David Mittelman *Editor*

Stress-Induced Mutagenesis

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For Sarah, Joseph, Joshua, and Sophia

Preface

In 1942, Julian Huxley referred to the cross-disciplinary, unified evolutionary theory as the *modern evolutionary synthesis*. In the early part of the twentieth century, Fisher, Haldane, and Wright—and later Mayr, Dobzhansky, and others—produced a revised model of Darwinian evolution that rationalizes Mendelian genetics in the context of natural selection. The presence of pre-existing heritable variation is key for selection to be effective and is a contingency of adaptability. At the same time, mutation is proposed to accumulate at a constant rate, regardless of selective pressures and environmental cues.

The discoveries of epigenetic inheritance and stress-induced mutation have challenged the claim of independence between mutation and selection processes. The ability of an organism to undergo genetic or epigenetic change in response to environmental stresses suggests an ability to alter the *rate* of mutation, which could take effect globally or at specific parts of the genome, and temporarily or permanently. Mutation rates can be altered globally and permanently by the presence of mutator alleles, temporarily due to transient events such as environmental stress, or locally at "hotspot" locations in the genome. Such mechanisms are clearly valuable from an evolutionary perspective, as it is advantageous for mutation to be restricted in both time and space, if most selectable mutations produce deleterious outcomes.

This volume compiles key evidence for stress-induced genetic and epigenetic mutation, integrating cross-disciplinary observations from a number of species and biological systems, including human. The observations have vast implications for evolutionary biology but also for human medicine. For example, genomic instability is now recognized as a hallmark of most cancers. Tumor resistance and recurrence are modeled within the context of clonal expansions. The comprehensive understanding of stress-induced mutagenesis and the processes underlying evolvability, studied across many biological systems, will enable gains in the treatment and management of cancer, as well as other human disorders that result from damaged or unstable genomes.

Stress-induced mutagenesis has been most widely studied in bacterial systems. In the first chapter, Ivan Matic describes the role (and regulation) of mutator alleles

and stress-induced mutagenesis pathways in the evolution of bacterial populations. The chapter by Susan Rosenberg and colleagues dissects the pathways to stress-induced mutagenesis, focusing specifically on the localization of mutagenic repair to double-strand breaks. This chapter provides some exciting new evidence that argues mutagenesis is not an inevitable consequence of DNA repair. This has been a long-standing point of debate. In his classic critic, "Adaptation and Natural Selection," George Williams argued that mutation rate is a "mechanical inevitability," the byproduct of physical limitations in the fidelity of DNA repair processes and not the product of natural selection. Rosenberg and colleagues now show that stress-induced mutagenic repair is activated by repair components that are not required for the proper resolution of a DNA break. They also discuss the localization of mutation, during stress, to DNA breaks, which minimizes the impact of deleterious mutations to the genome. In the third chapter, Eduardo Robleto and Ronald Yasbin describe transcription-coupled mutagenesis pathways that illustrate another way in which mutagenesis can be triggered temporally by environmental cues, and then localized to focused portions of the genome. In the fourth chapter, Milton Saier and colleagues present intriguing evidence that stress can activate transposons, which play roles in gene regulation and disease.

From the seminal work of Lindquist and Rutherford, the stress-activated Hsp90 chaperone is now known to participate in the canalization of traits, something first described by Conrad Waddington more than a half-century ago. Hsp90 normally functions to buffer client proteins against the effects of genetic variation. Severe environmental stress can overwhelm the chaperone's buffering capacity, causing previously cryptic genetic variation to be expressed. In Chap. 5, Douglas Ruden and colleagues share very exciting evidence that, in flies, Hsp90 can induce novel epigenetic changes in addition to exposing existing variation. Shunsuke Ishii and colleagues continue the discussion of epigenetics in a very compelling chapter that maps a molecular pathway by which the ATF-2 family of transcription factors facilitates the inheritance of stress-induced epigenetic changes.

As mentioned above, focusing mutagenesis in time and space minimizes the impact of deleterious mutations across the genome. Tandem repeats are an important source of functional variation that also fit these constraints. Tandem repeat mutation rate is modulated by global and local factors and triggered by temporal events such as stress. Many tandem repeats affect morphological, behavioral, and life-history traits through subtle and quantitative effects on gene function. Most interestingly, the incremental functional impact of repeat mutation even further decreases the frequency of catastrophically deleterious effects. In Chap. 7, John Wilson and colleagues outline key lines of evidence in human cells that reveal how repeat mutation is modulated by local and global factors, as well as stress, transcription, and DNA methylation.

In Chap. 8, Peter Glazer and colleagues outline the mechanistic details by which the genomes of human cells become unstable as a result of exposure to hypoxia, or oxygen deprivation. This is a particularly important stressor in the context of cancer, as developing tumors experience hypoxic stress prior to the recruitment of dedicated blood supplies through angiogenesis. The finding that hypoxia and other stres-

sors can destabilize cancer genomes is very significant, as it is likely that tumors draw upon this variation to adapt to their microenvironments and to resist drug treatment. In the following chapter, Jac Nickoloff and colleagues describe a mechanism for the fascinating and equally frightening observation that in human cells, delayed transgenerational genomic instability can be induced by low-dose radiation treatment. The possibility that radiotherapy can be a trigger for future cancer development raises important questions about the safety and appropriateness of such a therapy. In the related and following chapter, Carmel Mothersill and colleagues explore stress-induced bystander effects and highlight the relevance of this phenomenon for cancer and adaptive evolution. Denise Montell and colleagues present in Chap. 11 some rather surprising studies that document the reversal of the apoptotic process. They present evidence that dying cells with damaged genomes can revert to living and proliferating cells, in a process they cleverly term *anastasis*. One implication of this phenomenon is that it could be a possible mechanism for tumor cells to survive and even resist treatment. In Chap. 12, Yuri Dubrova documents provocative evidence, in rodents, for transgenerational instability induced by radiation or chemical toxins. The findings are particularly compelling as the genomic instability increases in subsequent generations. The emerging evidence for this phenomenon in humans is frightening, particularly in the context of the recent Fukushima Daiichi nuclear disaster in Japan.

The final chapter by Subhajyoti De summarizes the emerging revolution in high-throughput sequencing. The affordability and availability of high-throughput sequencing has created an unprecedented surge in the use of genomic data in basic, translational, and clinical research. The ability to rapidly sequence and analyze entire genomes or populations of genomes is transforming the study of mutagenesis and genome evolution. For the first time, rather than utilizing engineered assays or genomic markers, the complete and unbiased spectrum of stress-induced changes can be directly measured genome-wide. The implications for cancer are vast as well, since tumor sequencing now enables the identification of trigger mutations and as well as passenger mutations that could serve as targets for tumor susceptibilities.

I am so grateful to the chapter authors for their enthusiasm and for helping me assemble this volume. As a graduate student, “DNA Repair and Mutagenesis” was my bible. The textbook, helmed by Errol Friedberg, is one of the most complete resources for understanding the intricacies of DNA repair and genome stability. In preparing this volume I strived to produce a worthy and complementary resource documenting the evidence for stress-induced genetic and epigenetic mutation across all biological systems; and the implications of these processes to evolutionary theory and cancer genetics. The authors of these chapters are leaders in their respective disciplines, and I am incredibly thrilled and grateful that so many of them were able to collaboratively assemble what I think is one of the most comprehensive cross-disciplinary resources for this exciting and relevant field.

Specific thanks must go to Susan Rosenberg for encouraging me to tackle the daunting task of assembling this volume and to John Wilson for advice and support. Both are from Baylor College of Medicine and I have to acknowledge the Department

of Molecular and Human Genetics at BCM as an amazing environment in which to train and incubate ideas about science. I would like to thank my lab at my current institution, Virginia Tech, for their feedback and suggestions—and I would like to single out R. Matthew Ward, who carefully read through all the chapters with me, provided lots of valuable input and coauthored the last chapter. I also would like to thank Maureen Lawrence-Kuether, my administrative specialist, and Melanie Tucker and Meredith Clinton, both from Springer, for technical support and assistance during the writing process. Lastly, I would like to thank my wife, Kristen, for her patience, love, and support.

Blacksburg, VA, USA David Mittelman

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Chapter 1
Stress-Induced Mutagenesis in Bacteria

Ivan Matic

Abstract Under stress, high mutation rates can be advantageous because they increase the probability of generation of the adaptive mutations. Mutation rates can be modulated by changing the proportion of constitutive mutator versus non-mutator bacteria at the population level, or by inducing stress responses, which increase mutation rates transiently in individual cells. Constitutive mutator alleles are selected because they hitchhike with the adaptive mutations they generate. There are two nonexclusive hypotheses concerning the nature of selective pressure acting on the molecular mechanisms controlling stress-induced mutagenesis: stress-induced mutagenesis could be an unavoidable by-product of mechanisms involved in survival under stress, or stress-induced mutator phenotypes could be selected for in the same way as constitutive mutator alleles; that is, via hitchhiking with the adaptive mutations they generate. However, regardless of the nature of selective pressure acting on stress-induced mutagenesis, it is very likely that the resulting increased genetic variability plays an important role in the bacterial evolution.

Stress is a disturbance of the normal functioning of a biological system, provoked by environmental factors whose amplitude and persistence are such that they cause a reduction in growth rate and increased mortality (Bijlsma and Loeschcke 1997). Some organisms react to stress by inducing behavioral or physiological responses, while others increase production of genetically diverse offspring. For a long time, increased production of genetic diversity was not considered adaptive, because, given the huge population size of most bacterial species, it was thought the adaptation was rarely limited by the available genetic diversity. However, strong bottlenecks or stressful environmental conditions can severely reduce bacterial population size and, consequently, genetic diversity. Under such circumstances, it could be advantageous to have

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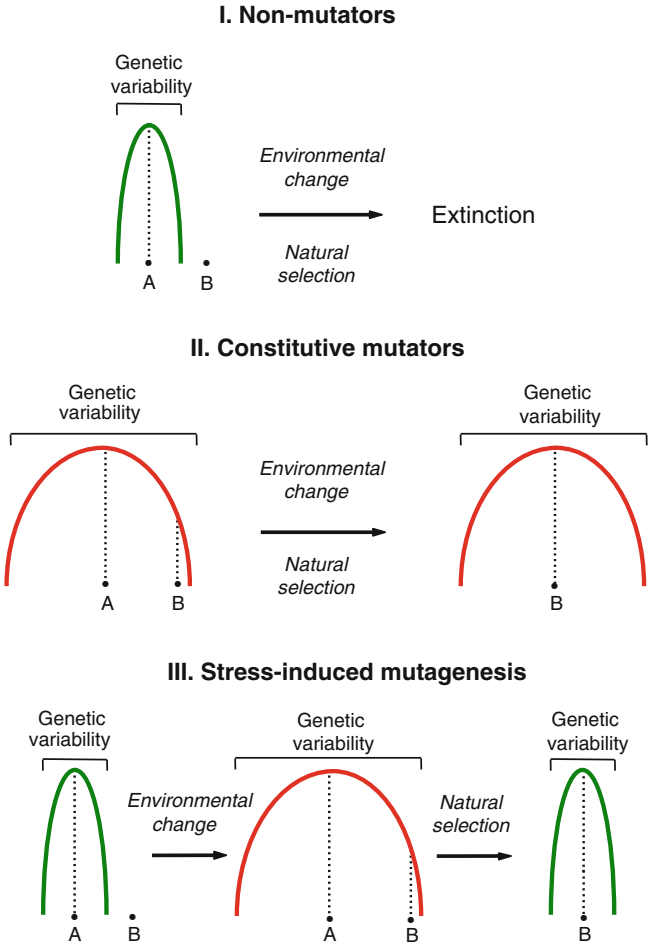

Fig. 1.1 Interplay between genetic variability and population evolvability. Allele A has maximal fitness under starting non-stressful environmental condition and consequently dominates population. Allele B is required for the adaptation to new environmental condition. (**I**) Because genetic variability is low, allele B is not present in the starting nonmutator population. When such population face environmental change, it goes to extinction. (**II**) Mutator population generates higher genetic variability. Consequently, allele B is present in the starting population. Upon environmental change, the individuals carrying allele B are favored by the natural selection. While this strategy increases probability for the rapid adaptation to the environmental change, on the longer run, the cost associated with high mutation rates is expected to cause the loss of fitness of the mutator cells. (**III**) The ability to regulate mutation rates in response to the environmental cues might be advantageous from the evolutionary point of view because the limitation of the increase in the mutation rates to stress phases should reduce the overall cost of a high rate of mutation and, in the same time, increase probability of generation of the adaptive alleles

high mutation rates (Fig. 1.1). Experimental (LeClerc et al. 1998; Mao et al. 1997) and theoretical (Boe et al. 2000) studies indicate that the observed frequency of strains with high constitutive mutation rates (mutators) in bacterial natural populations is much higher than would be expected from mutation/selection equilibrium alone.

This suggests that there are situations in nature where mutator phenotypes confer a selective advantage. Constitutive mutator phenotypes generally result from mutations in genes coding for DNA repair enzymes or proteins that assure the accuracy of DNA replication. These mutant genes are called mutator alleles. Mutagenesis can also increase during stress as a result of direct alteration of the DNA molecule, and/or from a genetic program that is induced under stress. Two stress responses are known to increase mutagenesis when induced: the SOS system induced by genotoxic stresses (Friedberg et al. 2006); and the RpoS-regulated general stress response triggered by many different stresses (Battesti et al. 2011). In this chapter, the role of molecular, ecological, and evolutionary factors involved in shaping mutation rates in bacterial populations, using the *Escherichia coli* species as an example, is discussed.

Selection of Constitutive Mutators

Newly arisen mutations can have very different impacts on the fitness of the organism, ranging from deleterious to neutral to beneficial. However, they appear at very different rates. For example, for *E. coli* K-12, the rate of deleterious mutations per genome per replication is at least $2–8 \times 10^{-4}$ (Boe et al. 2000; Kibota and Lynch 1996), while that of beneficial mutations is about 2×10^{-9} (Imhof and Schlotterer 2001). It is generally believed that the rate of spontaneous mutations results from a balance between the effects of deleterious mutations and the metabolic costs of reducing mutation rates (Drake et al. 1998). Indeed, the replication error rate in *E. coli* is low, i.e., about 10^{-10} per base per replication, and it seems that it cannot be reduced further (Drake 1993). Any variant that has increased mutation rates is expected to have reduced fitness due to increased production of deleterious mutations. However, when adaptation is limited by the available genetic variability, natural selection favors mutator cells. Mutator alleles are carried at high frequency through hitchhiking with the beneficial mutations they generate when the fitness gain provided by beneficial mutations counterbalances the fitness loss of the increased generation of deleterious mutations (Taddei et al. 1997a). The linkage between beneficial mutations and mutator alleles is particularly strong in bacteria because the rate of gene exchange in these asexual organisms is, in general, very low. Mutators are particularly favored when several beneficial mutations are required for the adaptation (Mao et al. 1997). For example, if the probability of generating each beneficial mutation is 10^2-fold higher in mutator populations than in non-mutator populations, then the probability that two beneficial mutations will be generated in 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 frequency of mutators depends on the total population size (Tenaillon et al. 1999), on mutator strength (i.e., the mutator mutation rate relative to the non-mutator mutation rate (Taddei et al. 1997a)), and on the rate of gene exchange (Tenaillon et al. 1999). It also depends on the stability of the environment. For example, mutator alleles are particularly advantageous upon a shift

in environmental conditions (Tanaka et al. 2003; Travis and Travis 2002). The dynamics of mutator selection depend also on environmental spatial heterogeneity, which can allow or prevent the 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 (Travis and Travis 2004).

Conditions favoring strong constitutive mutators must be frequent in nature because mutators have been found in populations of *E. coli* (Matic et al. 1997), *Salmonella enterica* (LeClerc et al. 1996), *Neisseria meningitidis* (Richardson et al. 2002), *Haemophilus influenzae* (Watson et al. 2004), *Staphylococcus aureus* (Prunier et al. 2003), *Helicobacter pylori* (Bjorkholm et al. 2001), *Streptococcus pneumoniae* (del Campo et al. 2005), and *Pseudomonas aeruginosa* (Oliver et al. 2000), with frequencies ranging from 0.1% to above 60%.

Mismatch-Repair Deficient Mutators

The vast majority of strong constitutive mutators found in the laboratory (*E. coli* (Sniegowski et al. 1997), *S. enterica* serovar Typhimurium (LeClerc et al. 1998), *Pseudomonas fluorescens* (Pal et al. 2007) and in nature (*E. coli* (Matic et al. 1997; Sniegowski et al. 1997), *S. enterica* (LeClerc et al. 1996), *N. meningitides* (Richardson et al. 2002), *P. aeruginosa* (Oliver et al. 2002)) have a defective mismatch-repair system due to the inactivation of *mutS* or *mutL* genes. Molecular characterization of *E. coli* and *P. aeruginosa* natural *mutS* and *mutL* mutants has revealed that these genes are inactivated by a variety of mechanisms: frameshifts, insertions, premature stop codons, and deletions (Oliver et al. 2002; Li et al. 2003). The mismatch repair system controls the fidelity of DNA replication by eliminating biosynthetic errors (Friedberg et al. 2006), and by participating in DNA lesion processing during transcription-coupled repair (Mellon and Champe 1996). 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 nonidentical DNA sequences (Matic et al. 1995). The mismatch-repair system involves several proteins, of which two—MutS and MutL—have been highly conserved during evolution. The MutS protein recognizes seven of eight possible base pair mismatches. Only C-C mismatches, which represent the least frequent replication error, are not recognized. In addition, the MutS protein 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 protein for various mismatches. MutL plays the role of "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 mutations (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 were not subjected to selective pressure (Mao et al. 1997; Boe et al. 2000). For *S. enterica* serovar Typhimurium, the frequency of mutators in the unselected population is even lower, $1–4 \times 10^{-6}$ (LeClerc et al. 1998). It was experimentally and theoretically demonstrated that the mismatch repair mutators do not have a selective advantage because of the absence of the metabolic load imposed by the production and activity of these DNA repair enzymes. If there were a selective advantage due to decreased metabolic load, then this 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 mutator/non-mutator population size is above a certain threshold. Such a threshold was observed for *mutS* (Giraud et al. 2001; Labat et al. 2005) and *mutT* (Chao and Cox 1983) mutators. This threshold is determined by the ratio of the frequency of mutants carrying beneficial alleles in mutator populations to the frequency in non-mutator population. In each population, the frequency of these mutants depends on the mutation rate and the population size (Le Chat et al. 2006). Therefore, mismatch repair deficient mutators are selected because they produce more adaptive mutations. This selective advantage occurs despite the fact that mismatch repair defective mutators start with a small selective disadvantage (about 1%) relative to non-mutators (Boe et al. 2000; Trobner and Piechocki 1984). 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 (Tröbner and Piechocki 1981).

Low spontaneous mutation rates are maintained by the activity of many molecular mechanisms that protect and repair DNA, as well as by the mechanisms that assure high-fidelity DNA replication. Inactivation of over 20 different *E. coli* genes can confer mutator phenotypes of different strengths (for a review, see Horst et al. 1999). So, why do the vast majority of strong mutators found in nature and in laboratory systems have defective mismatch-repair systems? One explanation for this phenomenon is that inactivation of the other genes involved in important aspects of DNA or RNA metabolism might have too high a cost for advantageous mutations to compensate. For example, competition experiments in chemostats have shown that *E. coli mutT* mutators can also be selected for by the beneficial mutations they generate (Chao and Cox 1983), but are never found in *E. coli* natural populations. This may be explained by the fact that inactivation of *mutT* gene, which codes for the protein that eliminates 8-oxo-G from the nucleotide pool (Friedberg et al. 2006), increases replication, but also increases transcriptional errors (Taddei et al. 1997b) and sensitivity to oxidative stress (Guelfo et al. 2010), which might considerably reduce the fitness of the mutant cell.

Another specific advantage of mismatch repair deficient strains over other mutator alleles, which might also explain their abundance in nature, is their hyper-recombination phenotype (Friedberg et al. 2006). Recombination can also increase adaptability by increasing genetic variability. Consequently, genotypes with increased recombination rates might be selected due to favorable genotypes

generated by the association of beneficial mutations from 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 (Funchain et al. 2001).

Counter-Selection of the Constitutive Mutators

Experimental and theoretical studies showed that the frequency of mutator strains in a population could rapidly increase to almost 100%. However, the majority of natural isolates are not mutators. A major factor that diminishes the fitness of constitutive mutators is the continuous production of deleterious mutations once adaptation is achieved (Giraud et al. 2001). A second factor is that neutral, beneficial, and deleterious mutations can impact fitness differently in different environments. Consequently, no single genotype is optimally adapted to all environments. For example, an adaptive mutation in one environment can be deleterious in another, a phenomenon called "antagonistic pleiotropy" (Cooper an. Lenski 2000). Therefore, migration from one environment to another might contribute to the reduction of mutator fitness in natural populations, as observed in in vivo laboratory experiments for *E. coli mutS* (Giraud et al. 2001) and *S. enterica* serovar Typhimurium *mutS* (Nilsson et al. 2004) mutators. Continuous passage through strong bottlenecks results in the accumulation of deleterious mutations due to genetic drift, a phenomenon, called Muller's ratchet, which is particularly deleterious to 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. Contrarily, 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 (Funchain et al. 2000).

Therefore, in the long run, the fitness cost associated with high mutation rates is expected to cause the elimination of the mutator genome with a consequential loss of adaptive mutations from bacterial populations. However, some adaptive mutations generated in mutator backgrounds can be saved either by horizontal transfer to a non-mutator background, or by a reduction in the mutation rate of the adapted mutator strain before the load of deleterious mutations becomes too high. This reduction of mutation rate might be achieved by the reversion of the mutator mutation, or by the acquisition of suppressor mutations as observed in the populations of *mutT* mutators (Tröbner and Piechocki 1984). The probability of acquiring 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 seem to happen frequently during *E. coli* evolution (Denamur et al. 2000).

Some bacterial species, like *H. influenzae* and *N. meningitides* (Moxon et al. 2006), possess mechanisms allowing them to permanently maintain high mutation rates at some loci, while at the same time avoiding the 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 within 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 reversion mutations. Such mutagenesis can increase bacteria fitness by allowing 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 the strong mismatch repair deficient mutators in the natural populations of *N. meningitides* and *H. influenzae* (Richardson et al. 2002; Watson et al. 2004).

Stress-Induced Mutagenesis

Because the cost of constitutive mutator alleles comes largely from deleterious mutations generated outside the adaptation phase, limitation of increased mutation rates to phases of adaptation could be particularly advantageous by reducing the overall cost of a high mutation rate (Fig. 1.1) (Bjedov et al. 2003; Tenaillon et al. 2004). The possibility of adapting the mutation rate to environmental conditions could be interesting from an evolutionary point of view. Another advantage of limiting the increase in the mutation rate to stressful periods is the fact that environmental stresses can alleviate the average deleterious effect of mutations (Kishony and Leibler 2003). Computer simulations have shown that stress-induced mutator alleles could be selected for almost as efficiently as constitutive mutators (Bjedov et al. 2003). 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, a population with a tenfold or 100-fold (relative to the non mutator mutation rates) stress-inducible mutator phenotype will adapt up to 15% or 38% faster, respectively, than a non-mutator population. An increase in the adaptation rate due to stress-inducible mutagenesis may also limit the selection of the constitutive mutator alleles. It was previously described that the fixation of an allele improving the rate of adaptation decreases the selection for other alleles improving the rate of adaptation (Tanaka et al. 2003). Simulations have shown that fixation frequency 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 generate. However, analysis of the molecular mechanisms involved in the control of the stress-induced mutagenesis suggests that mutagenesis might also increase as a byproduct of the survival strategy.

Molecular Mechanisms Associated with Stress-Induced Mutagenesis

In *E. coli*, different stresses increase the generation of mutations via different mechanisms. (1) 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, while methylating agents generate O^6-methyl-guanine (Friedberg et al. 2006). (2) Some environmental agents directly affect DNA, but also inhibit anti-mutator DNA repair enzymes, thus increasing mutation rates. For example, the nitric oxide produced by macrophages damages DNA and inhibits Fpg DNA glycosylase, O^6-methyl-guanine-DNA methyltransferase, and DNA ligase (Graziewicz et al. 1996; Laval and Wink 1994; Wink and Laval 1994). (3) DNA lesions, such as the pyrimidine dimers produced by the UV irradiation, which block replicative DNA polymerase, induce the SOS system, resulting in increased mutagenesis (Friedberg et al. 2006). (4) Different stresses—such as starvation, high osmolarity, low temperature and low pH—induce the RpoS-regulon (Battesti et al. 2011). The induction of this regulon increases the capacity of cells to resist different stresses and survive, but also results in increased mutagenesis (Bjedov et al. 2003; McKenzie et al. 2001). (5) Stresses have also been shown to induce the mobility of transposons and insertion sequences, which can lead to gene activation or inactivation (Levy et al. 1993).

Cases (3), (4), and (5) imply genetic control of the mutation rate. Concerning the selective pressure acting on such mechanisms, the case of insertion sequences and transposons is peculiar. It has been shown that transposons could be selected for as mutator genes by hitchhiking with the mutations they produce (Chao et al. 1983). Nevertheless, it is hard to tell whether transposon mobility is the result of a selection acting to enhance the chance of survival of the bacterial strain carrying them, or if it is the result of an inherently selfish nature of transposons. Transposon mobility can increase the opportunity for transmission to other bacteria by increasing their copy number in the chromosome, or by jumping on the conjugative plasmids and bacteriophages. Bacteria are likely to die under stress, but transposons might be transmitted before or after the cell death.

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 (Friedberg et al. 2006), which is induced in response to stresses that damage DNA and/or interfere with the replication catalyzed by the replicative DNA polymerase (Sassanfar and Roberts 1990). All these stresses increase the intracellular concentration of single-stranded DNA (ssDNA), the SOS inducing signal (Sassanfar and Roberts 1990). ssDNA is the substrate for the RecA protein, which

binds it to form a RecA-nucleofilament (RecA*). Depending on the nature of the DNA substrate, RecA loading requires either RecFOR or RecBCD complexes. The RecFOR complex facilitates the formation of RecA* on ssDNA gaps covered with SSB (Morimatsu and Kowalczykowski 2003), while the substrate for the RecBCD complex is a blunt, or nearly blunt, double-stranded DNA end, from which it produces ssDNA, on to which it loads RecA (Kowalczykowski et al. 1994). RecA* acts as a co-protease, promoting the self-cleavage of the SOS repressor LexA, thus inducing the SOS response (Little et al. 1980). At least 40 genes belong to the SOS regulon (Courcelle et al. 2001; Fernandez De Henestrosa et al. 2000). The timing of expression of different SOS genes is controlled by the affinity of the LexA repressor for the SOS boxes of those genes. The level and length of the induction depends on the amount and persistence of the single-stranded DNA in the cell. Once DNA lesions are repaired and replication restored, SOS functions are again repressed.

Most SOS functions are implicated in dealing with the DNA lesions. These functions can be loosely grouped into two categories: the elimination of DNA lesions, aims to restore the original genetic information, and the tolerance such lesions, which allows continuation of the genome replication without eliminating the lesions. Damage tolerance is a measure of last resort to rescue cells from DNA damage because persistent lesions block the replicative polymerase, which is potentially lethal event. Without it cells would become highly sensitive to death by either external or 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 (Rattray and Strathern 2003), most of which belong to the Y-family of DNA polymerases that are found in prokaryotes, eukaryotes, and archaea (Ohmori et al. 2001). *E. coli* possesses two Y family DNA polymerases, regulated by the SOS system, capable of performing translesion synthesis (TLS): PolIV and PolV, which are encoded by *dinB* and *umuDC* genes, respectively. Y-family DNA polymerases lack $3' \rightarrow 5'$ exonuclease activity, but have a more open catalytic site compared to the replicative polymerases, and have low processivity (Yang 2003). These features enable the Y-family DNA polymerase to successfully bypass lesions, but also compromise the accuracy of replication of an undamaged template. Lesion bypass can be either error-free or error-prone, depending on whether the correct or incorrect nucleotide, respectively, is incorporated opposite the damage (Friedberg et al. 2002).

The most ubiquitous branch of the Y-family of DNA polymerases, the DinB branch, is typified by *Escherichia coli* PolIV, human Polκ, and the archaeal Dbh/Dpo4 enzymes (Ohmori et al. 2001). Such remarkable conservation throughout evolution strongly suggests that the Y-family DNA polymerases from the DinB branch are extremely important for cell survival and fitness. In addition to SOS, the transcription of the *dinB* gene is controlled by RpoS, a sigma subunit of RNA polymerase, which regulates a general stress response (Layton and Foster 2003). PolIV is also regulated by the heat shock chaperone GroE (Layton and Foster 2005). Therefore, PolIV is a component of several cellular stress responses. When the SOS regulon is induced, the number of PolIV molecules rapidly increases to 250 and

2,500 per cell (Nohmi 2006). The overexpression of the *dinB* gene substantially increases spontaneous mutagenesis (Kim et al. 1997), probably by competing with PolIII for binding to the β-clamp (Lenne-Samuel et al. 2002). 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 (Brotcorne-Lannoye and Maenhaut-Michel 1986), and for the increased generation of mutations under carbon source starvation in the stationary phase (McKenzie et al. 2001; Tompkins et al. 2003; Foster 2000).

PolV, is regulated both transcriptionally and posttranslationally, and in addition needs other proteins in order to perform translesion synthesis (Sutton et al. 2000). PolV is a protein complex composed of three subunits: UmuC, and two truncated UmuD proteins. The transcriptional induction of the *umuDC* operon and the synthesis of the UmuC and UmuD proteins do not result in the production of an active lesion-bypass DNA polymerase. The UmuD protein must first undergo RecA* assisted self-cleavage (mechanistically similar to that undergone by LexA) which removes the N-terminal 24 residues and yields a UmuD' derivative. UmuD and UmuD' each form homodimers and, in addition, interact with each other to form a 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. Despite the fact that UmuC has catalytic DNA polymerase activity, the protein strictly requires UmuD' homodimer to function as a polymerase on damaged DNA. In addition to RecA's roles in SOS response induction and UmuD cleavage, RecA also plays a direct role in the PolV mediated TLS. PolV concentration increases from zero to about 60 molecules per cell only 1 h after SOS induction (Nohmi 2006). Thus, *E. coli* possess different mechanisms of control in order to ensure that active PolV is only present in cells that have suffered DNA damage that cannot be repaired otherwise. Like 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 (Friedberg et al. 2006).

Y family DNA polymerases are an excellent illustration of how molecular constraints on survival functions can lead to mutagenesis. They can bypass noncoding lesions that modify the structure of the DNA and block replicative polymerases, thus allowing survival. But because this bypass is performed with low fidelity, it introduces mutations. Hence, the maintenance of genetic integrity is sacrificed for survival. Why did such polymerases not evolve to be error-free—i.e., to add the proper nucleotide opposite the cognate DNA lesions? There are two possible, non-exclusive explanations. First, because each TLS polymerase recognizes several types of lesions, the reduced fidelity could be the optimal solution for the trade-off between the ability to bypass different lesions and the fidelity of the bypass. Another possible explanation is that the cost of the resulting deleterious mutations is lower than the selective cost associated with the activity of error-free DNA repair systems. Hence, there is no strong selective pressure to reduce the error-rate.

Some other SOS-associated phenomena that are not involved in DNA repair can also increase genetic variability in stressed bacterial populations; for example, 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 (Roberts and Devoret 1983). 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 also increased mutagenesis (Sedgwick and Goodwin 1985). Numerous SOS functions can be implicated in the genetic exchange. During interspecies conjugation, the DNA sequence divergence between genomes of different species slows down the RecA-mediated recombination steps, resulting in the induction of the SOS response by the RecA*. The induction of the SOS response enhances interspecies recombination via an overproduction of the proteins involved in homologous replication (Matic et al. 1995). Thus, interspecies conjugation acts as an intracellular stress inducer in the recipient cells. Paradoxically, DNA sequence divergence—a major component of the interspecies genetic barrier (Matic et al. 1996)—helps cells to partially overcome this obstacle by triggering the SOS response (Matic et al. 2000). 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 (Kannan and Dharmalingam 1990; Rinken and Wackernagel 1992).

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 (Hengge-Aronis 2002; Eisenstark et al. 1996). The outcome of the competition is influenced by the varying number 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 condition. The production of RpoS is regulated at every step of gene expression: transcription, translation, protein stability, and activity (Battesti et al. 2011). 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 small noncoding RNAs: *dsrA* RNA, *rprA* RNA, and *oxyS* RNA. In growing cells, RpoS levels are maintained at a low level due to degradation by the ClpXP protease in a reaction that is 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 RpoS concentration control mechanisms (Hengge-Aronis 2002; Peterson et al. 2005). 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 concentration, RpoS outcompetes the vegetative sigma factor, RpoD, and regulates transcription of hundreds of genes with unrelated physiological functions (Patten et al. 2004; Weber et al. 2005). Several factors have been shown to determine the outcome of this competition: Lrp (leucine-responsive regulatory protein) affects the selectivity of these two sigma factors for many promoters; Rsd (regulator of Sigma D), an anti-RpoD factor, controls the level of functional RpoD holoenzyme (Jishage and Ishihama 1998); and *rsd* gene expression is inversely correlated with growth rate. The intracellular concentrations of glutamate and polyphosphate, as well as decreased DNA superhelicity, have also been shown to enhance the activity of RpoS holoenzyme, and to repress that of RpoD in stationary phase *E. coli* cells. Because the RpoS regulon is not induced only in stationary phase, but rather responds to many different stress conditions, it is considered a general stress response (Hengge-Aronis 2000).

The induction of the RpoS regulon, which concerns about 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, heath shock, oxidative stress, and extreme osmolarity). Intriguingly, while the key priority of this regulon is to assure survival, conservation of original genetic information is not. For example, RpoS stimulates transposition of the *Pseudomonas putida* transposon Tn*465* during stationary phase (Ilves et al. 2001). The overproduction of the RpoS-regulated *hha* gene increases the frequency of transposition of insertion elements within the *E. coli* chromosome as well (Mikulskis and Cornelis 1994). Overproduction of the *rpoS* gene results in increased mutagenesis in growing cells (Yang et al. 2004). Furthermore, the RpoS regulon has been implicated in stationary phase mutagenesis in the *E. coli* and in *P. putida* (Saumaa et al. 2002).

Two molecular mechanisms have been described as being are responsible for the RpoS regulon-dependent increase of stationary phase mutagenesis: induction of the *dinB* gene, and downregulation of the mismatch repair system. Such noncanonical regulation, i.e., LexA independent, of the PolIV TLS DNA polymerase may help the cells to survive certain DNA damages without new protein synthesis. This could be a case with cytotoxic alkylating DNA lesions (Bjedov et al. 2007), which can accumulate in DNA because of RpoS-dependent downregulation of the *alkA* gene. This gene codes for the DNA glycosylase that removes replication-blocking 3-methyladenine and 3-methylguanine (Landini and Busby 1999). In stationary phase cells, the transcription of *mutS* and *mutH* genes and the concentration of MutS and MutH proteins decreases to very low levels via an RpoS- and Hfq-dependent mechanism, compared to the growing cells (Feng et al. 1996; Tsui et al. 1997;

Harris et al. 1997). Consequently, the activity of the mismatch repair system is reduced during stationary phase. The overproduction of the MutS protein—but not the MutL protein—in wild-type cells significantly decreases stationary phase mutagenesis (Bjedov et al. 2003). Similarly, overexpression of the MutS repair protein significantly decreased the rate of G:C T:A transversion mutation in stationary-phase wild-type, *mutY* and *mutM* strains (Zhao and Winkler 2000).

What would be the benefit of inactivating the mismatch repair system, a major contributor to DNA replication and recombination fidelity? Once again, there are two possibilities; either the downregulation of the mismatch repair system activity contributes to survival, or it is selected for because it facilitates adaptation via the beneficial mutations it generates, as is the case for the mismatch repair mutants. Currently, there is no definite answer, but the most likely hypothesis is that the contribution to survival of the downregulation of the mismatch repair activity may be as simple as the energy saved by not expressing these repair functions when nutrients are limited. The absence of the mismatch repair system surveillance results in increased mutagenesis, but the fitness cost of the resulting deleterious mutations is probably much less important than that incurred by energy "exhaustion" due to the production of "useless" proteins under starvation conditions.

Environmental Tuning of Mutation Rates Among *E. coli* Natural Isolates

The availability of essential nutrients—including carbon, nitrogen, and phosphorus—and frequency of different stresses both between different ecological niches. Therefore, it is not surprising that different *E. coli* ecotypes, which have different lifestyles, have different nutritional capabilities and respond differently to the same stresses. In many cases, such variability was shown to result from allelic variation of *rpoS* gene or from polymorphism of its regulatory elements (Ferenci 2003). For example, strains with high constitutive intracellular levels of RpoS protein metabolize fewer substrates and poorly compete for low concentrations of nutrients, and have increased stress resistance (King et al. 2004). Constitutively low intracellular concentration of RpoS has exactly the opposite effects on nutritional competence and stress resistance. These phenotypes are, to a large degree, the consequence of a competition between different sigma factors within the cell; e.g., the absence of RpoS allows a higher level of transcription of RpoD-dependent genes (Nystrom 2004). Therefore, nutritional limitations and environmental stress conditions impose conflicting choices that result in selection of the loss or modification of RpoS function in different environmental niches as a function of nutrient availability, and of the frequency and nature of stresses.

E. coli strains that have increased stress resistance due to high intracellular levels of the RpoS protein have also increased mutation rates (Yang et al. 2004; King et al. 2004). This can be explained by the RpoS dependent regulation of DNA repair genes discussed in this chapter. As we observed that there is a positive correlation