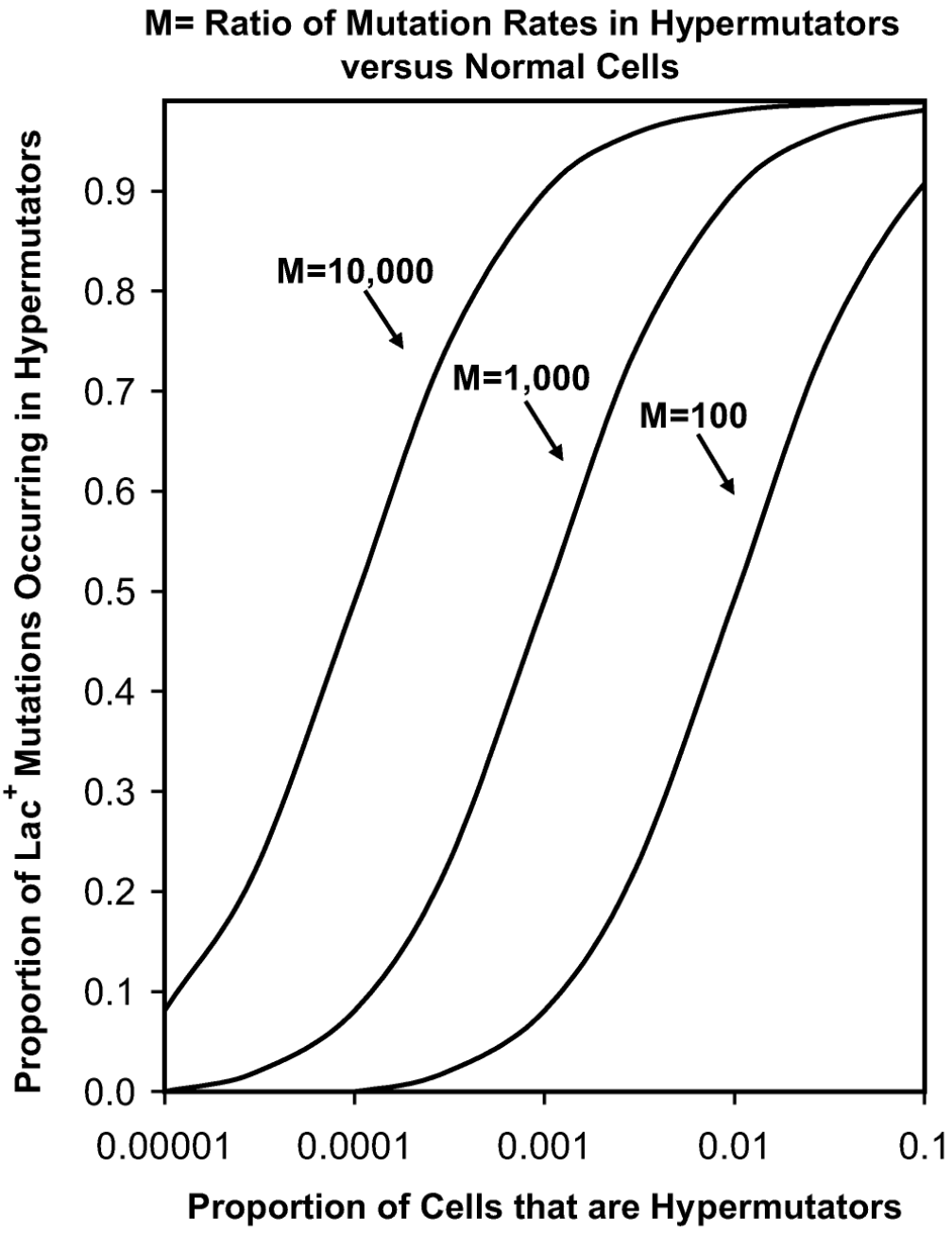

FIGURE 3.

The relationship between the proportion of Lac^+ revertants that occur in the hypermutating subpopulation (*Y axis*), the proportion of cells that are hypermutators (*X axis*), and the elevation in mutation rate in the hypermutating cells (*M*). The curves are base on the derivation by Cairns (1998).

TABLE 1

Definition of terms

Term	Definition
Directed mutation*	The appearance among cells under selection of mutations that relieve the selective pressure in the absence of the appearance of nonselected mutations.
Adaptive mutation	The appearance among cells under selection of mutations that relieve the selective pressure whether or not other nonselected mutations are also produced.
Stress-induced mutation	The induction of a general mutagenic state in response to stress. If the stress is starvation, then the equivalent term is “starvation-induced mutation”.
Transient mutation	A temporary state during which the mutation rate is increased, resulting in the accumulation of mutations throughout the genome. Could be the result of various inducible processes.
Hypermutation	A state during which the mutation rate is increased to extremely high, potentially lethal, levels resulting in the accumulation of mutations throughout the genome.
Stationary-phase mutation	A general mutagenic state that occurs in cells in stationary phase.
Stationary phase	The period of laboratory batch culture when the cells have run out of one or more nutrients. Also used to describe nutrient-limitation in natural populations.
Long-term stationary phase	The period of laboratory batch culture after the cells have entered stationary phase and after the majority (*e.g.*, 99% or more) have died. Characterized by balanced growth and death of the population.
Starvation	A general term describing the lack of one or more nutrients required for growth. Starvation can result from the normal depletion of nutrients during batch culture, or from a population being artificially suspended in nutrient-free medium.
Nutrient-limitation; nutritional-deprivation	Same as starvation, but often implies that only one nutrient is lacking.
Hunger	The state of cells when they are growing with suboptimal levels of nutrients, as for example in nutrient-limited chemostats.
Nutritional selection	A nutrient limitation that can be overcome by mutation; *e.g.*, Lac^- cells incubated with lactose as the only carbon and energy source.
Adaptive evolution	Evolution that proceeds by selection for improved fitness, in contrast to evolutionary changes caused by neutral mutations and genetic drift.

* Mutation and mutagenesis are used interchangeably.

TABLE 2

Examples of Stress-induced Mutation

Name[†]	Organisms	Type of Mutation	Selected Phenotype	Genetic Requirements	References
Starvation-induced Mu-mediated fusions	*E. coli*, Mu	Transposition	Growth on arabinose plus lactose	RpoS, ClpP, HNS[*]	(Cairns *et al.*, 1988; Foster *et al.*, 1994a; Gomez-Gomez *et al.*, 1997; Lamrani *et al.*, 1999; Maenhaut-Michel *et al.*, 1994; Maenhaut-Michel *et al.*, 1997; Mittler *et al.*, 1990; Shapiro, 1984)
Resting organisms in a structured environment (ROSE) mutagenesis	*E. coli*	Base substitutions	Resistance to rifampicin	CyaA. RecA. LexA[*], UvrB, Pol I, Not Pol V, Not RecBCD, Not RpoS	(Taddei *et al.*, 1995; Taddei *et al.*, 1997a)
Mutagenesis in aging colonies (MAC)	*E. coli*	Base substitutions	Resistance to rifampicin	RpoS, Crp, CyaA, RecA, MMR[*], Pol II, Not RecBCD, Not Pol I	(Bjedov *et al.*, 2003)
SOS-dependent spontaneous mutagenesis	*E. coli*	Base substitutions	Tryptophan prototrophy	RecA, Pol V	(Bhamre *et al.*, 2001; Timms *et al.*, 1999)
Stationary-phase mutagenesis	*P. putida*	Frameshifts, base substitutions, transposition	Growth on phenol	Pol IV, Pol V, RpoS, MutY, Not RecA, Not MMR[*]	(Ilves *et al.*, 2001; Kasak *et al.*, 1997; Saumaa *et al.*, 2002; Saumaa *et al.*, 2006; Tark *et al.*, 2005; Tegova *et al.*, 2004)
Stationary-phase mutagenesis	*B. subtilis*	Base substitutions	Amino acid prototrophy	ComA, ComK, Pol IV, MMR[*], Mfd, Not RecA, Not RpoS analog	(Pedraza-Reyes *et al.*, 2004; Ross *et al.*, 2006; Sung *et al.*, 2002; Sung *et al.*, 2003)
Adaptive mutation	*E. coli*	Frameshifts	Growth on lactose	Pol IV, RecA, RecBCD, RpoS, GroE, Ppk, Not Pol V	See text

[†] Usually the name used by the authors of the work.

[*] Loss or inactivation.