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review

Molecular mechanisms involved in the regulation of mutation rates in bacteria

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Abstract

Organisms live in constantly changing environments in which the nature, severity and frequency of environmental stresses are highly variable. Organisms possess multiple strategies for coping with environmental fluctuations. One such strategy is the modulation of mutation rates as a function of the degree of adaptation to the environment. When adaptation is limited by the available genetic variability, natural selection favors cells that have higher mutation rates in the bacterial populations. High mutation rates can be advantageous because they increase the probability that beneficial mutations will be generated. Constitutive mutator alleles are carried to high frequency through hitchhiking with beneficial mutations they generate. However, once adaptation is achieved, deleterious mutations that are generated by constitutive mutator alleles reduce the cellular fitness. For this reason, the possibility of adapting the mutation rate to environmental conditions is interesting from an evolutionary point of view. Stress-induced mutagenesis enables rapid adaptation to complex environmental challenges without compromising bacterial fitness because it reduces the overall cost of a high mutation rate. Here, we review the molecular mechanisms involved in the control of modulation of mutation rates in bacteria.

Stress is a disturbance in the normal functioning of a biological system that is provoked by environmental factors that cause a reduction in growth rate and increase mortality *(1)*. Some organisms react to stress by inducing behavioral or physiological responses, whereas others increase the production of genetically diverse offspring. For a long time, the increased production of genetic diversity was not considered as being adaptive because, given the large population of most bacterial species, it was thought that adaptation was rarely limited by the available genetic diversity. However, strong bottlenecks or stressful environmental conditions can severely reduce bacterial populations and, consequently, genetic diversity. Under such circumstances, it may be advantageous to have high mutation rates. Experimental *(2, 3)* and theoretical *(4)* studies indicate that, in nature, the frequency of bacterial strains with high constitutive mutation rates (mutators) is much higher than expected from a mutation/selection equilibrium. This finding suggests that there are situations in nature whereby being a mutator confers a selective advantage. Constitutive mutator phenotypes generally result from mutations in genes encoding DNA repair enzymes and proteins that ensure the accuracy of DNA replication. These mutant genes are called mutator alleles. Mutagenesis can also increase during stress due to a direct alteration of the DNA and/or a genetic response that is induced by stress.

The following two stress responses are known to increase mutagenesis when induced: the SOS system, which is induced by genotoxic stresses *(5)*, and the RpoS-regulated general stress response, which is triggered by many different stresses *(6)*. In this review, the role of molecular, ecological and evolutionary factors involved in shaping mutation rates in bacterial populations, using the *Escherichia coli* species as an example, will be discussed.

SELECTION OF CONSTITUTIVE MUTATORS

Newly arisen mutations can have markedly different impacts on the fitness of the organism, ranging from deleterious through neutral to beneficial. However, these mutations appear at very different rates. For example, in *E. coli* K-12, the rate of deleterious mutations per genome per replication is at least $2\text{-}8\times10^{-4}$ *(4, 7)*, whereas that of beneficial mutations is approximately 2×10^{-9} *(8)*. It is generally believed that the rate of spontaneous mutations results from a balance between the effects of deleterious mutations and the metabolic cost of reducing mutation rates *(9)*. The replication error rate in *E. coli* is low, i.e., approximately 10^{-10} per base per replication, and it appears that this rate cannot be reduced further *(10)*. Any variant with increased mutation rates is expected to have reduced fitness due to the increased production of deleterious mutations. However, when adaptation is limited by the available genetic variability, natural selection favors mutator cells. Mutator alleles are carried to high frequency through hitchhiking with the beneficial mutations they generate. The linkage between beneficial mutations and mutator alleles is particularly strong in bacteria because the rate of genetic exchange in these organisms is very low. Mutators are particularly favored when several beneficial mutations are required for adaptation *(3)*. For example, if the probability of generating each beneficial mutation is 10^2-fold higher in the mutator than in the non-mutator populations, the probability that two beneficial mutations will be generated in the mutator population is 10^4-higher than in non-mutator population.

The selection of mutator alleles also depends on many other parameters. For example, the increase in mutator frequency is dependent on the total population size *(11)*, mutator strength (i.e., the increase of the mutator mutation rate relative to the non-mutator mutation rate *(12)*), and the rate of gene exchange *(11)*. It also depends on the stability of the environment. For example, mutator alleles are particularly advantageous following a shift in environmental conditions *(13, 14)*. The dynamics of mutator selection also depend on the environmental spatial heterogeneity, which allows or prevents competition between the cells carrying different adaptive mutations. Therefore, theoretical modeling predicts that mutators will be particularly favored in temporally and spatially heterogeneous environmental conditions *(15)*.

Conditions favoring strong constitutive mutators are frequent in nature and have been found in populations of *Burkholderia cepacia* complex pathogens *(16)*, *Campylobacter jeuni (17)*, *E. coli (18)*, *Haemophilus influenzae (19)*, *Helicobacter pylori (20)*, *Neisseria meningitidis (21)*, *Pseudomonas aeruginosa (22)*, *Salmonella enterica (23)*, *Staphylococcus aureus (24)*, *Streptococcus pneumoniae (25)*, and *Yesinia pestis (26)*, with frequencies ranging from 0.1% to greater than 60%.

MISMATCH-REPAIR-DEFICIENT MUTATORS

The vast majority of strong constitutive mutators found in the laboratory (*E. coli (27)*; *S. enterica* serovar Typhimurium *(2)*, *Pseudomonas fluorescens (28)*) and in nature (*B. cepacia* complex pathogens *(16)*, *E. coli (18, 27)*; *S. enterica (23)*; *N. meningitides (21)*; *P. aeruginosa (29)*, *Y. pestis (26)*) have a defective mismatch-repair system due to the inactivation of either *mutS* or *mutL* genes. The molecular characterization of naturally occurring *E. coli* and *P. aeruginosa mutS* and *mutL* mutants has revealed that these genes are inactivated by a variety of the following mechanisms: frameshifts, insertions, premature stop codons and deletions *(29, 30)*. Mismatch-repair system control the fidelity of DNA replication by eliminating biosynthetic errors *(5)*, and by participating in the processing of DNA lesions during transcription-coupled repair *(31)*. In addition, the mismatch-repair system is involved in the maintenance of chromosomal structural integrity and in the control of horizontal gene transfer by preventing recombination between non-identical DNA sequences *(32)*. The mismatch-repair system involves several proteins, of which two, MutS and MutL, have been highly conserved during evolution. MutS recognizes seven of eight possible base pair mismatches. Only C-C mismatches, which is the least frequent replication error, is not recognized. In addition, MutS binds up to four unpaired bases, allowing for repair of frameshift errors. The efficiency with which different mismatches are repaired is determined by the affinities of MutS to various mismatches. MutL plays the role of the „molecular matchmaker" between MutS-mismatch complexes and other proteins involved in the repair process. The inactivation of *mutS* or *mutL* genes results in a strong mutator phenotype, with a 10^2-fold increased rate of transition (G:C->A:T and A:T->G:C) and 10^3-fold increased rate of frameshift mutations. In addition, *mutS* or *mutL* knockout mutants have a strong hyper-recombination effect, resulting in a 10^1-10^3-fold increase in the rate of chromosomal rearrangements.

Any bacterial population is expected to harbor a subpopulation of mismatch-repair mutants due to spontaneous mutations in the mismatch-repair genes. The frequency of mismatch-repair-deficient mutators was estimated to be less than 3×10^{-5} in cultures of *E. coli* K-12 that are not subjected to any selective pressure *(3, 4)*. For *S. en-*

terica serovar Typhimurium, the frequency of mutators in the unselected population is even lower, $1\text{-}4\times10^{-6}$ *(2)*. It was experimentally and theoretically demonstrated that mismatch-repair mutators do not have a selective advantage due to of the absence of the metabolic load imposed by the production and activity of these DNA repair enzymes. If a selective advantage was present due to decreased metabolic load, then the advantage should be independent of the initial ratio of mutator to non-mutator cells. However, this is not the case; the mutator outgrows the non-mutator strain only when the ratio of mutators to non-mutators is above a certain threshold. Such a threshold was observed for *mutS (33, 34)* and *mutT (35)* mutators. This threshold is determined by the ratio of the frequency of mutants carrying beneficial alleles in mutator versus non-mutator population. In each population, the frequency of these mutants depends on the mutation rate and the population size *(36)*. Therefore, mismatch-repair-deficient mutators are selected because they produce more adaptive mutations. This selective advantage occurs even though mismatch-repair-defective mutators start with a small selective disadvantage (approximately 1%) relative to non-mutators *(4, 37)*. The selective advantage of mutators over non-mutator strains is not restricted to *mutS* mutants but is also observed for *mutL* mismatch-repair-deficient mutants *(38)*.

Low spontaneous mutation rates are sustained by numerous molecular mechanisms that protect and repair DNA and by mechanisms that ensure high-fidelity DNA replication. Inactivation of more than twenty different *E. coli* genes can confer mutator phenotypes of different strengths (for a review see *(39)*). The following question is thus raised: why do the vast majority of strong mutators found in nature and the laboratory have defective mismatch-repair systems? One explanation for this phenomenon is that inactivation of other genes that are involved in important aspects of DNA or RNA metabolism might have too high a cost to be compensated by advantageous mutations. For example, competition experiments in chemostats have shown that *E. coli mutT* mutators can also be selected for by the beneficial mutations they generate *(35)* but are never found in natural populations of *E. coli*. This result may be explained by the fact that inactivation of *mutT* gene, which encodes the protein that eliminates 8-oxo-G from the nucleotide pool *(5)*, increases replication, but also, transcriptional errors *(40)*, and increases the sensitivity to oxidative stress *(41)*, which might considerably reduce the fitness of the mutant cell.

Another specific advantage of mismatch-repair-deficient strains over other mutators is their hyper-recombination phenotype *(5)*, which may explain their abundance in nature. Recombination can also increase adaptability by increasing genetic variability. Consequently, genotypes with increased recombination rates may be selected for simply because of the favorable genotypes generated through the association of beneficial mutations that have appeared in different individuals. Indeed, the frequency of mismatch-repair-deficient mutants has been reported to increase rapidly in *E. coli* populations by hitchhiking with the recombination events they generate *(42)*.

COUNTER-SELECTION OF THE CONSTITUTIVE MUTATORS

Experimental and theoretical studies have shown that the frequency of mutator strains in a population could rapidly increase to almost 100%. However, most natural isolates are not mutators. The continuous production of deleterious mutations following adaptation is a major factor that diminishes the fitness of constitutive mutators *(33)*. A second factor is that neutral, beneficial and deleterious mutations can affect fitness in different environments in a variety of ways. Consequently, no single genotype is optimally adapted to all environments. For example, an adaptive mutation in one environment can be deleterious in another, which is a phenomenon called „antagonistic pleiotropy" *(43)*. Therefore, migration from one environment to another might contribute to the reduction of the fitness of the mutators in natural populations, as observed in *in vivo* laboratory experiments for *E. coli mutS (33)* and *S. enterica* serovar Typhimurium *mutS (44)* mutators. Continuous passage through strong bottlenecks also results in the accumulation of deleterious mutations due to genetic drift. This phenomenon, called Muller's ratchet, is particularly deleterious for strong mutator populations. For example, when wild-type and *mutS* defective cells were passaged through single-cell bottlenecks, only 3% of the wild-type lineages had phenotypically detectable mutations after 40 cycles. By contrast, 4% of *mutS* lineages had died out, 55% had auxotrophic requirements, 70% had defects in at least one sugar or catabolic pathway, 33% had a defect in cell motility, and 26% were either temperature-sensitive or cold-sensitive lethal *(45)*.

Therefore, in the long run, the fitness cost associated with high mutation rates is expected to cause the elimination of the mutator genome with consequential loss of adaptive mutations from bacterial populations. However, some adaptive mutations generated in mutator backgrounds can be saved either through horizontal gene transfer to a non-mutator background or through a reduction in the mutation rate of the adapted mutator strain before the load of deleterious mutations becomes too high. The reduction of mutation rates might be achieved through the reversion of the mutator mutation, and by the acquisition of suppressor mutations as observed in the populations of *mutT* mutators *(46)*. The probability of the acquisition of these two types of mutations is higher in mutator backgrounds. The hyper-recombination phenotype of mismatch-repair-deficient strains might also facilitate the reacquisition of the functional mismatch-repair genes from non-mutator bacteria *via* horizontal gene

transfer. Such events have been reported to occur frequently during *E. coli* evolution *(47)*.

Some bacterial species, such as *H. influenzae* and *N. meningitidis (48)*, possess mechanisms allowing them to permanently maintain high mutation rates at some loci while simultaneously avoiding fitness costs associated with high genome-wide mutation rates. The hypermutability of these loci results from the mutational properties of repetitive DNA sequences located within the gene or its controlling elements. These genes code for evasins, LPS biosynthesis enzymes, adhesins, iron acquisition proteins, and restriction-modification systems. Repetitive DNA sequences experience high rates of insertion and deletion mutations through replication slippage, which results in alternating loss-of-function and reversions. Such mutagenesis can increase bacterial fitness by enabling evasion of the host's immune system. However, the type of variation produced by localized mutator activity might not always be sufficient for adaptation, as suggested by the presence of strong mismatch-repair-deficient mutators in natural populations of *N. meningitides* and *H. influenzae (19, 21)*.

STRESS-INDUCED MUTAGENESIS

Because the cost of constitutive mutator alleles comes largely from deleterious mutations generated outside the adaptation phase, limiting increases in the mutation rate only to phases of adaptation could be particularly advantageous because it may reduce the overall cost of a high mutation rate *(49, 50)*. For this reason, the possibility of adapting the mutation rate to environmental conditions is interesting from an evolutionary point of view. Another advantage of limiting the increase in mutation rates to stressful periods is that environmental stresses can alleviate the average deleterious effect of mutations *(51)*. Computer simulations have demonstrated that stress-induced mutator alleles could be selected for almost as efficiently as constitutive mutators *(49)*. The strength of the selected stress-induced mutator alleles is positively correlated with the strength of selection. Mutations produced under stress could represent a large proportion of overall mutations and may have evolutionary consequences. For example, stress-inducible mutators with 10-fold or 100-fold higher mutation rates relative to non-mutators will adapt up to 15%, and 38% faster, respectively. Increases in the adaptation rate due to stress-inducible mutagenesis may also limit the selection of the constitutive mutator alleles. It was also shown that the fixation of an allele that improves the rate of adaptation decreases the selection for other alleles that improve the rate of adaptation *(13)*. Simulations have shown that the frequency of fixation of a constitutive mutator decreases as a function of the strength of the stress-inducible mutagenesis. The relative decrease in the fixation of a constitutive mutator allele was almost perfectly correlated with the relative improvement of the adaptation rate due to stress-inducible mutagenesis. Therefore, computer simulations suggest that stress-induced mutagenesis could be the result of selection because of the beneficial mutations that such a process can potentially generate.

MOLECULAR MECHANISMS ASSOCIATED WITH STRESS-INDUCED MUTAGENESIS.

In *E. coli*, different stresses increase the generation of mutations *via* the following different mechanisms: (i) various chemical and physical agents can generate mutagenic miscoding DNA structures that cause DNA replication errors. For example, reactive oxygen species generate 8-oxo-guanine, whereas methylating agents generate O^6-methyl-guanine *(5)*. (ii) Some environmental agents directly affect DNA but also inhibit anti-mutator DNA repair enzymes, thus increasing mutation rates. For example, nitric oxide produced by macrophages damages DNA and inhibits Fpg DNA glycosylase, O^6-methylguanine-DNA methyltransferase, and DNA ligase *(52-54)*. (iii) Stresses have also been shown to induce the mobility of transposons and insertion sequences, which can lead to either gene inactivation or activation *(55)*. (iv) DNA lesions, such as pyrimidine dimers produced by UV irradiation, block replicative DNA polymerase and induce the SOS system resulting in increased mutagenesis *(5)*. (v) Different stresses, such as starvation, high osmolarity, low temperature and low pH, induce the RpoS-regulon *(6)*. The induction of this regulon increases the capacity of cells to resist different stresses and survive but also results in increased mutagenesis *(49, 56)*.

Case (iii) is peculiar because transposon mobility is the result of their inherently „selfish" nature. Transposon mobility can increase the opportunity for transmission to other bacteria by increasing their copy number on the chromosome, or by „jumping" and inserting into conjugative plasmids and bacteriophages. Bacteria are likely to die under stress, but transposons might be transmitted before or after cell death. Cases (iv) and (v), i.e., the SOS response and the RpoS-regulon, respectively, are of interest for this discussion because they involve genetic control of the increased mutation rate.

SOS RESPONSE

All living organisms possess inducible genetic networks capable of responding to, and coping with, genotoxic stresses. The paradigm for such a network is the *E. coli* SOS system *(5)*, which is induced in response to stresses that damage DNA and/or interfere with replication catalyzed by the replicative DNA polymerase *(57)*. All these stresses increase the intra-cellular concentration of single-stranded DNA (ssDNA), which induces the SOS response *(57)*. ssDNA is the substrate for the RecA pro-

tein, to which it binds and forms a RecA-nucleofilament (RecA*). Depending on the nature of DNA substrate, loading of RecA requires either RecFOR or RecBCD complexes. The RecFOR complex facilitates the formation of RecA* on ssDNA gaps covered with SSB *(58)*, whereas the substrate for the RecBCD complex is a blunt, or nearly blunt, double-stranded DNA from which it produces ssDNA on to which RecA is loaded *(59)*. RecA* acts as a co-protease, promoting the self-cleavage of the SOS repressor LexA, thus inducing the SOS response *(60)*. At least 40 genes belong to the SOS regulon *(61, 62)*. The timing of expression of different SOS genes is controlled by the affinity of the LexA repressor for the SOS boxes in the promoter region of those genes. The level and length of the induction depend on the amount and persistence of the single-stranded DNA in the cell. Once DNA lesions are repaired and replication is restored, SOS functions are again repressed.

Most SOS functions are implicated in dealing with DNA lesions. These functions can be loosely grouped into the following two categories: elimination and tolerance of the DNA lesions. Elimination of the DNA lesions aims at restoration of the original genetic information, whereas damage tolerance allows continuation of genome replication without elimination of the DNA lesions. Damage tolerance is a last resort to rescue cells from DNA damage because persistent lesions block the replicative polymerase, which is a potentially lethal event. Without damage tolerance, cells would become highly sensitive to killing by external and endogenously generated DNA-damaging agents. DNA lesions can be tolerated *via* different pathways, of which the two best studied are homologous recombination and replicative lesion bypass. Replicative lesion bypass requires specialized DNA polymerases *(63)*, most of which belong to the Y-family of DNA polymerases that are found in prokaryotes, eukaryotes, and archaea *(64)*. *E. coli* possesses the following two Y family DNA polymerases that are regulated by the SOS system and able to perform translesion synthesis (TLS): PolIV and PolV, which are encoded by *dinB* and *umuDC* genes, respectively**.** The characteristics of the Y-family DNA polymerases are as follows: the lack of the 3''5' exonuclease activity, a more open catalytic site compared to the replicative polymerases and low processivity *(65)*. These features enable the Y-family DNA polymerase to successfully bypass lesions, but they also compromise the replication accuracy of an undamaged template. Lesion bypass can be either error-free or error-prone, when the correct or incorrect nucleotide, respectively, is incorporated opposite the damage *(66)*.

The most ubiquitous branch of the Y-family of DNA polymerases, the DinB branch, is typified by *E. coli* PolIV, human Polκ, and the archaeal Dbh/Dpo4 enzymes *(64)*. The remarkable conservation of these polymerases throughout evolution strongly suggests that the Y-family DNA polymerases from the DinB branch are essential for cell survival and fitness. In addition to SOS, the transcription of *dinB* is controlled by RpoS, a sigma subunit of RNA polymerase, which regulates the general stress response *(67)*. PolIV is also regulated by the heat shock chaperone GroE *(68)*. Therefore, PolIV is a component of several cellular stress responses. When the SOS regulon is induced, the concentration of PolIV rapidly increases from 250 to 2500 molecules per cell *(69)*. The overexpression of *dinB* substantially increases spontaneous mutagenesis *(70)*, probably through competition with PolIII for binding to the β-clamp *(71)*. PolIV is a low fidelity enzyme with a misincorporation frequency in the range of 10^{-3} to 10^{-5}. In stressed cells PolIV was shown to considerably contribute to mutagenesis. For example, PolIV is responsible for the untargeted mutagenesis of non-irradiated lambda phage in UV irradiated cells *(72)* and the increased generation of mutations under carbon source starvation and stationary phase *(73–75)*.

PolV is regulated both transcriptionally and posttranslationally and requires additional proteins to perform translesion synthesis *(76)*. PolV is a protein complex composed of the following three subunits: UmuC and two truncated UmuD proteins. The transcriptional induction of the *umuDC* operon and the synthesis of UmuC and UmuD do not result in the production of an active lesion bypass DNA polymerase. UmuD must first undergo RecA* assisted self-cleavage (mechanistically similar to that undergone by LexA) which removes 24 residues from the N-terminal and yields an UmuD' derivative. UmuD and UmuD' each form homodimers and, in addition, interact with each other to form an UmuD UmuD' heterodimer that is more stable than either of the homodimers. All three classes of dimers interact with UmuC and greatly influence its action. Even though UmuC contains catalytic DNA polymerase activity, the protein requires the presence of the UmuD' homodimer to function as a polymerase on damaged DNA. In addition to the roles RecA plays in the induction of the SOS response and the cleavage of UmuD, RecA also has a direct role in PolV-mediated TLS. PolV concentration increases from zero to approximately 60 molecules per cell one hour following SOS induction *(69)*. *E. coli* possess different mechanisms of control to ensure that active PolV is present only in cells that have suffered DNA damage that cannot be repaired otherwise. Similar to PolIV, PolV is a low fidelity enzyme with a misincorporation frequency in the range of 10^{-3} to 10^{-4}. In addition, PolV is predominantly error-prone when promoting TLS. Consequently, inactivation of the *umuDC* operon eliminates mutagenesis induced by many genotoxic agents *(5)*.

The Y family DNA polymerases are an excellent illustration of how molecular constraints on survival functions can lead to mutagenesis. They can bypass non-coding lesions that modify the structure of the DNA and block replicative polymerase, thus promoting survival. However, because bypass is performed with low fidelity,

mutations are introduced. Hence, the maintenance of genetic integrity is sacrificed for survival. Why did such polymerases fail to evolve to be error-free, i.e., to add the proper nucleotide opposite the cognate DNA lesions? There are two possible nonexclusive explanations. First, because each TLS polymerase recognizes several types of lesions, the reduced fidelity could be the optimal solution for the tradeoff between the ability to bypass different lesions and the fidelity of the bypass. Another possible explanation is that the cost of the produced deleterious mutations is lower than the selective cost associated with the activity of error-free DNA repair systems. Therefore, there is no strong selective pressure to reduce the error-rate.

Other SOS-associated phenomena that are not involved in DNA repair can also increase genetic variability in bacterial populations undergoing stress, such as the increased transposition frequency of Tn*5* and Tn*10* and the induction of temperate bacteriophages, such as lambda, 434, 21, P22, f80 and coliphage 186 *(77)*. Induction of bacteriophages results in cell lysis, but bacteriophages can transfer host genes to new cells. Some conjugative plasmids carry genes encoding PolV orthologs that are even more active than those encoded by the host chromosomes, and therefore can confer increased cellular resistance to genotoxic agents but can also lead to increased mutagenesis *(78)*. Numerous SOS functions are implicated in genetic exchange. During interspecies conjugation, differences in the DNA sequences between genomes of different species slows down the RecA-mediated recombination steps, resulting in the induction of the SOS response by RecA*. The induction of the SOS response enhances interspecies recombination *via* an overproduction of the proteins involved in homologous replication *(32)*. Thus, interspecies conjugation acts as an intracellular stress inducer in the recipient cells. Paradoxically differences in the DNA sequence, which is a major component of the interspecies genetic barrier *(79)*, helps cells to partially overcome this obstacle by triggering the SOS response *(80)*. The SOS response-dependent restriction alleviation can increase the frequency of transduction and conjugation. Furthermore, it has been found that double-strand exonuclease (ExoV) is inhibited in SOS-induced cells, which might confer a hyper-recombinogenic phenotype *(81, 82)*.

RPOS-REGULATED GENERAL STRESS RESPONSE

RpoS is one of seven *E. coli* RNA polymerase sigma factors, which compete for the association with the core polymerase subunit *(83, 84)*. The outcome of the competition is influenced by the varying numbers of each sigma factor and by different molecules that can affect the binding of sigma factors to the RNA polymerase. Each sigma factor coordinates the transcription of a set of genes, thus allowing fine control of adaptation to different physiological conditions. The production of RpoS is regulated at each of the following steps of gene expression: transcription, translation, protein stability, and activity *(6)*. Transcription of the *rpoS* gene is controlled by the cAMP receptor protein and through the signaling of ppGpp and polyphosphate. The *rpoS* mRNA is translated at low levels because the long 5' untranslated region of the *rpoS* transcript folds into a stem-loop that occludes the ribosome-binding site. The stability of this *rpoS* mRNA secondary structure is modulated by a cascade of interacting factors, including Hfq, HU, H-NS (histone-like nucleoid structuring protein), LeuO (transcription regulator), and by small noncoding RNAs such as those encoded by *dsrA*, *rprA* and *oxyS*. In growing cells, RpoS is maintained at a low level due to degradation by the ClpXP protease in a reaction promoted by RssB (proteolytic targeting factor) and inhibited by the chaperone DnaK. RssB activity is modulated by three proteins, IraP, IraM, and IraD, produced under specific stress conditions that interact with RssB and prevent RpoS degradation. Various stress conditions differentially affect the mechanisms controlling RpoS concentrations *(83, 85)*. Thus, a reduced growth rate results in increased *rpoS* transcription, whereas high cell density, high osmolarity, low temperature, phosphorus starvation and low pH stimulate the translation of already present *rpoS* mRNA. Low pH, carbon source starvation and high temperature modulate RpoS proteolysis.

When present at high concentrations, RpoS is able to outcompete the vegetative sigma factor, RpoD, and regulates transcription of hundreds of genes with unrelated physiological functions *(86, 87)*. Several factors have been shown to determine the outcome of this competition. Lrp (leucine-responsive regulatory protein) affects the selectivity of RpoS and RpoD for many promoters. Rsd (regulator of Sigma D), an anti-RpoD factor, controls the level of functional RpoD holoenzyme *(88)*. Expression of *rsd* is inversely correlated with growth rate. Intracellular concentrations of glutamate and polyphosphate and decreased DNA superhelicity have also been shown to enhance the activity of the RpoS holoenzyme and repress that of RpoD in stationary phase *E. coli* cells. Because the RpoS regulon is not induced only during stationary phase, and because it responds to many different stress conditions, it is considered a general stress response *(89)*.

The induction of the RpoS regulon, which involves approximately 10% of the *E. coli* genes, results in morphological and metabolic modifications and provides resistance to a variety of stresses (e.g., resistance to UV, heat shock, oxidative stress and extreme osmolarity). Intriguingly, although the main priority of this regulon is to assure survival, conservation of original genetic information is not a priority. For example, RpoS stimulates transposition of the *Pseudomonas putida* transposon Tn*465* during stationary phase *(90)*. The overproduction of RpoS-regulated *hha* increases the frequency of transposi-

tion of insertion elements within the *E. coli* chromosome as well *(91)*. Additionally, the overproduction of *rpoS* results in increased mutagenesis in growing cells *(92)*. Furthermore, the RpoS regulon has been implicated in stationary phase mutagenesis in *E. coli* and *P. putida (93)*.

The following two molecular mechanisms have been described as being responsible for the RpoS regulon-dependent increase of stationary phase mutagenesis: induction of the *dinB* gene and downregulation of the mismatch-repair system. Such non-canonical regulation, i.e., LexA independent, of the PoIIV TLS DNA polymerase may help the cells survive certain DNA damages without new protein synthesis. This could be the case with cytotoxic alkylating DNA lesions *(94)*, which can accumulate in DNA because of RpoS-dependent downregulation of *alkA* which encodes the DNA glycosylase that removes replication-blocking 3-methyladenine and 3-methylguanine *(95)*. In stationary phase cells, the transcription of *mutS* and *mutH* and the concentration of MutS and MutH decreases to very low levels *via* a RpoS- and Hfq-dependent mechanism compared to growing cells *(96-98)*. Consequently, the activity of the mismatch-repair system is reduced during stationary phase. The overproduction of MutS, but not MutL in wild-type cells significantly decreased stationary phase mutagenesis *(49)*. Similarly, overexpression of the MutS repair protein significantly decreased the rate of G:C→T:A transversion mutations in stationary-phase wild-type, *mutY* and *mutM* strains *(99)*.

What would be the benefit to bacteria of RpoS-mediated downregulation of the mismatch-repair system, which is a major contributor to the fidelity of DNA replication? One hypothesis is that downregulation of mismatch-repair activity contributes to survival simply by saving energy by not expressing the proteins necessary for mismatch-repair during stationary phase when there is no active DNA replication. However, it has been shown that RpoS downregulates mismatch-repair activity also in actively growing cells when they are exposed to sub-inhibitory concentrations of antibiotics *(100)*. This effect is achieved by a RpoS-controlled small RNA, SdsR, which mediates MutS depletion. SdsR pairs with *mutS* mRNA and induces its cleavage by the house-keeping endonuclease RNase III. The absence of efficient mismatch-repair system surveillance leads to increased mutagenesis, which increases the probability of generating adaptive mutations.

CONCLUDING REMARKS

Organisms live in constantly changing environments in which the nature, severity and frequency of environmental stresses are highly variable. Therefore, organisms possess multiple strategies to cope with environmental fluctuations. One such strategy is the modulation of mutation rates as a function of the degree of adaptation to the environment. Mutation rates are low when the environment is stable and high when the environment is unstable and stressful. High mutation rates can be advantageous because they increase the probability that adaptive mutations will be generated. Mutation rates can be modulated by changing the proportions of constitutive mutators vs non-mutators at the population level and by inducing stress responses, which increase mutation rates transiently in individual cells. It has been demonstrated that constitutive mutator alleles are selected because they hitchhike with the adaptive mutation they generate. Computer simulations have shown that stress-induced mutagenesis can also be selected in a wide range of conditions because it is advantageous at both the individual and the population levels *(101, 102)*. Simulations also showed that stress-induced mutagenesis facilitate a rapid adaptation to complex environmental challenges without compromising the population fitness in a stable environment because it breaks the trade-off between adaptability (the capacity of populations to adapt to new complex conditions) and adaptedness (the ability of populations to remain adapted to existing conditions). Finally, the fact that stress-induced mutagenesis may play an important role in bacterial evolution was also strongly supported by the high similarity between the mutational imprints of the RpoS-dependent stress-induced mutagenesis observed in the laboratory and naturally evolving genomes *(103)*.

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